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FOREWORD

The Welsh School of Pharmacy, Cardiff University, is the only school of pharmacy in Wales and is one of the top schools of pharmacy in the UK. Its research has been independently judged to be predominantly of international standing, and more than half is recognised as world-leading or internationally excellent. The quality of teaching and learning has also been deemed "excellent", by the National Teaching Quality Assessment. Our students graduate well-prepared and satisfied, as seen by the consistently high pass rate in the pharmacist registration examination and high ranking in the National Student Survey respectively.

These attributes mean the School attracts large numbers of well-qualified UK, EU and international applicants for its undergraduate and postgraduate degrees. This year saw the first intake of students reading for the Cardiff MPharm degree at Taylor's University in Malaysia, in a partnership arrangement where students spend their first two years at Taylors University and can then transfer to Cardiff to complete their studies.

The Welsh School of Pharmacy recognises the importance of research-led learning and teaching. All of our MPharm students undertake a significant, independent Masters level research project in the final year of the four year degree, and present and defend their research at the Poster Day. The abstracts of these projects are collected here (unless intellectual property and/or other issues preclude publication).

Research at the School is structured into four disciplines, with many interdisciplinary and external collaborations. Our research encompasses medicinal chemistry, pharmacology and physiology, drug delivery and microbiology, and pharmacy practice and clinical pharmacy, thus impacting on many aspects of healthcare and pharmaceutical sciences throughout the UK and the world. Further information on the School's research activities and degree programmes, along with contact details for academic staff can be found at http://www.cardiff.ac.uk/phrmy.

This is the 11th edition of the abstract book, and this year we are pleased to include, for the first time, abstracts from many of our 30 graduating PhD and Masters students.

Within this publication the student is the first named author, and collaborators and supervisors follow. An alphabetical list of authors appears in the index. The following may be used to cite an abstract:
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I am grateful to my colleagues for their assistance in collating this book, most especially to Dr Dai John, past editor, and Dr Keith Brain.

Rebecca Price-Davies
July 2011

Developments in aseptic manufacturing for biotechnology and medicinal products

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During the past 20 years many advances have been made in the development of clinical nutritional support for patients[1]. Parenteral nutrition (PN) delivered from containers made out of a variety of plastic materials such as copolymer Ethylene-Vinyl Acetate (EVA) and Poly-Vinyl Chloride (PVC) allows the provision of all essential nutritional components. The use of these plastics for PN doesn't come without problems, for example, the water vapour and oxygen permeability of these plastics may lead to instability of the PN formulation and therefore reduced the clinical effectiveness of the PN infusions. Another example is the instability of readily oxidisable nutrients, such as vitamins (the least stable nutrient being ascorbic acid), this being a major limiting factor in the shelf life of complete PN admixtures. The aim of this project was to compare the suitability of EVA and new Poly Propylene (PP) containers for the storage and delivery of PN over a time period-mimicking hospital clinical practise for PN storage and delivery, since there is a growing demand for an increase in the shelf life of PN admixtures.

The water vapour and oxygen barrier capability of the EVA and PP containers was compared by assessing the profile of a simple infusion solution of potassium chloride and calcium chloride, by measuring its pH, % dissolved oxygen, potassium and chloride concentrations and weight loss of the solutions over period of 3 weeks. After each week's storage within a fridge, they were then left for 24 hours at room temperature. During manufacture and filling some containers were nitrogen purged to see if this had any effect on the measurements taken. Some containers were also overwrapped with aluminium and plastic covers to see whether this had an effect on the water vapour and oxygen barrier properties of the containers. HPLC was used to investigate the stability of water soluble vitamins (using the Solivito® N preparation) stored in the same EVA and PP material containers over a storage period of 6 days at room temperature.

The results obtained suggest that the PP containers have better water vapour and oxygen barrier properties compared to EVA containers. PP is well known in the manufacturing industry for its good water vapour barrier properties[2&3]. The water soluble vitamin results show that the degradation of ascorbic acid was more significant in the PP containers compared to the degradation in the EVA containers. Some of the results are not as expected and further studies need to be carried out as research into this area is at early stages.

The studies undertaken as a part of this project are not clear enough to make a true decision on which type of plastic material, EVA or PP, is most suitable for the storage and delivery of PN. Although PP containers are more expensive than EVA, this may not be an issue in the long term as PP could provide PN solutions with a longer shelf life and thus make them more cost effective than multi-layered containers. In the near future, they could be the new infusion containers to be used by the NHS.

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Evaluation of the effect of poly[Ethyl Acrylate-co-Acrylic Acid] on fibroblasts *in-vitro*

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The management of chronic wounds represents a major health burden and places a substantial drain on healthcare resources.[1] Although a variety of factors will contribute to the formation of a chronic wound, Whilst within the wound bed, bacteria will respire and ferment, the fermentation products reducing the wound pH to as low as 5.45.[3] Consequently, pH-sensitive polymers could be a useful strategy to combat infection, the low pH activating the polymer. Poly [Ethyl acrylate-co-acrylic acid] (CJ-RR) is a synthetic polymer which has displayed a pH-dependent ability to disrupt the membranes of bacterial cells, hence could be of potential benefit as a wound healing agent. The aim of this study was evaluate the effect of polymer CJ-RR on two different fibroblast cell lines, at pH 5.5 and pH 7.

In the series of experiments, authentication tests such as spectrophotometry and NMR were performed to evaluate the solubility and purity of the polymer respectively. A range of CJ-RR polymer concentrations were prepared in Dulbecco's modified eagle medium (DMEM) and their pH was determined using a pH probe. The cytotoxicity of CJ-RR was evaluated by employing 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) *in-vitro* assay, cell viability being determined following 24 hours and 72 hours incubation.

The ^{1}H and ^{13}C NMR spectra obtained showed a close resemblance to that of previous years, concluding that the final product obtained was infact CJ-RR. The spectrophotometric spectra revealed that CJ-RR was fully soluble in DMEM and that no impurities were present within the sample. From the pH assessment, it was clear that higher polymer concentrations caused a greater decrease in solution pH due to weakly acidic properties of the polymer. The pH of 20mg/ml of CJ-RR was 6.35, in comparison to pH 7.98 for DMEM alone. The results obtained from the MTT assay demonstrated that CJ-RR exhibited time-, concentration and pH-dependent properties. Further experimentation showed that the two different fibroblast cell lines, HCA-2 and T7 hTERT, under evaluatio had different responses to CJ-RR. The proliferation of T7 hTERT appeared to be dependent on pH and length of exposure to CJ-RR whereas HCA-2 displayed more pH and concentration dependent properties. With regards to concentration, it was observed that lower CJ-RR concentrations of around 0.625mg/ml had a stimulatory effect on fibroblast proliferation. However, it was determined that the optimum concentration for HCA-2 proliferation occurred at 10mg/ml. Time-dependent toxicity was also observed, prolonged exposure times of 72 hours proving to have more of a detrimental effect on the cells than 24 hours. Finally, increased cell viability occurred at pH 7. Despite this, fibroblast proliferation was still observed at pH 5.5.

Cell viability at pH 5.5 indicates that the CJ-RR polymer is in fact pH-sensitive and able to induce fibroblast proliferation within the acidic milieu of an infected wound. The time-dependent toxicity related to 72 hours exposure suggests the need for an additional antibacterial to be used in conjunction with CJ-RR. This will reduce the risk of bacterial resistance developing in addition to reducing the concentration of the CJ-RR polymer required. Further work is required to determine the exact concentration of CJ-RR required to achieve maximum fibroblast proliferation. Furthermore, the structure and mechanism of action of CJ-RR needs to be established. This study has produced positive results with regards to the potential of CJ-RR to be used as an agent to aid wound healing.

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Novel gemcitabine protides: design, synthesis and biological evaluation

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Cancer remains one of the major causes of death worldwide. Despite its disadvantages cytotoxic chemotherapy remains leading treatment for some cancers. A good example would be gemcitabine or 2',2'-difluoro-deoxycytidine (dFdC) which is first line treatment for pancreatic and non-small cell lung cancer. This antimetabolite is very effective in solid tumours both as monotherapy and in combination with cisplatin or paclitaxel to also treat advanced bladder, ovarian and metastatic breast cancers.[1] Gemcitabine is a prodrug, it requires equilibrative nucleoside transporter (ENT) 1 and 2 to enter cells as well as three phosphorylations by various intracellular kinases to become active drug metabolites – gemcitabine triphosphate (GemTP) and diphosphate (GemDP). Main mechanism of action is incorporation of GemTP instead of deoxycytidine triphosphate (dCTP) into the DNA stand plus additional inhibition of DNA polymerase. Both pathways leading to cancer cell growth G1/S-phase arrest and apoptosis. Moreover dFdC exhibits unique self-potentiation mechanism as GemDP inhibits ribonucleotide reductase essential for synthesis of dCTP. Also GemTP inhibits deoxycytidine deaminase which converts gemcitabine monophosphate (GemMP) into inactive uridine derivative that is consequently excreted. Hence rate of formation and concentration of GemTP is maintained at high which correlates to increased activity.[2] However there are few mechanisms of resistance via transporters or deoxycytidine kinase (dCK) downregulation and overexpression of the deaminase enzyme. These lead to reduced or no response to dFdC in some cancer patients. This problem can be partially overcome by gemcitabine ProTides-phosphoramidates of dFdC that enter cancer cells by passive diffusion and are cleaved by carboxypeptidase Y and HINT to GemMP. Therefore the first rate limiting dCK phosphorylation step is bypassed as well as there is no need for ENTs.

ProTides or phosphoramidates consist of the parent drug- gemcitabine and phosphorochloridate which contains aromatic and amino acid (AA) ester groups, attached to the drug at 5' position on the sugar moiety. This project was aimed to synthesise novel dFdC ProTides using naphthlyl (Naph) and phenyl (Ph) as aromatic; L-alanine (L-Ala) and dimethylglycine (DMG) AA with pentyl, hexyl and cyclopentyl groups as AA ester. Steps required for production of a ProTide summarised in the table below[3]:

Reaction	Main reagents/solvents	Product (s)	Column eluent
Gemcitabine protection	DBDC/1,4-dioxane and H_2O	3' Boc gemcitabine	DCM/Methanol
AA ester synthesis	Boc AA, alcohol,DCC,DMAP/DCM	N-Boc AA ester	Hexane/Methanol
Deprotection of AA ester	Para toluene sulfonic acid/ EtOAc	AA ester tosyl salt	NA
Phosphodichloridate synthesis	$POCl_3$, naphthol, anhydrous TEA (triethylamine)/ Et_2O (diethyl ether)	Naph phosphodi-chloridate (NPDC)	Hexane/ EtOAc (ethyl acetate)
Phosphorochloridate sy.	AA ester salt, NPDC, TEA/DCM	Phosphorochloridate	Hexane/ EtOAc
ProTide synthesis	3' Boc gemcitabine, tBuMgCl, phosphorochloridate/ anh. THF	Boc protected (3') ProTide	DCM/Methanol
ProTide deprotection	DCM, TFA,$NaHCO_3$/ EtOAc	Free ProTide	DCM/Methanol

Six pure ProTides were successfully made by carrying out steps above. Each reaction product was confirmed using appropriate NMR. Final compounds however were analysed using proton (^{1}H), carbon (^{13}C), fluorine (^{19}F) and phosphorus (^{31}P) NMR as well as HPLC and mass spectrum. This was to confirm that there is no active impurities present i.e. gemcitabine, which can affect biological evaluation. 5 out of 6 compounds were tested on L1210 (leukaemia) and HeLa (cervix cancer) cell lines and displayed very potent anticancer properties as they inhibited proliferation in both cell lines at submicromolar and subnanomolar concentrations respectively. cLog P had no correlation with anticancer activity however it could be seen that cyclopentyl as an ester group and DMG as AA decreased anticancer activity of ProTides, therefore in the future studies it will be the best to use L-Ala and alkyl chain in ester to synthesise potent compounds. CPF31 is a lead clinical compound that contains benzyl group in ester moiety, it was less potent in L1210 cell than 3 of the novel ProTides. Hence in the future there compounds may be considered as an alternative to CPF31 in certain cancers. In conclusion the aim of this project was achieved as novel gemcitabine ProTides were designed, synthesised and evaluated and it is hoped this contributed to current and future ProTide research.

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Mechanisms of action of tryptamine on gastrointestinal contractility

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Tryptamine naturally occurs in trace quantities in the body, particularly the brain and is endogenously produced from the decarboxylation of L-tryptophan. It is also present in our diet, in foods such as meat, cheese, chocolate and various wines[1] and has been implicated in various diseases, particularly schizophrenia. In the past, a number of theories have been proposed in an attempt to explain the mechanisms of action of tryptamine in the gastrointestinal tract. Various studies have shown tryptamine to stimulate contractility of the ileum. Research has also demonstrated tryptamine to be capable of exerting sympathomimetic effects on the body, acting at sympathetic nerves to release noradrenaline. This would in fact cause gastrointestinal relaxation rather than the observed contractions. Other research has found that since tryptamine is a derivative of 5-hydroxytryptamine (5-HT), the two substances would act on the same receptors.[2] The aim of this project was to identify the mechanisms by which tryptamine mediates contractility of the ileum by comparing its actions with 5-HT.

Concentration-response curves of tryptamine and 5-HT were performed on electrically stimulated sections of ileum to establish the receptors involved in producing contractions. The responses measured were: baseline contraction, twitch height and contractile height. Ritanserin, a 5-HT_{2A} antagonist was used to determine its effect on the contractile responses brought about by 5-HT and tryptamine.

A dose-dependent increase in baseline contraction, significantly inhibited in the presence of ritanserin, was observed for both tryptamine and 5-HT, indicating 5-HT_2 receptor activation. Additionally, tissue desensitisation, most likely involving 5-HT_2 receptors, was found to occur in response to tryptamine as well as 5-HT. However, in contrast to 5-HT which caused twitch height reduction with increasing concentration, tryptamine induced dose related increases that were partially blocked by ritanserin. The inhibitory effect of 5-HT on twitch response height was markedly counteracted by ritanserin. This difference in the effect of tryptamine on twitch height indicates the existence of an underlying mechanism distinct from 5-HT receptors.

In conclusion, tryptamine exerts its effect on gastrointestinal contractility by more than one mechanism. From the results obtained in this project, it is evident that tryptamine essentially acts via 5-HT_2 receptors to trigger baseline contraction, but activates a different receptor type to mediate acetylcholine release and hence twitch response. The proposed target belongs to a family of G-protein-coupled receptors that have been characterised in the recent literature and are known as trace amine associated receptors (TAARs).[3,4] The findings of this present study may pave the way for future research into dietary tryptamine and its relation to TAARs in the gastrointestinal tract. Further experiments would need to be carried out to confirm the presence of TAARs in guinea pig ileum.

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Investigating levodopa-induced dyskinesia using manganese-enhanced magnetic resonance imaging and 3D Brain Atlas

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Parkinson's disease (PD) is a neurodegenerative disease affecting mainly motor function which is improved using levodopa (L-DOPA). However, chronic L-DOPA treatment can cause levodopa-induced dyskinesia (LID).[1] Limited therapeutic options for LID pushes the need for more studies on LID to be done. Manganese-enhanced magnetic resonance imaging (MeMRI) is an imaging technique which allows neuronal activities and brain architectures to be investigated.[2] Manganese (Mn^{2+}) is taken up by activated nerve cells via voltage-gated calcium transporters. The accumulated Mn^{2+} causes intensity increase as detected by MRI scanners.[2] The present study evaluates the use of the combined technique of MeMRI with 3D brain atlas in a model of PD/LID and investigates the neuronal activity pattern in brain regions following acute or chronic L-DOPA treatments.

MRI scans were carried out on a hemiparkinsonian model of PD/LID: baseline scan or T0 (after chronic treatment, without Mn^{2+}), 1st scan or T1 (with Mn^{2+}), 2nd scan or T2 (with Mn^{2+} and acute treatement). The Paxinos and Watson rat brain atlas[4] was integrated to fit the MRI software used, modified and overlaid on the scans to measure the intensity and differences in intensity between T0, T1 and T2. The brain regions-of-interest (ROIs) are the within the basal ganglia and the cerebral cortex. Due the gradient bias, the data was normalized using mean factors calculated from the control brain regions.

The brain regions showed highly variable trends with large standard error of the mean. Without L-DOPA treatment between T0-T1, most ROIs in the control and PD groups have higher Mn^{2+} uptake on the non-lesioned side than the lesioned side, while ROIs in the LID group on chronic L-DOPA showed the opposite trend. Between T1-T2, not all ROIs in the PD group on acute L-DOPA treatment have improved Mn^{2+} uptake on the lesioned side. There was also a smaller increase in Mn^{2+} uptake during T1-T2 compared to T0-T1 in all rat groups. Mn^{2+} level in the substantia nigra of the control rats dropped significantly between T1-T2. This observation was repeated in hippocampus regions.

The observations between T0-T1 indicate that while untreated PD causes lack of basal ganglia and cerebral cortex activation, LID causes abnormally high activation in these ROIs.[1] Lower increase of Mn^{2+} uptake between T1-T2 could be caused by the cumulative neurotoxic effect of Mn^{2+} or Mn^{2+} saturation in the neuronal cells.[2] The significant drop of Mn^{2+} level in the substantia nigra could be caused by efflux mechanism driven by the surviving GABA-ergic substantia nigra *reticulate.*[3] This combined technique seems to be more sensitive to hippocampus instead of the basal ganglia or cerebral cortex. This could be due to their larger and more defined area for intensity measurement, suggesting that the combined technique have more potential in the study of Alzheimers. However, this combined technique is still potentially useful in PD and LID study provided that appropriate methodology improvements are made to improve its robustness and sensitivity.

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A comparison of final year medical students' and junior doctors' views on their training and ability to prescribe antibiotics

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Doctors and hospitals rest a heavy reliance on antibiotics. At any one time, approximately one third of hospital patients are receiving antimicrobial therapy[1]. Media reports on hospital-acquired infections and bacterial resistance regularly incite public anxiety. News headlines such as ''Young Doctors 'putting lives at risk' through lack of training in prescribing drugs'[2] highlight the customary view that medical students are not prepared adequately for prescribing. This project was conducted in Ysbyty Gwynedd (Bangor) and aimed to investigate final year medical students' and junior doctors' in views on their training and ability to prescribe antibiotics. In addition, different education and training, in terms of formats already received by medical students and junior doctors on antimicrobial prescribing was documented and their preferences and perceptions of each of these were assessed.

Ethics approval for the study was obtained. A multi-method approach combining the use of semi-structured interviews, a quantitative questionnaire and a case-based assessment was used. Background interviews were conducted with a purposive sample of four hospital staff. Using a literature review and *ad verbatim* interview transcripts, a questionnaire and a confidence-rating case-based assessment was designed in collaboration with antimicrobial pharmacists. After piloting, review and amendment, it was distributed to 41 participants during three non-antimicrobial-related teaching sessions for self-completion by fifth year medical students and junior doctors.

All interviewees believed medical students had little confidence in prescribing. The questionnaire achieved a response rate of 97.5% (n=40). Junior doctors expressed stronger agreement in confidence than medical students in prescribing antibiotics in secondary care. (P<0.001) The majority of medical students (70%) disagreed or strongly disagreed that their medical education had adequately prepared them for antibiotic prescribing and the majority of respondents (62.5%) strongly disagreed or disagreed that it contained enough teaching on antibiotic prescribing. In total, 95% of respondents strongly agreed or agreed that the BNF and microbiologists increased their competence, as did 85% in relation to the antimicrobial pharmacist. In total 87.5% strongly agreed or agreed that workshops led by infection specialists increased competence. In the assessment junior doctors had a higher proportion of answers correct (70.5%) in comparison to medical students (60%). Of these answers, junior doctors had a higher proportion (49%) of 'usable data' (both correct and confident) compared to medical students (19.1%).

The study achieved a respectable response rate of 97.5% and helped bridge gaps in literature. Although there is room for improvement in antimicrobial prescribing amongst junior doctors, a certain level of knowledge illustrated by the percentage of correct answers was possessed by the majority. However, medical students were not confident in prescribing and expressed general dissatisfaction with the medical undergraduate education in preparing them to prescribe. It is apparent that medical schools are not compensating for the loss of clinical pharmacology[3] teaching vital for antibiotic prescribing. In contrast to the current literature[4], the majority of junior doctors in Ysbyty Gwynedd are generally confident in antimicrobial prescribing. Identifying the questions in the case-based assessment that frequently received incorrect answers establishes educational needs that can be addressed. As for the hospital trust the study was conducted it in, it is now their decision as to whether they will consider the areas of improvement highlighted by this study. Strategies to address these needs can include utilising workshops held by multidisciplinary teams, which are clearly favoured by participants. Further studies using larger numbers of subjects from a variety of medical schools and hospitals is required to confirm whether the findings from these studies are representative of medical students and junior doctors in the UK.

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Permeation study of ketorolac across an *ex-vivo* porcine bladder model

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Intravesical drug delivery involves the direct administration of drugs into the bladder[1]. This route of drug delivery is useful in urinary conditions as it minimises systemic side effects and gives a greater exposure of target tissues to drugs[2]. There is a general lack of scientific data about tissue concentrations that are achieved with therapeutic concentrations of intravesical medication[3]. This study aims to assess the barrier function of the bladder by assessing the permeability of ketorolac and oxybutynin through a full thickness porcine bladder wall. In addition this study will investigate the tissue concentrations of ketorolac required to deliver a therapeutically active concentration within tissue.

The bladder permeability, of varying concentrations of ketorolac and oxybutynin were tested using a Franz Cell permeation study. Tissue samples were taken and separated into two layers to evaluate the tissue concentrations within the urothelial and muscle layers, while samples from the receptor phase allowed permeation through the whole bladder wall to be determined.

From the results it was evident that ketorolac is able to permeate into the bladder wall and yield appreciable drug concentrations in a dose dependent manner. We were also able to obtain data on the tissue concentrations that are obtained with a clinically relevant dose of ketorolac. Higher ketorolac concentrations within the urothelium layer were observed which indicated that the urothelium was the rate-limiting barrier in the permeation of ketorolac into the bladder wall. Further studies indicated that a greater proportion of ketorolac is deposited within the muscle layer than in the urothelial layer after both layers reached steady state concentrations.

This study has revealed the tissue concentrations that are attained from a clinically relevant dose of ketorolac. Furthermore this study has shown that the concentration of ketorolac permeated into the bladder wall increases with increased dose applied to the urothelium.

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A qualitative assessment of manufacturers' usage instructions for household rubber gloves

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Rubber gloves are a popular and useful way of avoiding direct contact from wet work, chemicals and unhygienic materials that may be found in the domestic environment. They are primarily used in cleaning activities. Gloves for occupational use are required to be tested by manufacturers and employers to ensure fitness for purpose[1]. However, consumers are faced with a selection of gloves from a variety of outlets. Glove manufacturers include storage instruction for users and provide warnings of conditions known to accelerate degradation, such as moisture, sunlight and heat. The project aim was to establish the relevance of these instructions to the gloves' functionality.

The first objective was to imitate the damage likely to occur to rubber gloves in a household environment by exposing glove samples to a range of typical conditions. Samples cut from four glove brands were distributed into test groups which then underwent measured exposures to bleach, detergent, white spirit and light. A negative control group and a dark control group were also used. The second objective was to compare physical properties between brands and between treated and control glove samples. Permeability was tested in Franz diffusion cells using the test permeant benzoic acid. Receptor phase samples were analysed for benzoic acid concentrations by HPLC-UV. Tensile strength was measured in terms of the load required to displace the sample by a specified distance. Thickness measurement required the use of a micrometer.

No permeation was measured through negative controls so any permeation observed through test samples was assumed to be attributable to rubber degradation. Permeation was seen through at least three out of four brands after exposures to each test agent. Tensile strength and thickness measurements supported the permeation data by supplying more evidence of change to materials; these changes were more significant in samples exposed to chemical agents. The greatest total receptor benzoic acid concentration was seen through samples exposed to bleach and all brands were affected by this agent. Interesting results were observed with the increasing frequencies of glove exposure to white spirit applications; degradation was expected to happen to the greatest extent in samples exposed to the most white spirit but these did not show more permeability or change in tensile strength or thickness. Some brands appeared to be more resistant to the effects of test agents than others.

Data show that manufacturers' instructions are valid and relevant. Using a wide range of typical agents was a strength of the study as it allowed scope for exploration and commentary. One brand's manufacturer did not include any guidance for use even though the data showed that it was not the most resistant. Apart from including the word 'rubber' in the package description, no brand made any reference or warning regarding allergy to latex, which affects an increasing population[2,3]. Limitations of the study design include lack of repetition and the assumption that the glove samples did not have holes or punctures caused by mechanical trauma. The unrepresentative 'immersion' of samples and exposures to single agents only potentially made the glove damage observed atypical. Improvements to the study include performing repetitions, testing barrier integrity and testing gloves that have been worn by volunteers.

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Extended analysis of the factors used to predict the permeability coefficient (k_p)

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Despite its barrier role, drugs cross skin with rate (flux (J)) given by the product of a permeability coefficient (kp) and the concentration of drug on the surface (C).

$$J = k_p * C^{1}$$

The permeability coefficient has the units of distance/time representing the rate at which the drug moves within the barrier. In the pharmaceutical industry, this is important as it aids safe dosing regimes that give drug concentrations within the therapeutic window. For this reason, many researchers have investigated k_p in an attempt to create a quantitative structural permeability relationship (QSPR). A number of QSPR's[2] and factors have been proposed, with the current gold standard being the equation suggested by Potts and Guy in 1992[3]. Despite the array of factors suggested as predictors of kp there is limited evidence that all the factors have been pooled into one analysis to find the most important and relevant factors. The aim of this study is to investigate factors that have been published in an attempt to find the most important predictor combinations for the permeability coefficient, and find a possibly novel QSPR which is reliable, robust and based upon readily available predictor properties.

A database was created which contained two hundred and twenty two compounds with published experimental values for k_p. Data was confined to reports of invitro kp values across human skin for aqueous donor/receptor compartments and non-ionised drugs. (Species: Human, Vehicle: water). Molecular properties used as predictors: Molecular weight (MW), octanol/water partition coefficient (P), aqueous solubility as mole/l (S), hydrogen bond donor and acceptors group numbers (HB,HA), Abraham scaled values of donor and acceptor H-bonding capacities (α,β), Hildebrand solubility parameter (δ), were calculated or collected from various sources. The database was then refined to a final balanced array, which only contained records that had a full dataset for all the predictors. This enabled a true comparison of the predicting powers of the various predictor combinations. Regression analyses and other statistical tests were performed using Minitab16 software.

The results indicated that logP, logS and molecular weight were the most relevant factors for calculating k_p. These led to further which followed three lines or enquiry. An analysis using logP and molecular weight (Potts and Guy predictors from 1992), an analysis using logP, logS and molecular weight (major investigation point) and an analysis which considered all eight predictors (secondary investigation as five of eight predictors not relevant to k_p). Analysis showed there was a slight improvement to the R^2 adjusted value when logS was added to the based on MW and logP – those used in the "Gold Standard" QSPR of Potts and Guy which is currently widely used. Further paired t-tests indicated there was no statistical difference between either the Potts and Guy QSPR and the extended Potts and Guy which considers logS.

There is thus no evidence that any improvement is gained by introducing predictors other than MW and logP, although the original Potts and Guy equation should be modified to figure 1 as more data are available and Potts and Guy used some data now known to be unreliable.

Figure 1: logkp (cm sec^{-1}) = -2.12 - 0.00838 MW + 0.606 LogP Mean

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Investigations into transungual barrier properties

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The nail acts as a barrier protecting the soft tissues of the fingers and toes from damage.[1,2] It is this property that is problematic when targeting topical drug treatment to the nail, consequently resulting in treatments being limited and of little success.[2] Extended treatment durations, difficult regimens and relapses are common.[2,3] Diseases of the nail are an increasing problem with onychomycosis and psoriasis being predominant.[1-3] It is evident that further research and development in the treatment of nail disease is required. The aim of this research was to establish the effects of differing conditions including humidity and hydration along with the effects of the possible penetration enhancers cysteine, hydrogen peroxide urea, sodium dodecyl sulfate (SDS), thioglycolic acid and urea on transungual penetration. In addition, attenuated total reflectance – fourier transform infrared (ATR-FTIR) studies have been utilised. Objectives were to gain a greater understanding of the changes that occur within the nail's structure to facilitate improvements in current treatments.

Electrical resistance data was utilised as an indication of possible alterations within the structure thereby affecting penetration. Resistance measurements were taken both through and along the nail plate over time with application of an alternating current. In vitro samples were investigated in dry, hydrated and controlled humidity environments. They were also exposed to the intermittent application of a current and additionally investigations under mimic in vivo set-up. Resistance readings were obtained for samples pre-treated with possible penetration enhancers. Electrical capacitance was also studied. Furthermore, treated samples were examined by ATR-FTIR in an attempt to identify structural changes.

Resistance data of hydrated samples demonstrated an initial increase in resistance to a peak value, before unexpectedly declining to an average 'baseline' value, similar to that obtained with dry nail samples at around 157 kilo ohms (KΩ). Despite this incomprehensible data, the phenomenon was reproducible throughout. Comparable peak values were obtained for readings both along and through the nail. Set-up mimicking in vivo conditions indicated a relatively stable baseline, this relating the changes observed in resistance to the nail drying. With samples subjected to controlled hydration conditions, the highest resistance values were experienced at the lowest humidity. It has been demonstrated that results obtained are not a direct consequence of either applying the current, nor a result of the nail acting as a capacitor, this having been previously proposed.[4] Finally significant changes were observed in both resistance curves and ATR-FTIR spectra where nails had been pre-treated with possible penetration enhancers.

Phenomenon observed with hydrated resistance data was unexpected with no obvious explanation. A previously proposed theory of the nail acting as a capacitor[4] was invalidated, however, with no alternative explanation for the results. Evidence suggests changes could be attributed to alterations within the nail, specifically transformed geometry due to variation in water content. Clear distinctions can be seen in results obtained with pre-treated samples, suggesting possible alterations in penetration of the nail plate, warranting further investigations on a more comprehensive scale. Additionally results have concluded the possible beneficence and utilisation of infrared spectroscopy in possible identification of enhanced penetration of the nail plate.

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Design and presentation of a computer-assisted learning (CAL) package on diabetes mellitus type 2: pathophysiology and treatment

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Internet-based technology and its accessibility have facilitated many possibilities for providing pharmacy education using modern strategies. Computer Assisted Learning (CAL) encompasses a range of computer-based packages, which are focused on delivering interactive and flexible instruction usually on a specific topic. They are designed in such a way that they can help to provide students with a learning experience that can be utilised in addition to, or as an alternative to traditional teaching methods[1]. Previous studies evaluating this method have shown that individuals can learn at a similar level compared to conventional teaching[2]. The aim of this study was to create a CAL package that provided comprehensive didactic instruction on diabetes type 2: pathophysiology and treatment and evaluate its effectiveness as a teaching medium.

The package was constructed using Microsoft PowerPoint® software and included material about the pathophysiology of diabetes type 2, its treatment, the related complications, and the treatment of the complications within the remit of the pharmacist. The information was gathered from current clinical guidelines and reference books, to ensure that the package reflected the most up-to-date evidence-based practice. The package was designed using a professional colour scheme that only incorporated other colours for clarity, made extensive use of diagrams and images to aid understanding and it included a multiple choice quiz at the end. The value of the package for teaching purposes was appraised by final year students based on assessing three areas of the package, presentation, content and overall impression. The online software, Google Docs®, was used to provide a questionnaire which employed a 5-point Likert scale and a facility for free text comments. The Google Docs® software recorded the results into a Microsoft Excel® spreadsheet which was used for the statistical analysis of the Likert scale responses.

The CAL package was well received by the undergraduate cohort. The response rate was 23 final year students out of a possible 103 (22%). Package Presentation: respondents agreed the package used an appropriate colour scheme which allowed the information to be easily understood (82%, n=19) and the diagrams and images helped them to understand the information (74%, n=17). Comments provided by the respondents were related to the success of the classic theme and the diagrams facilitated the learning process. Suggestions made regarding improvement, involved the use of darker text on the light background in some areas. Package Content: respondents agreed that the package covered all the relevant information (76%, n=18) but should have clearly indicated the role of the pharmacist (74%, n=17). Proposed improvements included a more in-depth information provision regarding diet and the physiological role of insulin. Overall Impression: respondents confirmed that the package complemented the course (88%, n=21); would benefit their practice as a pharmacist (96%, n=22); was useful if there was weakness with a topic (91%, n=21); and a self test section was useful (100%, n=23). The comments provided referred to the value of the package for revision and improvement of the package by increasing the quantity of questions for self testing.

The majority of the final year students believed that the package was well presented, comprehensive, and was a practical teaching aid. However, several improvements were suggested, such as using darker text on the light background in some areas, including more detailed content in some areas and provision of more questions in the self test section. In conclusion, the package was a good reference tool that should be available to accompany lectures and to expand student choice of learning.

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Antibiotic prescribing practice: an evaluation of junior doctor and medical students' perceptions of their educational needs in comparison with their measured actual needs and of the success of different educational programmes

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Antibiotics are frequently prescribed across many different medical disciplines often by junior doctors. Ineffective antibiotic prescribing can have serious affects on morbidity and mortality, therefore it is essential junior doctors are proficient in effective antibiotic prescribing which can often be a complex process[1,2]. A prerequisite to which is as an understanding of the theory behind prescribing and therefore antibiotic prescribing education is delivered to both medical students and junior doctors[2]. Concerns raised regarding junior doctor's prescribing competence have produced calls for changes to education[3,4]. The project aim is to compare the educational needs of junior doctors and medical students at different stages of their career to inform further developments in antibiotic education. This project is in collaboration with two others projects in the same Health Board.

Following ethics approval, semi-structured interviews with key informants and information from a literature review were used to inform the development of a questionnaire. The self completion questionnaire was semi-structured, containing mainly closed questions utilising attitude statements with 5 point Likert scales. Design was collaborative with the sister projects. A multiple choice prescribing assessment with negative marking and a maximum score of 33, was designed to test prescribing competence and confidence and created by the project supervisors. The questionnaire and prescribing assessment were combined and piloted then data was collected during non antibiotic teaching sessions. The prescribing assessments were marked by the supervisor, data was then anonymised, coded and entered into SPSS and Excel spreadsheets for analysis.

All three interview respondents believed that inadequate knowledge amongst junior doctors is leading to ineffective prescribing and that changes to education interventions can potentially have a positive impact on prescribing. Fourteen completed questionnaires and prescribing assessments were returned. Eleven of these were from first year junior doctors and three were from second year junior doctors. Answers showed all respondents had received undergraduate lectures as part of their antibiotic education. Respondents considered on ward teaching from microbiology to be the most useful teaching method and the BNF and microbiology consultants the most useful resource with almost all (n=13, n=14, n=14) agreeing they were useful. All respondents reported they were confident with antibiotic prescribing in secondary care and assessing patients for the signs of sepsis. A majority (n=11) thought undergraduate antibiotic teaching should be increased. Scores in the prescribing assessment ranged from 32 out of 33 to -19 with an average of 7.6.

Overall, the questionnaire results illustrated that the respondents had mixed views on their antibiotic education. Prescribing assessment scores were very low but this may not be a true indicator of low prescribing competence due to the high impact of negative marking. The most significant limitation of this study was the low number of respondents. It is essential junior doctors are competent and confident in antibiotic prescribing and this study shows that competence may be lacking among some junior doctors. Further research is needed to ensure future developments to antibiotic education have maximum benefit on antibiotic prescribing competence.

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The synthesis of perillaldehyde derivatives as potential anticancer agents

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There are over 3000 species of plants that could provide chemotherapeutic effects. More than 60% of the current antitumour agents are derived from natural source.[1] Perillaldehyde (PA) is an essential oil extracted from Perilla Frutescens leaf. PA has been reported to exhibit broad spectrum antimicrobial properties. Apart from that, PA is found to be a potential calcium channel blocker which led to vasodilatation of the aorta. Recent study reported that PA showed antioxidant properties by upregulating thioredoxin (Trx) gene expression.[2] Oxidative stress is associated with the process of carcinogenesis. Antioxidant systems like Trx are found in the body to scavenge ROS. We proposed that perillaldehyde would have antitumour properties by interacting with the thioredoxin systems. However, the α, β- unsaturated aldehyde is the main issue to be concerned in PA. It is reactive and can bind covalently to proteins, nucleic acid, lipids and enzymes. The aldehyde group is potentially cytotoxic and genotoxic and is infrequently present in drug molecules.[3]

In this study, novel perillaldehyde derivatives with different functional groups were synthesised to overcome the selectivity and toxicity problem. Oxime ethers and oxime esters were chosen to mask the reactive aldehyde moiety. The first synthetic route was conversion of Perillaldehyde to oxime (KTC1) by treatment with hydroxylamine. KTC1, perillartine is used as a sweetener in Japan. KTC1 was used as starting material for the synthesis of KTC 2 to KTC 6 compounds. KTC1 was transformed to KTC 2 and 3 by etherification with alkyl halide. On the other hand, KTC 4, 5 and 6 were generated by esterification of KTC1 with acyl halide. Compounds were characterised by NMR analysis and mass spectrometry.

Experimental results:

Comp	Reagents and conditions	Yield (%)	cLogP	Melting Point (°C)
KTC 1	PA, $NH_2OH.HCl$,CH_3COONa, EtOH, stirring at 70°C, 1hr	76	2.80	86-88
KTC 2	KTC 1, MeI, KOH, DMSO, stirring at rt., 35min	60	2.93	-
KTC 3	KTC 1, BnCl, KOH, DMSO, stirring at rt., 35min	64	4.24	-
KTC 4	AcCl, Et_3N, DMAP, stirring at rt., overnight	55	3.22	50-52
KTC 5	Benzoyl chloride, Et_3N, DMAP, stirring at rt., overnight	59	4.66	90-92
KTC 6	p-Nitrobenzoyl chloride, Et_3N, DMAP, stirring at rt., overnight	34	4.40	144-147

All compounds formed are thought to be *Trans* isomers. It was justified by the single peak at C_{10}- H position of the NMR spectra and the comparison with existing analytic data for PA oxime.[4] The Michael acceptor (MA) that responsible for antitumour activity was retained in all five novel compounds formed. The MA of the analogues has potential to interact with nucleophilic thiol groups (Selenocysteine and Cysteine) of the proteins (Trx reductase, KEAP1, ErbB2 kinase, Hsp and p53 tumour suppressor). The bioavailability of compound KTC 2-6 is predicted to obey Lipinski rule of five. These novel analogues were all synthesised by one-step reaction from PA oxime. Five novel perillaldehyde oxime ether and oxime ester analogues were successfully synthesised. These perillaldehyde derivatives were designed as anticancer agents by the potential of interaction with thiol group in tumour cells. Antitumour evaluation is required to allow direct comparison with existing antitumour agents.

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Factors influencing choice of postgraduate pharmacy programme

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Continuing Education (CE) is a lifelong process building upon the foundations of existing skills and knowledge, with the aim to continuously improve standards of care[1]. Pharmacy is changing as a profession, with higher expectations and more responsibility presented to community and primary care pharmacists[2]. Higher Education Institutions (HEIs) offer postgraduate courses as a form of CE. Unlike hospital pharmacy, the link between studying a postgraduate course and career progression is unclear; therefore this study aims to investigate the motivational factors of community and primary care pharmacists to study a postgraduate course and factors that influence the choice of study.

Participants of the study were current students on the Welsh School of Pharmacy Diploma in Pharmacy Clinical Practice (Community and Primary Care), which will be referred to as the Diploma. Purposive sampling was conducted to select between eight to ten students of the current cohort of eighteen Diploma students. Characteristics considered during sampling were gender, the number of years of practise, the year of study on the postgraduate programme and the location and type of pharmacy for which the student currently works. A qualitative approach was adopted and an interview schedule produced and piloted face-to-face on one past student of the Diploma. Students identified were invited to participate in a telephone semi-structured depth interview (SSDI). Interviews were audio recorded, transcribed verbatim and thematically analysed. Themes identified were used to inform the development of appropriate scales and items to include in a questionnaire.

A total of seven SSDIs were conducted (including pilot), five were telephone and two face-to-face interviews. Seven broad themes were identified which were further divided into sub-themes. The broad themes identified were as follows: Motivational factors; barriers to studying; approach to finding the right course; factors influencing choice of study; application and enrolment; positive features of the course and ideas for the promotion and marketing of the course.

This qualitative approach yielded a range of views from students' perspectives and informed the development of appropriate scales and items to include in a questionnaire. Not all students identified by purposive sampling responded resulting in only one male student. There was no representation from a pharmacist working in a small independent pharmacy. Further work is needed in the piloting and formatting of questions to develop a final questionnaire. This can be distributed to all current and past students of the WSP Diploma to investigate the factors which influence their decision to commence postgraduate study and the choice of postgraduate programme. The questionnaire could also be adapted to be distributed more widely to all community pharmacists in Wales to investigate barriers to enrolling on a postgraduate programme.

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Evaluation of the education, training and development postgraduate taught module for qualified pharmacists

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In 2010, a new postgraduate module enrolled its first cohort, for module PHT734: Education, Training and Development. This new module was produced by the MSc in Clinical Pharmacy in collaboration with the WCPPE, to offer work based education and training advice for diploma tutors, pre-registration tutors and teacher practitioners. The course ran from July 2010 until November 2010 and the module participants (n=9) were required to attend two 1 day study days and to complete a portfolio and an assignment on the topic of their choice[1]. The aim of the study is to evaluate the education, training and development module and to discover whether the module was 'fit for purpose'.

The study used the 'triangulation method' where both qualitative and quantitative data was collected, to help prove the validity of the study data [2]. Therefore, the study was conducted in two parts, firstly an assessment of the participants portfolios and assignments, followed by a semi-structured qualitative interviews with the participants. The participant's portfolios and assignments underwent analysis for several key topics mainly concentrating on the participants self needs analysis and personal development needs at the beginning (time 0) and the end of the module (time 1). The participants learning styles, key learning points on reflection, and their chosen assignment topic were also investigated. Data collated from the portfolios and assignments was entered into a Microsoft Excel® database. Semi-structured qualitative interviews were also conducted either via telephone or face-to-face. Interviews were conducted to discover the participant's views of the module and whether they had experienced a change of practice. The interviews were tape recorded and transcribed verbatim into a Microsoft Word® document and thematic analysis was conducted.

Of the nine pharmacists enrolled on the module, seven of the participants consented for an interview and eight participants consented for their individual portfolio and assignment to be analysed. The analysis of the portfolios found that the participants self needs analysis improved between time 0 and time 1. Applying adult learning, determining students learning styles and assessment methods were the needs that the participants identified as having most improved on while being enrolled on the module. The participants identified feedback, learning styles, lesson planning and assessment methods as points they needed to develop on the first personal development plan at time 0. However, the points identified on the personal development plan for time 1 included assessment methods which was the only point identified at time 0. After undertaking a Honey and Mumford Learning Styles test, half of the participants identified with the same learning style (Reflector). Furthermore, half of the students also identified the same personality style (ESFJ) after completing a Myers Briggs test. The assessment of the student's assignments also discovered that seven out of the eight participants choose feedback as the topic of their assignments. The interviews concluded that the majority of the participants considered the portfolios to be useful, although it involved a lot of work. Very few participants had any knowledge of learning styles before the module, although now all participants could give examples where they have changed their practice. Most participants spoke of implementing lesson plans and gave examples of where they had implemented new teaching methods. All of the participants suggested they would recommend the module to a friend and they believed it was 'fit for purpose', although there were suggestions made about the number of study days.

All the participants showed an improvement in their self needs analysis and in their self-confidence after completing the module. In addition the results have shown that pharmacists do not identify with a single learning style and this has been reflected in the study by Austin [3]. The working practices of all the participants were shown to have changed due to this module, therefore the module achieved its aim, and was 'fit for purpose'. The General Pharmaceutical Council are introducing new standards for pre-registration tutors [4], a module such as this will ensure all tutees receive the same standard of teaching from their tutors. Further work with the next cohort of students may need to be considered to confirm whether more teaching of assessment methods is required. Consequently, a recommendation for the module could be to supplement the study days with online modules.

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Pharmacology of cannabinoids in LPS-induced inflammation in isolated ileum

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Cannabis sativa, more commonly known to people as the marijuana plant is the source of cannabinoid compounds that mediated their pharmacological effects via CB_1 or CB_2 receptors.[1] Cannabinoid receptors are located throughout human and animal bodies, with the receptors located in the brain and gastrointestinal tract being of particular scientific interest.[2] Inflammatory Bowel Disease (IBD) is a chronic relapsing condition consisting of Crohn's Disease (CD) and Ulcerative Colitis (UC), the cure and aetiology of them is unknown.[3] Lipopolysaccharide (LPS) is a major constituent of the cell wall of gram-negative bacteria and is frequently used in research to mimic inflammation and infection in tissue,[4] producing a suitable pseudo inflammatory response for an *in vitro* study. The main aim of this project was to further investigate the use of a potent cannabinoid WIN55,212-2 (WIN) to protect the LPS induced dysfunction of the electrical field stimulation (EFS) evoked contractile response in the isolated guinea-pig ileum. Any protective effect mediated by WIN would be pharmacologically characterised to determine if a specific cannabinoid receptor is involved.

Isolated ileum was incubated in an organ bath consisting of Kreb's solution and mounted on a stainless steel electrode that induced contraction by EFS. Tissue preparations were left to stabilise for 30mins before experimental procedures began. Initial aims, were to establish the effects of WIN and LPS on the EFS-evoked contractions over a two hour incubation period. Later studies involved 20 minute incubation of rimonabant and AM630, CB_1 and CB_2 selective antagonists respectively, to determine the pharmacological action of WIN. Statistical significance was determined using a one sample Student's t-test for comparison of one mean to the hypothetical mean of 100 and an unpaired Student's t-test for comparison of two means.

LPS (16ng/ml) induces dysfunction on the EFS-evoked contractions such that after a two-hour incubation period contraction was reduced to 31.3±8.5% initial contraction. Pre-treatment with WIN ($1x10^{-8}$M) significantly reduces dysfunction induced by LPS (71.5±12.7% initial contraction), providing a protective effect. Rimonabant, CB_1-selective antagonist did not antagonise the protective effect of WIN on LPS, but potentiated the protective effect (85.0±7.3% compared to 71.5±12.7% initial contraction). Whereas, the CB_2-selective antagonist, AM630 inhibited the protective effect of WIN on LPS (54.2±6.2% compared to 71.5±12.7% initial contraction). To characterise the pharmacological effects of the antagonists, each antagonist was incubated in turn with WIN. Both antagonists showed to have large direct effects causing tissue relaxation rather than inhibiting the relaxation induced by WIN. AM630 showed a significant (P<0.05) direct functional relaxation for almost the two hour incubation.

LPS induced a pseudo inflammatory response, resulting in dysfunction on EFS-evoked contractions. WIN ($1x10^{-8}$M) has shown to have a protective effect on the pseudo inflammatory response that is induced by LPS on the isolated guinea-pig ileum. Rimonabant, showed no effect on the protective effect of WIN. Therefore, CB_1 receptors are not involved in the WIN protective effect. Whereas, AM630 showed to inhibit the protective effect of WIN, suggesting that protective effect of WIN is via CB_2 receptors. However, both of the antagonists have shown to cause direct relaxation on the tissue contractions, rather than indirectly acting to protect the LPS dysfunction by inhibiting WIN. Therefore, the protective effect of WIN through CB_2 receptors is inconclusive. Thus, data observed in this study shows the protective effect of WIN is neither mediated via CB_1 nor CB_2 receptor subtypes. The specific pharmacological signalling pathway for WIN still has to be fully determined, further studies with selective agonists and antagonists of CB_2 and other known receptors should assist to determine the mechanism of WIN. However, this study has shown that the development for cannabinoid-based therapeutics as anti-inflammatory agents for conditions such as IBD, is a potential for further investigations.

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Phosphorylation status of the kinases Akt and GSK-3 during myocardial ischaemia/reperfusion

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Myocardial ischaemia is the result of compromised blood supply to an area of the myocardium[1]. Reperfusion injury is the term used to describe the additional injury caused by restoration of blood flow during reperfusion. Furthermore, this injury is attributed to opening of the mitochondrial permeability transition pore (mPTP)[1]. The heart has an intrinsic adaptive response to ischaemia, which activates the reperfusion injury salvage kinase (RISK) pathway, consisting of signalling from PI3k-Akt and ERK/12 (extracellular regulated kinase 1/2), which prevents mPTP opening and calcium overload[1]. Natriuretic peptides including ANP and BNP are endogenous hormones which cause vasorelaxation and natriuresis[2]. When administered during reperfusion, they are cardioprotective independent of their hormonal effects by acting on the NPR-A receptor stimulating the production of cGMP[3]. cGMP activates PKG which prevents mPTP opening indirectly through a signalling pathway which was thought to be independent of the RISK pathway[3]. However, it was recently shown that wortmannin (PI3K inhibitor), the soluble guanylate cyclase (sGC) inhibitor ODQ and the ERK1/2 inhibitor PD98059 abolished ANP's protective effect, implying PI3K-Akt-eNOS-NO-sGC signalling was involved[4]. There is currently only pharmacological evidence to support this and no direct biochemical evidence to corroborate these findings. Therefore, we hypothesized that treatment with the natriuretic peptide BNP during early reperfusion after a period of ischaemia will result in increased phosphorylation and therefore activation of the protective Akt and inactivation of the injurious GSK-3. These effects will be blocked by wortmannin, the inhibitor of the upstream kinase PI3k.

Isolated hearts were perfused according to Langendorff. Regional ischaemia was introduced by inserting a ligature behind the left descending coronary artery and forming a snare to allow reversible occlusion. There were two groups: ischaemia/reperfusion and normoxic perfused hearts. Within these two groups there were four subgroups: treatment with 10mM BNP, treatment with 100nM wortmannin, treatment with BNP and wortmannin and time-matched controls. All hearts underwent 20 minutes of stabilisation and then hearts in the normoxic-perfusion group were perfused with drug treatment for fifteen minutes. Hearts in the ischaemia-reperfusion group then had 35 minutes of ischaemia; treatment was perfused five minutes before the end of ischaemia then during 10 minutes of reperfusion. The proteins Akt, p-Akt (serine 473), GSK-3 and p-GSK-3 (serine 9) were detected using Western blotting. Optical densitometry was used to quantify the blots, assessed as ratios of phosphorylated:total protein expression normalised to average control. Statistical analysis was performed with PRISM statistical analysis software using One-Way ANOVA plus Newman-Keuls post hoc test. Values are expressed as mean ± standard error of the mean (SEM).

In ischaemia/reperfusion, BNP did not significantly increase the expression ratio with both GSK-3 and Akt compared to control (Akt: control 1, BNP 1.14 ± 0.36, GSK-3: control 1.00, BNP 1.30 ± 0.15, both ns), but treatment with BNP and wortmannin did result in a significant decrease in expression ratio compared to BNP alone with both proteins (Akt: BNP 1.14 ± 0.36, BNP + wortmannin 0.20 ± 0.12, $P<0.05$. GSK-3: BNP 1.30 ± 0.15, BNP + wortmannin 0.67 ± 0.11, $P<0.01$). In normoxic perfusion hearts, BNP treatment resulted in a marked increase in the expression ratio by approximately threefold with both proteins compared to controls (Akt: control 1.00, BNP 2.94 ± 0.38, $P<0.0001$. GSK-3: control 1.00, BNP 3.12 ± 0.97, $P<0.05$). Treatment with BNP plus wortmannin also resulted in a significant decrease in the expression ratio compared to just BNP with both proteins (Akt: BNP 2.94 ± 0.38, BNP + wortmannin 0.53 ± 0.10, $P<0.0001$. GSK-3: BNP 3.12 ± 0.97, BNP + wortmannin 0.28 ± 0.10, $P<0.05$).

The results of the present study suggest that in ischaemia/reperfusion, BNP does not significantly increase the expression of p-Akt or p-GSK. Treatment with wortmannin lowered the expression of both phosphorylated proteins compared with BNP alone. However, in normoxic perfused hearts, BNP treatment does produce a significant increase in p-Akt and p-GSK-3, with wortmannin reducing this effect, suggesting that GSK-3 is downstream of Akt. Whether GSK-3 is the point of convergence of the PI3K-Akt and ERK1/2 pathways still remains uncertain.

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Review of the use of filters during the manufacture and administration of parenteral nutrition in the United Kingdom

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Parenteral Nutrition (PN) is increasingly being used to provide nutritional support to patients who have some form of intestinal failure[1]. It has been estimated that an intensive care patient often receives more than 10^7 foreign particles greater than 2µm in any given 24 hour period as a result of receiving intravenous therapy[2]. In 1994 the Food and Drug Administration (FDA) issued a safety alert referring to 2 deaths and at least 2 cases of Adult Respiratory Distress Syndrome (ARDS) following a peripheral infusion of three-in-one PN admixtures[3]. In response to this alert, the British Pharmaceutical Nutrition Group (BPNG) set-up a working group to establish whether, UK pharmacists were filtering their PN so as to prevent such clinical events occurring. A questionnaire was sent to BPNG members during March 1998, the results found that 43% of the centres surveyed were not using any sort of filter during the administration of PN[1]. Ten years have passed since the BPNG guidelines were established; the purpose of this project was therefore to repeat the 1998 survey of filter usage, in an attempt to establish if the guidelines were being followed or whether filter usage during the manufacture and administration of PN to patients in hospital has changed.

In order to achieve this goal, a structured questionnaire was designed to explore current BPNG members' usage of filters during the manufacture and administration of PN. The questionnaire was based on items generated from the previously study and from published literature on the subject matter. Each of the members was sent the questionnaire via email (Microsoft Word attachment), and was asked to return the questionnaire within one month of receipt. Returned questionnaires were then analysed using the computer programme; Statistical Package for the Social Sciences© (SPSS) version 16.0, for trends etc.

147 emails were sent out, with a total amount of 23 questionnaires returned, giving a response rate of 15.65%. Of the 23 respondents, 3 were unable to provide data on filter usage and another 3 indicated they did not produce PN on-site and therefore were unable to answer the questionnaire. On analysing the remaining questionnaires it was found that the most frequent type of filter used during the manufacturing process was a 5µm filter, which was used for particulate removal as the solution was injected into the final container. It was noted that lipid solutions were only filtered by 1.2µm sized filters, whilst aqueous and amino acid/glucose solutions were mainly filtered using 0.2µm filters, as recommended. The majority of filters were changed on a 24 hour cycle, while filters that were changed less often were thought to have been the more advanced positive charge endotoxin retaining filter although this was not totally clear from the responses received. It was noted that 78% of filters attached during the administration of PN were attached at ward level, while 60% of respondents reported having a problem with filters when administering PN to patients.

The results confirm BPNG members are attempting to adhere to guidelines published on the use of filters during the administration and manufacture of PN. Although many of the centres surveyed were using filters correctly, many were using a 15µm filter that is not recommended in the BPNG guidelines. Further research would involve conducting a larger study to test reliability and the development of a combined questionnaire with Australia and New Zealand.

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A study of the effect of storage conditions on parenteral nutrition additives

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Malnutrition is a condition where the body suffers adverse effects due to deficiency of nutrients such as protein, energy sources, vitamins and minerals. It is a common condition that can both cause or be a result of ill health. Malnutrition can be treated by parenteral nutrition (PN) which is the delivery of nutrients intravenously[1]. PN is either peripherally administered, delivered into a vein outside the chest or abdomen area for short term use, or alternatively through a central line for long term use. A central line is a plastic tube which is inserted into a large vein near the patient's heart.[2] PN admixtures usually consist of water, electrolytes, proteins, carbohydrates, and lipids. Other essential vitamins, trace elements and minerals which exist in solutions or lipid emulsions can be added to the admixtures or given separately and are known as parenteral nutrition additives. Parenteral nutrition additives are usually only added to PN fluids shortly before use as they can be highly unstable.[3] Additrace®, Peditrace® are parenteral additives consisting of water soluble trace elements and Solivito® N contains a mixture of water soluble vitamins. Vitlipid® N Adult is a concentrated emulsion of lipid soluble vitamins. The aim of the study is to investigate whether light, darkness, refrigeration and room temperature storage conditions will have an effect on the chemical and physical properties of a number of frequently used parenteral nutrition additives, to assess the feasibility of syringe storing additives.

Syringes were aseptically prepared in a laminar flow unit and the individual effects of light, darkness, room temperature (18-25°C) and refrigeration (2-8°C) on the physical and chemical properties on each of the test additives were investigated. Changes in chemical and physical properties were assessed by way of turbidity, microscopy, laser diffraction, pH and osmolality testing. Syringes were stored according to their experimental condition and samples taken daily for a period of 7 days. Syringes in the light condition were stored on a window sill and dark condition syringes were wrapped in foil and placed into a covered cardboard box. The syringes for both the room temperature and refrigeration conditions were placed flat on a tray on a laboratory bench or a pharmacy refrigerator respectively.

Some significant changes were observed for both turbidity and microscopy testing in all conditions, not however, for all additives. Turbidity testing showed significant results for all additives with exception of Peditrace® in the light condition and Additrace® in the refrigeration condition. Only the lipid additives demonstrated significant changes in microscopy and these results were observed in all conditions. Osmolality, laser diffraction and pH testing demonstrated smaller changes, which were not defined as significant, in all storage conditions.

All experimental conditions (light, darkness, refrigeration and room temperature) showed similar results. The turbidity and microscopy results suggest that the effect of syringe-storing additives for periods of seven days or more may mean that the additives are clinically unacceptable for patient administration. Further research would need to be done on a larger scale to check if the study results are a true reflection of the additive stability.

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Design and synthesis of novel Bcl-3 inhibitors

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Bcl-3, a newly discovered oncoprotein characterised as a member of the inhibitory ankyrin repeat domain IκB family, has been implicated in the pathogenesis of aggressive and highly resistant Her-2 positive breast cancer, demonstrable by Bcl -/- mice that show fewer percentage of metastatic grade tumours and improved survival prognosis[1]. Aberrantly involved in oncogenesis, Bcl-3 upregulates the dissociation of p50 from cytoplasmic p105-p50 interacting moieties[2], potentiating its migration to nuclear regions, where the binding of Bcl-3 to either p50 or p52 homodimers activates transcription via the transactivation domain of Bcl-3[3]. Bcl-3 acts to upregulate the transcription of cyclin D1, activating its promoter via the NF-κB binding site interaction[3]. Bcl-3 expression additionally diminishes induction of tumour suppressor protein p53 in response to DNA damage, simultaneously amplifying the effect of its inhibitor hdm1[4].

Previous molecular modelling studies have identified an inhibitor of the Bcl-3-p50/p52 protein-protein interaction, such that the aim of the project was to modify a previously identified 'hit' scaffold via molecular modelling, offering improvement on its docking score, inverted U-shape conformation, and current effective amino acid interactions with residues arg811, arg769, ser800, ser807 and his806, selecting the most promising candidate compounds for chemical synthesis.

We herein report the design and synthesis of pyrrolidine-2-carboxamides and propanamido benzamides as potential novel inhibitors of the Bcl-3 and p50/p52 protein-protein interaction. All compounds synthesized showed desirable amino acid interactions, and concise conformations, and bear the functional groups a)anthranilamide b) R proline amino acid linker or two carbon alkyl chain c)para positioned chlorine, hydroxyl or dimethoxy, considered fundamental in achieving optimal active site fit.

Methods for the synthesis of desirable 4-chloro and 3,4- dimethoxy carboxamide compounds employed a sulfonylation reaction, with a subsequent amide coupling reaction as the final step. A Method involving EDCI was unsuccessful and hence was disregarded as a potential second step method, with the thionyl chloride method proving the most effective. Chemical yields were generally low, calling for further optimization of reaction conditions however; successful methods were identified for both steps, with one final product isolated in testament of this. Future work will focus on determining the biological activity of this compound. Conversely, first step s-alkylation for the production of the benzamide compounds calls for improvement, with respect to circumvention of undesirable disulfide bond formation.

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Investigating the numeracy skills of undergraduate students entering the MPharm programme at the Welsh School of Pharmacy

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Basic numeracy skills are essential to undergraduate students entering the MPharm degree. Without these skills more complex pharmaceutical calculations would be difficult.[1] Despite this, lecturers are reporting a noticeable reduction in the basic numeracy skills of entrants to undergraduate degree programmes. Variation in the numeracy ability of undergraduate students may be due to a number of factors which include; highest mathematical qualification held on entry to university, increasing numbers of international and mature students entering university, and mathematical anxiety.[2-4] Numeracy diagnostic tests are used to assess basic numeracy ability and are becoming an increasingly popular way of evaluating the skills of University entrants'. Diagnostic tests can be used to identify weaknesses and to tailor teaching and learning. The aim of the present study was to investigate the numeracy skills of undergraduate students entering the MPharm programme at the Welsh School of Pharmacy (WSP).

Ethical Approval for this study was obtained from the WSP's Ethics Committee. A bespoke, contextualised pilot diagnostic numeracy tool was designed by the study team in 2010 to evaluate the numeracy skills of students entering Pharmacy/Medicine degree programmes. First year MPharm undergraduates (n=145) from WSP (n=117) and Taylors University (TU) (n=28) sat the paper based diagnostic tool in week two of the academic year 2010-2011. The diagnostic tool comprised 25 medicines-based questions, covering six calculation domains. Students had 45 minutes to complete the diagnostic test. Calculators were not permitted. Students were asked to indicate a level of confidence in their answer for each question. Students also completed an accompanying questionnaire designed to evaluate the numeracy diagnostic tool and collect demographic data.

Four students from the WSP did not take the diagnostic test giving a response rate of 141/145= 97%. The mean final score for the student population was 20/25 (80%). Ten percent of students got a final score of below 50%. Students with A-level mathematics had a mean score of 20/25 and those without A-level had a mean score of 18/25. There was a statistical difference (Mann Whitney U test: $p \leq 0.05$) between final scores of students with A-level mathematics and those without. Students from Southeast Asia and Australia got significantly higher final results when compared to students from the rest of the world. Unit conversion was found to be the weakest calculation domain.

The pilot numeracy diagnostic tool successfully identified the weaknesses of individual students in specific numeracy domains. The results support published literature of problems in undergraduate numeracy skills and the wide range or numeracy ability that exists between students entering higher education.[2,4] Of those undergraduate students entering the MPharm programme at the WSP in 2010-11 those with a pre-university education from Southeast Asia and Australia possessed greater numeracy skills and were more confident in their abilities. Undergraduates entering the WSP in 2010-11 with A-level mathematics or equivalent possessed greater numeracy skills and were more confident in their abilities than students without A-level mathematics. Future work will translate the numeracy tool to an electronic format.

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Freeze-thawing effects on serum antibody response to anthrax protective antigen - an ELISA based method

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Vaccination is the most cost effective form of protection against deadly pathogens such as *Bacillus anthracis*, the causative agent of anthrax. Whilst effective, the current UK anthrax vaccine (anthrax vaccine precipitated - AVP) remains the subject of considerable debate due to concerns over safety linked to adverse reactions and the public perception of risk[1]. In a recent study of the human immune response to AVP researchers found that the quality of the antibody response to Protective Antigen (PA), the principal protective component of the vaccine, reduced upon storage - samples assayed at the start of the study gave ten-fold higher results than those obtained for the same samples when they were tested many years later.[2,3] This lead them to hypothesise that prolonged storage combined with repeated cycles of freezing and thawing may have lead to degradation in the ability of the antibodies in serum samples to recognise PA. This study will determine if repeated cycles of freezing and thawing degrade the ability of antibodies from an AVP immunised individual to recognise PA.

Aliquots of sera from vaccinated and non-vaccinated individuals were exposed to varying numbers of freeze-thaw cycles: 1, 10, or 20, and the effect on polyclonal antibody specificity was determined using a 96 well indirect ELISA in which recombinant PA was employed as the well-adsorbed antigen. Horseradish peroxidase-conjugated mouse anti-human IgG was used as the secondary antibody with a solution of ABTS and hydrogen peroxide providing the chromogenic substrate. The optical density (OD_{405}) was determined using a Dynex Opsys MR Microplate Reader with a 405nm filter. An IgG control curve was constructed by coating wells with serial dilutions of Human IgG (Sigma) and was used to determine PA IgG concentrations. To ascertain the effect of freeze-thawing on a more defined population of antibodies a peptide competition ELISA was employed, in which chemically synthesised peptide sequences from regions of PA known to bind protective antibodies were mixed with serum samples to determine the degree of peptide specific binding.

Data appeared to show no effect of freeze-thaw exposure on antibody response following the indirect ELISA method, however following the peptide competition ELISA data suggested that increasing freeze-thaw exposure reduced antibody response. All results obtained (sample dilution, whether peptide present, number of freeze-thaw cycles, and OD) were analysed using Minitab software, and an ANCOVA was performed. It is quite clear that dilution will have a profound effect on OD and ANCOVA essentially controls this known effect allowing observation of the effects of peptide addition, exposure to freeze-thaw cycles, or a combination of both on antibody response. Freeze-thaw cycles were shown to affect the antibody response of human serum with a statistical significant value of $p= 0.089$ for all data collected and analysed. Whilst this does not allow for a 95% confidence interval, such a value is suggestive of an effect of freeze-thaw exposure on antibody response.

This study has failed to prove a significant effect of freeze-thawing on PA-specific antibody response of serum samples from individuals vaccinated against anthrax. It is, however, suggestive that there is an effect of freeze-thaw cycles on PA-specific antibody response. To further investigate the hypothesis, more replicates of each assay should be undertaken, the sample size should be increased and the number of freeze-thaw cycles increased (however, at this point it is worth considering that it is highly unlikely one serum sample would be subjected to such a high number of freeze-thaw cycles). This suggests that other environmental factors could be responsible for the reported ten-fold reduction in antibody titres[2]. With a larger timeframe and increased funding the aforementioned further investigations could be carried out to gain a more representative understanding of the possible effects of freeze-thawing serum samples on PA-specific antibody responses.

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How are Foundation Year One doctors (FY1) at Ysbyty Gwynedd prepared for their role as prescribers?

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The undergraduate medial degree should equip students with the appropriate technical skills and scientific background they will need for practice as a Foundation Year One doctor (FY1).[1] However, even though prescribing is a core clinical skill practised regularly by all FY1s, evidence suggests many are not sufficiently well-prepared for this role.[2,3] Ysbyty Gwynedd has developed a Prescribing Competency Programme (PCP) to try to ensure satisfactory prescribing skills development in FY1s. This study aimed to gather views about the PCP from key stakeholders and Foundation Year doctors to identify if it was relevant and beneficial and suggest improvements.

Ethics approval for the study was obtained. Seven semi-structured, individual interviews were conducted with three individuals involved in the programme, with two FY2s who completed the PCP in the previous year and with two current FY1s. Interviews were audio-recorded and transcribed *ad verbatim.* Key themes were identified and used alongside literature reviews to design two questionnaires, one for FY1s, and one for their supervising consultants. Questionnaires were piloted prior to distribution. The chi-square test (SPSS) was used to compare responses between FY1s and consultants.

Interview results identified that FY1s did not feel sufficiently well-prepared for prescribing at the start of their foundation year. They gave positive views on the PCP. Key stakeholders in the PCP felt it was their responsibility to facilitate additional teaching on prescribing and had noticed an improvement in prescribing skills since the programme was started at Bangor. The teaching was undertaken through a series of lectures, workshops and assessments. A total of 16 FY1s completed and returned the questionnaire (response rate 100%) and responses were received from 10/18 supervising consultants (55.6%). All but one FY1 (93.3%) rated the monthly pharmacy teaching sessions in the PCP as 3/5 or above and 100% agreed that the assessment was helpful in preparing them for their role as prescribers. The most commonly suggested improvement to the PCP were to add more clinical situations and to provide further detail for some topics. Only three (30%) consultants disagreed that they had seen an increase in FY1s confidence and competency this year compared to previous years and only three agreed that FY1s generally complete prescriptions clearly and accurately when they begin their post. The most frequently reported topics which required further FY1 training were basic pharmacology and therapeutic drug monitoring amongst the FY1s, and drug interactions and drug dose calculations amongst the consultants. There were significant differences between FY1 and consultants' responses to each area requiring further training apart from pharmacokinetics.

Limitations include recruitment from one hospital only and at one point in time so that it is not possible to generalise findings more widely. Responses could have been subject to social desirability bias where respondents may have responded in a way they perceive as being socially acceptable. However, confidentiality and anonymity was assured to minimise this. Future work could investigate how FY1s are prepared for their role as prescribers across other health boards and results compared with this site. This study confirms FY1s lack of preparation in prescribing and the need to provide sufficient learning opportunities with a robust assessment such as the PCP. The PCP has proved successful and valued by both FY doctors and other staff members at Ysbyty Gwynedd.

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The effect of static magnets on the partitioning and permeation of lidocaine

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The concept of 'Magnetophoresis' is described as utilising a magnetic field in order to increase the permeation of molecules[1]. The underlying basis of this is utilising diamagnetism, in which a substance that is diamagnetic will be repelled *away* from a magnetic field; therefore, applying a magnetic field could theoretically 'push' molecules across biological membranes and barriers, namely the skin, to increase permeation. The skin is a significant barrier to chemicals[2], meaning that permeation enhancers are often incorporated into transdermal formulations. Therefore, should the use of a static magnet show any significant impact on permeation, they could prove to be a useful physical permeation enhancer, being unpowered (therefore low cost), and could easily be incorporated into transdermal patch designs. Murthy et al. reported a significant increase in the permeation and partitioning of lidocaine hydrochloride and lidocaine base (which they report to be diamagnetic) in the presence of a magnetic field compared to those tested in the absence[1]. The claimed effects could be considered difficult to believe, as the repulsive force is likely very weak, and the static magnets were found (by Murthy et al[1]) not to induce any changes to the structure of the skin, a mechanism which is proposed for the enhanced permeation effects seen under the influence of a pulsed electromagnetic field[3]. The overall aim of this project was to try and recreate the data/results reported by Murthy et al. based on the descriptions given in their article. The most significant effect reported was with lidocaine hydrochloride, so this salt was tested rather than both the salt and base form.

Octanol / H_2O partition studies were carried out using 2ml vials, which contained 750µl of a lidocaine hydrochloride aqueous solution, and an equal volume of octanol. Similar to this, silastic – aqueous partition studies were set up in the same manner, with an aqueous solution of lidocaine and 1cm^2 of silastic. The vials were mixed by hand, then placed on a roller mixer and left to equilibriate, before sampling the aqueous phase to determine the total amount partitioned. The experiments ran for various times and test vials were exposed to various polarities and orientations of the magnets. Permeation studies were conducted using 8ml Franz-diffusion cells, using dialysis membranes and silastic® as the barriers in which the lidocaine hydrochloride would permeate across. Magnets of differing polarities and orientations were placed over the donor phase. The receptor phase of the cells was sampled at 2, 4 and 6 hours. All samples (permeation and partition) were analysed by HPLC.

Although the experimental set-ups and procedures were based on, and very similar to those reported by Murthy et al. they failed to reproduce their findings. Both the permeation and partition studies showed either no or very little difference between control and test groups, suggesting that the application of a static magnetic field has no bearing on the partitioning or permeation of lidocaine hydrochloride, despite the orientation or polarity of the magnets tested. The 2kg, 2.5kg and 4kg magnets used in the investigation of the partitioning studies did not produce the increase in partitioning reported, despite being stronger than the 300mT magnet used by Murthy et al. Likewise, the permeation studies showed essentially no effect, despite a total force of 10kg (four 2.5kg magnets) used and various polarities and orientations tested.

In conclusion, the data reported by Murthy et al. was not reproducible based on the descriptions given in the article; therefore, it is unlikely that any significant effect on partitioning and permeation occurs in the presence of these relatively low-strength magnets, suggesting that incorporation of a static magnet into a transdermal patch would not be beneficial. However, the conclusions drawn apply only to static magnets (of the strengths mentioned), and to lidocaine hydrochloride. The findings do not exclude the possibility that other types of magnetic field may have benefit, e.g. pulsed, nor does it exclude that the magnets may have a more significant effect on other molecules, or that a static magnet of a higher strength may have produced more significant results.

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An evaluation of the administration of subcutaneous insulin at mealtimes in acute inpatients at Betsi Cadwaladr University Health Board – North East Wales

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Since its initial discovery in 1675, diabetes mellitus (DM) remains a chronic and incurable metabolic disorder and its prevalence is growing at an exponential rate[1]. The treatment of type 1 diabetes (absolute insulin deficiency) is currently managed via the administration of exogenous insulin, commonly delivered subcutaneously (S/C)[1,2]. Many patients with Type 2 diabetes have also progressed to treatment with insulin. NICE stipulates the use of short-acting insulins (SAIs) in specific situations such as hyperglycaemia after a meal[3]. The manufacturers' licence specifically states the times in which these therapies should be administered. These timings may be difficult to adhere to in secondary care due to the unfamiliarity and lack of training of insulin regimens and administration[4]. The project aims to evaluate whether SAIs are being administered appropriately to patients at a North Wales hospital and also to determine any training and educational needs.

The ethically approved study consisted of three parts which utilised a triangulated approach. Part one consisted of non-participant observation to investigate when in-patients were receiving their SAI in relation to mealtimes in addition to how the insulin was delivered and who was administering. Part two involved the administration of a brief questionnaire to nursing staff on the observed in-patient ward(s). This healthcare professional questionnaire (HCPQ) was designed to gain an understanding of nurses' knowledge regarding SAIs and to determine whether their answers were reflected in their daily practice. The third part was a point prevalence study (PPS) to provide a 'snap-shot' view of DM patients and their insulin regimes at the hospital. The PPS involved visiting various wards to determine what types of insulin patients were using and how their treatment was being delivered. All findings were analysed using SPSS and Excel software.

A total of 72 observations were completed in the study from nine patients, whereby 75.7% (n=56) of observations consisted of a SAI analogue. The average administration time was 1-5 minutes before a meal (n=20) and 6-10 minutes before a meal when using a soluble biphasic insulin (n=3). Some meals (n=17) arrived 30 minutes later than expected. The HCPQ identified that the majority of nurses were qualified (n=10) and administered insulin 2-5 times daily (n=10). Seventy five percent of nursing staff (n=9) could not fully identify SAIs although 92% (n=11) could identify the administration timing of a SAI analogue. The PPS estimated the inpatient population of patients with diabetes managed on insulin to be 4.6% (n=21) if included wards were at full occupancy (n=457).

The evaluation indicated that the administration of soluble biphasic insulins was not in accordance with medicine licensing. The inappropriate timing of this insulin could also be translated to nurses' lack of awareness of when biphasic (both biphasic isophane and the newer biphasic analogue type) insulins should be given. In addition, the arrival of meals was often erratic in comparison to the specified meal timings laid out by the hospital, impacting on whether insulin had been appropriately administered or not in relation to a meal. Study findings justify recommendations to improve the delivery of S/C insulin from a multi-disciplinary perspective. These include the implementation of team-education and training to staff and patients, the highlighting of patients with diabetes to catering services and the provision of universal health care guidelines.

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Does caveolin-dependent endocytosis have a role in Alzheimer's disease?

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Alzheimer's disease (AD) is a debilitating incurable neurodegenerative disorder.[1] The amyloid cascade hypothesis is believed to be the central pathology in the disease. This involves an overproduction of β-Amyloid (Aβ) arising from the cleavage of amyloid precursor protein (APP) by β-secretase and γ-secretase. This leads to the formation of Aβ plaques which cause neuronal cell death, which ultimately leads to dementia.[2] Evidence suggests that caveolin-dependent endocytosis is involved in the formation of these plaques and ultimately AD. This pathway is characterised by a specific form of lipid raft which resides at the cell membrane, called caveolae. The protein caveolin is integral for inducing their formation.[3] Several lines of evidence have found APP, β-secretase and γ-secretase to reside in the caveolae, suggesting their involvement in amyloidogenic cleavage.[4] The aim of this study was to knock down caveolin-1 using sequence-specific siRNA, and subsequently measure the effects of this on intracellular APP levels, and extracellular Aβ levels. It was hypothesised that a decrease in caveolin-1 would cause a decrease in APP processing and therefore a decrease in Aβ.

Experiments were performed in astrocytoma MOG-G-UVW cells which were transfected with *cav-1* siRNA. Western Blotting was used to determine caveolin-1, APP and caveolin-3 levels in the *cav-1* siRNA-treated cells, whereas ELISA was used to determine APP and Aβ levels. Immunocytochemistry on fixed cells with fluorescent microscopy was also used to determine the effects of the siRNA on the location and expression of the various proteins, in addition, an MTS assay was used to investigate the effects of *cav-1* siRNA on MOG-G-UVW cell viability.

For caveolin-1 expression, both western blotting and immunocytochemistry showed a highly statistically significant reduction in the *cav-1* siRNA treated cells in comparison to the control, demonstrating the efficacy of the siRNA technique. Additionally MTS data confirmed that *cav-1* siRNA did not significantly affect MOG cell viability. For APP, western blotting, immunocytochemistry and ELISA showed no statistically significant differences in the *cav-1* siRNA-treated cells in comparison to control cells. For Aβ40, preliminary ELISA data showed an increase in *cav-1* siRNA-treated cells (n=1). For caveolin-3, western blotting showed no difference in the siRNA-treated cells however immunocytochemistry showed a change in caveolin-3 distribution. In the GFP-treated cells, large accumulations of labelling were visible, however, these accumulations were more fragmented and dispersed in the siRNA-treated cells, with additional punctuate labelling in the cell.

These results disprove the initial hypothesis as decreased caveolin-1 did not affect APP but appeared to cause increased Aβ levels. This indicated a different role for caveolin-dependent endocytosis in AD. This pathway may have a regulatory role in amyloidogenic cleavage. β-secretase and γ-secretase reside in the caveolae, therefore enzymatic activity is compartmentalised and, as a result, so too is amyloidogenic cleavage. Alternatively, the caveolin-dependent pathway could regulate Aβ processing through its trafficking of APP. Another theory is that this pathway regulates Aβ clearance. In relation to caveolin-3, these results suggest a "compensatory mechanism" between the caveolins. The distribution was altered, suggesting that the cell redistributed caveolin-3 to account for the decreased caveolin-1. In conclusion, although much of the data from this study is preliminary in nature, it is apparent that caveolin-dependent endocytosis has a role in AD. However, the precise role still needs to be elucidated and hence further experiments are needed in order to define fully the role of caveolin-dependent endocytosis in Alzheimer's disease.

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Investigating the numeracy skills of undergraduate medical students entering their first year at Cardiff University

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It has been widely reported that the basic numeracy skills of many new undergraduates have fallen below an acceptable level[1,2]. This is particularly concerning for those students whose academic study precedes a vocational career in which numeracy competence is vital. Student confidence in their numeracy skills has also been questioned and mathematics anxiety[3] has been recognised as a problem in both secondary and higher education. This study aimed to explore the numeracy competence and confidence of students, entering the first year of the Medical degree programme at Cardiff University.

Ethical approval was obtained from the Welsh School of Pharmacy's Ethics Committee. A bespoke validated medicines-based diagnostic numeracy tool, previously developed by the study team, was used to evaluate the competence of medical students, in various numeracy domains, in a non-compulsory teaching session. The diagnostic tool also enabled students to indicate their level of confidence in each of their answers. The tool contained 25 questions to be answered in 45 minutes. A questionnaire was also included to capture demographic data. The use of calculators was not permitted. All raw data from the tool, and the accompanying questionnaires, were entered into SPSS 18. Data was re-coded for analysis.

The response rate was 61.4% (180/293). Only 12.2% of students achieved full marks. International students (mean score: 22.32) were more competent than home students (mean score: 20.69). Students with a pre-University A-level (or equivalent) mathematics qualification outperformed those with a GCSE only ($p < 0.05$). Males were not more competent than females but were more confident in their ability. The numeracy domains with the lowest scores were unit conversions, as well as questions which involved a method consisting of two or more steps.

The results of this study suggest that the pre-University experiences of international students may equip them with better numeracy skills than that of students educated in the UK. The results also suggest that those students that study mathematics to A-level are more confident and competent in basic numeracy skills. This may inform the admissions criteria and/or the teaching methods used in Medical degree programmes. The higher confidence level of male students suggests that maths anxiety may be more prominent in females. This may inform future teaching strategies.

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The effect of biocide potentiation with a range of essential oils on *Pseudomonas aeruginosa*

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Disinfection plays an important role in infection prevention and contamination control in our daily lives and environments, both as a part of environmental management and in the processing of medical equipment. However, the range of disinfectants available for use in healthcare is restricted, and their spectrum of activity is limiting. A recent study[1] demonstrated the potential for paraben preservative activity to be enhanced by the inclusion of essential oils. This project was designed to explore the potential for enhancing the activity of existing antimicrobial agents to disinfection levels. The aim was to confirm the potentiation of paraben preservative activity by the inclusion of an essential oil delivery system, investigate the general application of the delivery system to a diverse range of biocides, and establish the mechanism by which this potentiation is achieved in the delivery system. It was hypothesised that potentiation is a result of biocide partition into oil micro-droplets, resulting in its direct delivery to the microbial surface at high concentrations.

The assessment of biocide and essential oil activity involved performing serial dilutions and viable counts[2]. A stock bacterial suspension was prepared and 100µl pipetted into 900µl of biocide/biocide and oil combination. At time intervals of 1, 5, 10, 15, 20 and 30 minutes a 100µl sample from this suspension was taken and neutralised in 900µl of Letheen broth. Six ten-fold serial dilutions were performed at each time point and three 10 µl drops of each dilution were pipetted onto the surface of pre-poured TSA plates. The plates were incubated at 37 ^{o}C for 24 hours for subsequent viable counts. Determination of partition coefficients for propyl paraben and chlorocresol followed a modification of the "Shake Flask Method"[3]. 1 ml of n-octanol was added to 4ml of HEPES buffer containing a known concentration of biocide, the concentration in the aqueous phase after vigorous mixing was determined using a UV spectrophotometer. Benzalkonium chloride followed the same method but the concentration present in the aqueous layer was estimated by a modification to the Orange II method[4]. For each biocide a calibration graph was prepared. No feasible method for experimentally determining the partition coefficient of propionic acid was available for this project so calculated values were used with validation for using a non-experimental approach being comparison of experimental values with calculated values for the other biocides.

Potentiation of biocide effect was observed with all essential oils in the presence of propyl paraben. In combination cinnamon oil produced the greatest log reduction (>5), with wintergreen (3.45-4.26), fennel (2.32-2.65), and peppermint (1.44-2.20) causing respectively less log reductions ($P<0.001$). Potentiation was only seen with chlorocresol (1.87-3.82) and benzalkonium chloride (1.42-2.20) in the presence of wintergreen oil although the other oils produced an apparent additive effect ($P<0.05$). Potentiation was not noted with propionic acid in combination with any oils, indeed the biocide was inhibited, conceivably the partition into the oil reduces its bioavailability. Propyl paraben had the greatest lipophilicity, Log P 2.04 (±0.31), this is comparable to the log P values obtained through calculation ($P>0.05$). Chlorocresol had a similar hydrophilic/lipophilic balance, Log P 1.90 (±0.17), although being slightly less lipophilic in nature. Benzalkonium chlorides partition coefficient value was 0.39 (±0.21), indicating an intermediate hydrophobic/lipophilic balance which mirrored that of propionic acid, Log P 0.33 (±0.17). The results suggest the hypothesised mechanism of delivery, based on biocide partitioning into the oil, is correct.

The application of the new delivery system, involving the inclusion of an essential oil, on a diverse range of biocides has demonstrated disinfection potential with propyl paraben, chlorocresol and benzalkonium chloride. However, it has been found the essential oil used in the delivery system is very important. Potentiation was most noticeable with wintergreen oil, which has the highest specific gravity of the essential oils tested. The mechanism of delivery appears dependent on the partition coefficient of the biocide. Propyl paraben had the highest partition coefficient value (2.04) and the greatest potentiation, whereas benzalkonium chloride which had the lowest partition coefficient value (0.39) had the least. Propionic acid was inhibited in the presence of oils; it differs in its chemical structure and its mechanism of antimicrobial action to the other three biocides. Overall, this project has proved that the oil based delivery system has the potential to enhance the activity of selected antimicrobial agents and extend their disinfectant capability.

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Specials – what role can/do Welsh hospital pharmacy production units play in the manufacture/supply of medicinal products to the primary care sector?

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'Specials' are medicines that have been made to satisfy an individual patient's need, and are usually manufactured as a formulation that is not available as a licensed product[1]. Specials' manufacturers play a vital role in catering for the needs of certain patients groups, but costs to the NHS are spiralling. This is mainly due to exploitation in both the supply and pricing of specials[2]. It was therefore considered timely to explore the manufacturing capabilities of hospital production units in Wales, in order to consider whether a 'Specials' manufacturing service could be provided to the primary care sector.

Following ethical approval obtained from the WSP ethics committee, the target population was identified as the 13 members of the Welsh Aseptic and Production Pharmacists (WASPP) committee. A self-administered e-mail questionnaire was considered the most appropriate data collection tool based on time constraints, geographical spread of the hospitals and capability to draw statistical conclusions[3]. With assistance on technicalities from Principal Pharmacist, Paul Spark from the St. Marys Pharmaceutical Unit, relevant themes for the questions were formulated to produce a semi structured questionnaire. The final draft was assessed for face and content validity before the Microsoft Word was attached to an e-mail and sent. Respondents were given one week to reply and follow-up e-mails were sent on two separate occasions to non-responders.

The questionnaire response rate was 54%. There was significant variation between resources available, and hence the services that each hospital unit was able to provide. For example, 4 out of 7 hospitals held a manufacturing 'Specials' license' (MS), therefore product manufacture capability varied considerably. This was reflected in the range and quantity of products manufactured by each hospital, for instance, MS holder, Wrexham Maelor hospital manufactured the most products. Despite the fact that the University Hospital of Wales advertised its services; none of the hospitals were engaged in supplying specials to the primary care sector. Only Wrexham Maelor could comment on how they priced their products which revealed that they could be manufactured at a considerably lower cost than commercial suppliers. With exception of Wrexham Maelor, each hospital stated that they required considerable investment in key areas in order to increase their manufacturing capacities. The hospitals' surveyed manufacture products entirely for the supply to patients in secondary care.

There are undoubted potential benefits of utilising Welsh hospital manufacturing units to provide medicinal products to the primary care sector. Evidence suggest that some hospitals would be 'able to provide a limited service', that would serve as the best value for money option for the NHS. Oral liquids are the top prescribed specials in primary care and Welsh hospital production units must consider manufacturing these, if they are to supply to community pharmacies. A lack of formulation and stability expertise, as well as the infrastructure to deliver products and provide customer support stands as a challenge to this prospect. A government programme (QIPP) is aiming to drive down the NHS spend on 'Specials', and until government intervention takes place, Welsh hospital production units will play an insignificant role[4]. Could the encouragement of community pharmacists to use the option of procurement from Welsh hospital production units hold the key to increasing their role?

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Synthesis of triazine inhibitors of Rad6B

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Rad6B is an E2-conjugating enzyme which is an essential component of the post-replication DNA repair pathway.[1] It is, however, over-expressed in human breast carcinomas, resulting in abnormal mitosis, aneuploidy,[2] chemoresistance[3] and stabilisation of the oncogene, β-catenin.[1] Two compounds, TZ008 and TZ009, have been found to be active against Rad6B; however, the synthesis of these s-triazine-based inhibitors has proven inefficient and improvement is required in order for further studies on these compounds to take place.[4] The main objective of this investigation was to try to improve the synthesis of compounds TZ008 and TZ009 by improving the yield of the second step of this reaction, the triazine cyclisation step between a substituted biguanide and ethyl glycolate.

The three step method previously used for the synthesis of these compounds involved synthesising a substituted biguanide and reacting it with ethyl glycolate before the resulting triazine was reacted with 4-nitrobenzoyl chloride.[4] For this investigation the previous method was altered slightly, by trying different protecting groups to prevent the free hydroxyl group of ethyl glycolate from causing self-polymerisation and thus reduced yield. First to be investigated was triisopropylsilyl (TIPS) ether, followed by benzyl ether. This added two steps to the synthesis, the addition and removal of a protecting group. An alternative reaction order was also investigated in which the biguanide was first reacted with 4-nitrobenzoyl chloride before being reacted with ethyl glycolate in the triazine cyclisation step.

^{1}H NMR analysis confirmed the generation of phenylbiguanide and 4-methylphenyl biguanide (intermediates 1a and 1b) was successful (yield 53% and 38% respectively). ^{1}HNMR analysis also confirmed both protecting groups were successfully added to ethyl glycolate. TIPS protection (generation of intermediate 2a) resulted in a yield of 107%, suggesting some impurity was still present, whilst benzyl protection (generation of intermediate 2b) had a moderate yield of 58%. ^{1}H NMR analysis of TIPS protected generation of the triazine (generation of intermediate 3a) suggested intermediate 3a may have been successfully made, however ^{13}C NMR analysis was inconclusive and the attempt to remove TIPS resulted in the production of an unidentified product. ^{1}H NMR analysis of benzyl protected generation of the triazine (generation of intermediate 3b) suggested the benzyl group had fallen off during the reaction. The attempt to remove the benzyl group confirmed this, producing a very similar ^{1}H NMR spectrum. Further investigations showed that the compound left when the benzyl protective group was cleaved was not the triazine-alcohol required for the final step of the synthesis. The alternative order for the reaction was also unsuccessful. 4-nitrobenzoyl chloride was successfully added to ethyl glycolate in a quick reaction with a moderate yield of 41%; however ^{1}H NMR analysis of the cyclisation step showed the reaction had been unsuccessful, probably because the benzyl-ester was not stable in the strongly basic reaction conditions.

The compounds TZ008 and TZ009 are the first reported small molecule inhibitors of Rad6B activity and as a result they have the potential to help provide further information about the actions of Rad6B and probe the biochemical and biological implications of its inhibition. It is therefore highly important that an efficient route of synthesis is found for these compounds. This investigation was not able to provide this efficient route, however it ruled out potential strategies and highlighted the importance of further study into the synthesis of these compounds and others like them.

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Investigating the numeracy skills of undergraduate students entering the MPharm programme at Brighton University

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As healthcare professionals involved in the administration of medicines, pharmacists need to demonstrate high levels of competence in their numeracy skills.[1] Concerns over the numeracy skills of pharmacy undergraduates have increased in recent years.[1,2] Numeracy diagnostic tools can be used to identify within individual weaknesses and can help staff tailor learning material accordingly.[3] This study aimed to pilot a paper based numeracy diagnostic tool, and to explore the competence and confidence levels of first year pharmacy students in Brighton University School of Pharmacy.

Ethical approval was obtained from Brighton School of Pharmacy. A pilot contextualised numeracy diagnostic tool was constructed by members of the project team in 2010 following semi structured interviews, focus groups and a small piloting exercise. The validated tool contained 25 medicines-based questions covering 6 numeracy domains. In addition a confidence assessment box was included alongside each question. An accompanying semi-structured questionnaire was constructed to obtain demographic information and students' opinion on the tool. The pilot tool was administered to a cohort of 130 first year pharmacy students at Brighton University School of Pharmacy. Students were given 45 minutes to complete the tool without calculators. Following this students were asked to complete a consent form and questionnaire. Results were then collected and analysed.

The tool was completed by 81% (105/130) of eligible participants and 53% (56/105) completed the questionnaire. The mean mark was 17.85 (out of 25) and the range was 3-25.Overall, students showed low levels of competence in the unit conversion questions, averaging 62 % (2.48 out of 4) in this particular domain. Twenty five percent (26/105) and 22% (23/105) of students gave answers that were 1000 fold or more away from the correct answer in two of the unit conversion questions. In the percentage section of the tool, 75% (15/20) of male students achieved a mark of either 4 or 5 out of 5, compared to 46%(16/35) of females. Limited qualitative feedback was received (n=6).

Results confirmed that students' confidence and competence varied in different numeracy domains. Differences in students' pre-university background had a minimal affect on their numeracy skills. Male students in the study demonstrated both high levels of competence and confidence in their numeracy skills compared to female students. The introduction of a numeracy test proved to be a valid and reliable method of highlighting weaknesses in students' numeracy skills. This study proved that pharmacy undergraduates competence and confidence in dealing with numbers is a concern that needs to be addressed. Future work aims to pilot the test in other Schools of Pharmacy and develop a computer based tool alongside additional material to support those weaker students.

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Mechanism of hyperactivity in three month old YAC128 transgenic model

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Huntington's disease (HD) is a devastating autosomal dominant neurodegenerative disorder. A YAC128 transgenic model has been utilised in this study who have been shown to demonstrate a hyperactive phenotype at 3 months followed by a hypoactive phenotype from 6 months[1]. It has been hypothesised that this hyperkinesia is comparable to chorea witnessed in HD patients. The aim of this study is to identify any alteration in dopamine biosynthesis, with tyrosine hydroxylase (TH), or levels in c-fos and FosB which may contribute to hyperactivity phenotype witnessed in the 3 month old YAC128 model.

Isolated brains were sliced and stained with Cresyl violet, and standardised peroxidase based immunohistochemistry for TH, c-fos and FosB proteins was performed.

YAC128 demonstrated no significant loss in neuronal cell bodies in either the striatum or cortex compared to WT littermates. TH expression in dopaminergic terminals of the striatum and cortex were unchanged in YAC128, however there was a significant difference noted in TH-positive cell bodies of the Substantia Nigra/ Ventral Tegmental Area (SN/VTA). c-fos expression was down regulated in the cortex and ventral striatum of YAC128 mice compared to WT littermates. FosB expression remained unchanged between the two groups although there was a slight increase witnessed in the lateral, dorsal and ventral striatum of the YAC128.

The absence of cell loss in either the striatum or cortex suggests that hyperkinesia is a not a result of direct cell loss, although neuronal dysfunction cannot be ruled out. Results imply that a compensatory mechanism[2] is present in the dopaminergic neurones of the nigrostriatal pathway as TH-positive cell bodies are reduced in the SN/VTA whereas TH expression remains unchanged in the striatum. Decreased c-fos expression implies a possible reduction in neuronal activity. For the cortex this could signify diminished corticostriatal neuronal pathway acting upon the medium spiny neurones of the striatum. For the ventral striatum this would signify that diminished activation of the direct and indirect pathways may occur, with hyperkinesia being produced due to particular dysfunction of the indirect pathway[3]. Unchanged FosB levels suggest no significant alteration in neuronal plasticity.

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MPharm students' views on what they consider to be the attributes of a good lecturer at the Welsh School of Pharmacy

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The Welsh School of Pharmacy at Cardiff University is one of twenty-six Schools of Pharmacy in the UK[1]. In 2009 WSP was ranked joint first for overall satisfaction out of all the schools of pharmacy in the UK. There are over forty members of School lecturing staff (this term includes teacher-practitioners but not visiting lecturers)[2]. Fifty three percent of the current Cardiff MPharm consists of teaching in lecture theatres (mainly lectures but also seminars). The aim of this study was to explore the views of MPharm students on what they believe are the attributes of a good lecturer.

Ethics approval for the study was obtained. MPharm students were given a previously-developed questionnaire[3], consisting of 22 likert statements, to complete. Students indicated their levels of agreement or disagreement with the statements by ticking the appropriate box. The data obtained were entered into SPSS 18 and were analysed using Kruskal-Wallis and the Mann Whitney U statistical tests. The Mann Whitney U test was used to compare responses between first and fourth years. Two focus groups were conducted with MPharm IV students to develop a better understanding of the results obtained. The focus groups were audio-recorded with consent, and were transcribed. All participants were made anonymous in the transcript.

There was an overall response rate of 85.1% from all MPharm students. The highest response was from first years (97.5%) followed by third year students (92.9%), then second year students (88.9%) and the lowest response was from the fourth year students (61.2%). No students indicated disagreement with the following statements, *'a good lecturer should be ready to start on time*', '*a good lecturer is easily accessible for academic support outside the classroom'* and '*a good lecturer provides appropriate feedback in a timely manner'.* Statistically significant differences in responses between first and fourth years were obtained. For example, for *'a good lecturer provides a lot of information 'one way' with no interaction from students'* first year students indicated a higher level of disagreement (84.8%) compared to the fourth year students (69.8%), ($U = 2793$, $p = 0.013$). Another example where there was a difference was *'a good lecturer is one who lectures for 50 minutes without giving a break at all.'* First year students indicated a higher level of disagreement (78.8%) compared to the fourth year students (38.0%). The focus groups were valuable as they highlighted possible reasons to why such differences were observed.

The study was useful in identifying a number of attributes that Cardiff University MPharm considered positive. As focus groups were only carried out with fourth year students and with only eight participants the results may not be generalisable. That is, not participants may have held different views. It may be useful to carry out focus groups with the other year groups and with a larger number of individuals. Nevertheless the focus groups were valuable as they provided possible reasons for the differences between first and fourth year responses. Future work may involve surveying MPharm students at other UK schools of pharmacy.

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Evaluation of the administration of subcutaneous insulin at mealtimes in acute inpatients at Betsi Cadwaladr University Health Board

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Short-acting insulin (SAI) is a common part of therapy for diabetics. It is administered subcutaneously at mealtimes to coincide with the rise in glucose from meals. For efficacy, it has to be appropriately timed according to its pharmacokinetic profile;[1] preventing poor glycaemic control which in turn could lead to vascular complications such as retinopathy.[2] A previous Australian study[3] found poor results; although in subacute care, of insulin administration not being appropriately timed in relation to patients' meals. Anecdotal evidence of such inappropriate timings was reported in a hospital in the BCUHB, North Wales, UK. We therefore investigated the incidence of correct timing of administration on acute wards in this hospital. The knowledge of nursing staff regarding SAI was also assessed by means of a structured questionnaire. Additionally, a point prevalence study was carried out on a single day to capture a snap-shot view of the total number of in-patients in the entire hospital on some form of insulin therapy.

By non-participant observation, records were made of the time interval between food intake by the patient and insulin administration by nurses or patients. A structured data-collection tool was constructed from themes that arose from literature[3] and group discussions with diabetes team (nurses and pharmacists). Non-probability, purposive sampling was used to recruit 15 patients. They were observed at fixed mealtimes (breakfast, lunch and tea) over 18 days. Using a structured questionnaire with six fixed choice questions, the knowledge of nurses was assessed in relation to SAI nomenclature and timing requirements in relation to meals. Themes arose from group discussions amongst the diabetes team. A total of 28 questionnaires were distributed to all nursing staff on duty were over three consecutive days. A point prevalence study was carried out to assess the prevalence of the various classes of insulin within the hospital.

Set criteria based on the individual preparation's pharmacokinetic profile were set with a few minutes additional allowance given. Administration of Novomix®30 and NovoRapid® should be 15 minutes before or after food intake[1] and for Humulin® M3, 20 to 45 before food.[4] Results were compared to these. Three insulin brands were encountered in the observation study (Novomix®30, 17%; Humulin® M3, 19%; and NovoRapid®, 62%). Of the106 sets of observations completed, 98 were analysable. Of these, 41 (42%) were not administered within the correct time window. Nurse factors, probably high workload were responsible for 37 of the 41 incorrectly timed doses. Where nurses were involved, administration was more likely to be outside the recommended time window ($p=0.001$). Patients were administered insulin on average 8±16 minutes after food intake began. Administration of Humulin® M3 was especially problematic, with 95% of observed administrations being incorrectly timed. Of the 28 questionnaires distributed, 27 (96%) were returned and analysed. A score of at least six correct questions, out of a possible nine, was attained by 67%. Increased exposure to insulin in practice correlated with improved knowledge of nomenclature and timing of SAI ($P=0.053$). Respondents were students, qualified nurses or sisters. In overall performance, sisters' knowledge did not appear much more than that of qualified nursing staff; student nurses knew considerably less than qualified nurses and sisters ($p < 0.05$). The point prevalence found 30 patients on insulin in the entire hospital; 60% of these were on some form of SAI as part of their therapy; most commonly NovoRapid ® (20%) and Novomix® 30 (17%).

The aims were achieved successfully; findings were consistent with a previous study and showed that many patients receive their insulin at incorrect times and are thus at risk of hypoglycaemia. [3] This is likely due to high workload and not enough nursing staff having sufficient knowledge of the importance of correctly timed SAI; as seen from the questionnaire study. Suggestions for new protocols to be drawn up by nursing staff themselves and regular re-education of patients and nurses is important. Further observational studies involving more patients and hospitals will be valuable and increase generalisability. Focus groups held with groups of nurses could shed light on reasons for non-administration of insulin doses within the correct time window.

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Microneedle-mediated intradermal delivery of a pDNA vaccine against H5N1 influenza

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Vaccination using plasmid DNA (pDNA) is an emerging technology which shows great potential due to its ability to induce both cell-mediated and humoural immune responses.[1] The design and production processes allow rapid turnaround of novel pDNA vaccines. Pandemic influenza possesses a tendency to undergo antigenic shift, producing new viral strains. This creates the need to regularly generate new vaccines- pDNA vaccines are ideally placed to meet this demand. Microneedle (MN) devices are in development as a method of painlessly delivering vaccines to the skin[2]- an ideal vaccination target due to its immune cell population[3] and the presence of keratinocytes which can express pDNA. This study will examine the ability of a novel pDNA vaccine against H5N1 A/Viet Nam/1203/2004 avian influenza (pCAGGS-HA) to be expressed in keratinocyte cells and *ex vivo* human skin, with special attention given to MN devices as a potential delivery method.

MNs were supplied by collaborators at Georgia Institute of Technology. MN coating studies were performed using fluorescent formulations and pDNA solution to determine the effect of changes in the formulation's viscosity and the drying time allowed during dip-coating respectively. Fluorescently coated MN arrays were applied to *ex vivo* human skin to show the release of formulation following a range of application times. A trans-epidermal water loss (TEWL) studies was also performed to confirm the breaching of the stratum corneum by coated and uncoated MN arrays. Transfections in cultured keratinocyte cells and in *ex vivo* human skin were performed with reporter plasmids as surrogates for the pCAGGS-HA plasmid. Finally, pCAGGS-HA (supplied by collaborators at Emory University) was delivered to keratinocytes and *ex vivo* human skin which were cultured prior to RNA extraction. RT-PCR was then used to ascertain the presence of the plasmid vaccine's expression.

It was found that increasing the viscosity of the coating formulation improved MN coverage levels whilst reducing variability. An increase in drying time between dips during the dip-coating process was shown to coat a greater total amount of formulation onto MN arrays, though at a greater cost in terms of formulation volume lost to evaporation and operator time. Maximal release of formulation from MN arrays was seen after 60 seconds residence time in skin. *Ex vivo* human skin showed a slight rise in TEWL following MN insertion. Reporter plasmid transfection was successful in both cells in skin, though pDNA delivered coated onto MN arrays was ineffective in *ex vivo* skin. RT-PCR on pCAGGS-HA-treated cell/skin RNA samples produced inconclusive results due to unforeseen issues with primer specificity being too low to discern between the plasmid signal and the constitutively expressed RNA in samples.

The results of this study reinforce previous studies on the ability of coated MN arrays to deliver a formulation to the skin.[4] Improvements were made in dip-coating by altering the viscosity of formulations and drying time used, though further studies are required to optimise these conditions in order to make MN coating a reliable process. Studies into MN application methods are also required to ensure that MN arrays are inserted to their fullest extent in skin to allow maximal formulation release. The poor rate of transfection seen using reporter plasmid coated onto MN arrays suggests that formulation studies may be required to ensure that the pDNA is both delivered and expressed efficiently. The primer issues prevented the expression of pCAGGS-HA from being properly identified. There was, however, some evidence that the vaccine produced immunostimulation- particularly when co-delivered with H5-representative protein. A refinement of the PCR analysis is required before the expression of pCAGGS-HA and therefore its viability as a vaccine can be concluded.

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Drug discovery and black tea: investigating the non-polar fraction for antibacterial activity

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The health benefits of tea are mainly attributed to its polar components[1]. This project focuses on the non-polar fraction of black tea, which has so far been poorly elucidated. Tea is derived from the plant, *Camellia sinensis.* This projects attempts to determine the relationship between the growing conditions of *Camellia sinensis* and the chemical composition of the non-polar fraction. The objectives of this project were to fractionate black tea; identify the components contained within the non-polar fraction; chromatographically compare black tea derived from plants grown in lowland and highland cultivars of Sri Lanka; and work towards developing an assay to assess the antibacterial activity of the non-polar components.

Lowland and highland tea strains were fractionated, using solvents of increasing polarity. Ground tea leaves were stirred in petroleum ether and filtered. The filtrate was the first crude fraction. The residue was resuspended in ethyl acetate, stirred and filtered to obtain the second fraction. The process was repeated for methanol and hot water solvents to obtain five crude fractions in total for each strain. The petroleum ether fraction was then resolved into bands using preparatory TLC. The colours and R_f values of the bands were compared to those found in scientific literature in order to identify the non-polar components[2]. The fractions of each strain were subject to three bacterial assays. An agar well assay involved impregnating filter paper disks with a solution of each fraction. These disks were placed onto a bed of *S. aureus,* incubated and inspected for signs of bacterial inhibition. The agar well assay was modified to incorporate DMSO to increase component solubility in the agar. A third bacterial assay, called the Miles-Misra method, was utilised to bypass permeability problems[3].

Beta-carotene, chlorophylls a and b, pheophytins a and b, lutein and zeaxanthin pigments were identified within the resolved TLC plates. No significant chromatographical differences were observed between lowland and highland strains. No distinct zones of bacterial inhibition were observed for either of the two agar well assays. Tea component concentrations within the agar were considered insufficient. Dissolution was more problematic for the non-polar components since the agar was aqueous-based. However, it was found that DMSO could be added to tryptone soya agar at a concentration, which improved the solubility of the polar components of tea without causing solvent-mediated bacteriostasis. All samples that were tested using the Miles-Misra Method also failed to show antibacterial effects, including the positive control, EGCG. The *bacterial count to sample concentration* ratio was deemed too large to detect antibacterial activity.

The fractionation enabled the categorisation of components of similar polarities. The non-polar components were successfully identified. Chromatographical comparisons between lowland and highland tea strains were made and significant progress was made regarding the development of an antibacterial assay. Although no conclusive antibacterial data was obtained from the assays, the main complications and limitations of the assessment of non-polar tea components of black tea were indirectly exposed during the investigation.

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Identification of kinase genes as potential therapeutic targets for the treatment of EGFR+ve and EGFR-ve fulvestrant (Faslodex) resistant breast cancer

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Acquired resistance to anti-hormonal agents such as fulvestrant is a major problem in the treatment of breast cancer, hallmarked clinically by aggressive disease relapse.[1] Furthermore, some tumours are intrinsically resistant to such agents. Further understanding of the mechanism underlying resistance to anti-hormonal agents is needed to provide new improved therapeutic targets. Clinical material representing anti-hormone resistance is scarce and only limited model systems are available for exploration. However, a novel late stage fulvestrant resistant (FasR) cell line has been developed at the Welsh School of Pharmacy. The FasR cells were developed by prolonged fulvestrant treatment of anti-hormonal responsive MCF7 breast cancer cells in order to better reflect clinical treatment timeframe. This has been recently cloned to give unique FasR EGFR+ve and FasR EGFR-ve cell lines for comparative mechanistic study of fulvestrant resistance.[2] Kinases regulate many transduction pathways and are often malfunctioning in tumour cells[3] . Preliminary data indicated that the expression of kinases PFKM, PRKCI, WEE1, C17orf75 , cMET, NTRK1, FGFR4 could be differentially expressed between the FasR clones and the parental MCF7 model. The aim of this project was to verify and decipher these kinases as they could potentially reveal new therapeutic targets to resolve the treatment of fulvestrant resistant EGFR+ve and EGFR-ve breast cancer.

The mRNA expression profile of PFKM, WEE1, PRKCI, C17orf75, cMET, NTRK1 and FGFR4 were analysed across the FasR clones (and versus MCF7) by using GeneSifter microarray analysis of Affymetrix data. Semi-quantitative RT-PCR was optimised for each gene and applied to verify any differential expression of the seven kinases at the mRNA level across the cell lines. To begin to investigate whether the genes could feasibly contribute to the behaviour of the FasR clones, an ontological interrogation in relation to key cancer end points was undertaken for each kinase using online bioinformatic resources including Pubmed, Genecard and Genedecks. The clinical impact of the genes in relation to anti-hormonal resistance was assessed using online KM plot software, a publically available free clinical microarray database that enables researchers to evaluate breast cancer outcome versus their gene of interest, in this instance focussing on an ER+ve, anti-hormone treated (tamoxifen) patient sub-set.

GeneSifter analysis verified by PCR showed PFKM, PRKCI, WEE1, C17orf75 and cMET were increased in the FasR EGFR- clone whilst FGFR4 was induced in the FasR EGFR+ clone. Following GeneSifter and PCR analysis, NTRK1 was not further explored due to equivocal expression in both FasR clones. Some discrepancies between MCF7 versus the resistant models were displayed, however, PFKM and cMET were significantly increased in the FasR EGFR-ve clone and FGFR4 in the FasR EGFR+ clone versus MCF7. Ontological studies suggested PFKM, PKCI, WEE1, cMET and FGFR4 were linked with cancer endpoints that could feasibly contribute to fulvestrant resistant growth and invasiveness, although very little was known for C17orf75. Clinical profiling of the kinase genes demonstrated a potential relationship with anti-hormonal resistance for all six genes, equating with their discovery in the resistant models.

This project has successfully verified gene expression profiles of the kinases PFKM, PRKCI, WEE1, C17orf75 , cMET and FGFR4 in novel fulvestrant resistant EGFR+ve and EGFR-ve breast cancer models (versus MCF7), with parallel deciphering of their gene ontology and clinical evaluation also indicative of a potential relationship with anti-hormonal resistance. Based on these results, the project is supportive of further exploration of these kinases as future targets for the treatment of EGFR+ve and EGFR-ve fulvestrant resistant breast cancer.

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Tissue eosinophilia in allergic airways disease and its relationship with bronchoalveolar lavage

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Allergic asthma is a chronic airways disease characterised by numerous hallmarks. However, it is airways eosinophilia in response to allergen inhalation that has been described as its most striking pathological feature.[1] Much current knowledge of asthma pathophysiology has been acquired through investigations in ovalbumin (OVA)-sensitised models with notable attention paid to changes in airways inflammatory cell numbers.[2] A tool used almost universally to investigate such changes is bronchoalveolar lavage (BAL).[3] Despite widespread use, evidence confirming BAL as a method capable of accurately reflecting tissue eosinophilia is lacking. The aim of the study was to develop a histological method that allowed for accurate enumeration of tissue eosinophils after exposure to a variety of allergen and viral challenges, with a view to establishing the ability of BAL to accurately depict tissue cell numbers.

Tissues were generated by *in vivo* investigations carried out at Cardiff University into optimisation of an OVA-sensitised model of asthma, determination of the threshold fluticasone propionate (FP) dose required to attenuate the asthmatic response, and the pulmonary effects of parainfluenza virus type 3 (PIV-3).[4] BAL was performed on the left lung following sacrifice. For histology, tissues were processed and paraffin-embedded according to standard protocols. Haematoxylin and eosin staining produced poor optical contrast between eosinophils and background tissue and notable neutrophil staining. In contrast, a relatively eosinophil-specific Sirius red protocol[5] exhibited high target cell specificity with excellent optical contrast and hence was the stain of choice. Stained sections were viewed using a light microscope at 200x magnification and each of three bronchioles per tissue section centred within the field of view. Eosinophils were enumerated via two methods; per field of view; and per bronchiole. The mean eosinophil count of three bronchioles was used for subsequent analysis. Means were compared using ANOVA with *post-hoc* Tukey-Kramer. Correlations were determined using Spearman's rank. P-values <0.05 were accepted as significant.

Strong and significant positive correlations were observed between BAL and tissue eosinophil numbers for all data sets and both methods of tissue eosinophil enumeration. This included analysis of all experimental groups ($r_s = 0.86$; $P = 0.0006$). One exception was observed for OVA model optimisation protocols where the correlation between BAL and tissue eosinophils counted per field of view did not reach significance ($r_s = 0.77$; $P = 0.1028$). Correlations were stronger and more significant for all data sets when eosinophils were enumerated per bronchiole, with the number of eosinophils per bronchiole found to be strongly dependent on bronchiole diameter ($r_s = 0.76$; $P = 0.001$). PIV-3 induced a modest but significant increase in tissue eosinophils which was mirrored by BAL ($P<0.05$ and <0.01 respectively). In OVA-challenged groups, influx of eosinophils into BAL fluid was significantly inhibited by 0.5 $mg.ml^{-1}$ FP ($P<0.001$). However, inhibition in tissue did not reach significance. For model optimisation groups, OVA challenge induced dense and widespread tissue eosinophilia that extended beyond the airways. The number of eosinophils in both BAL fluid and tissue increased upon increasing protocol intensity. However, many comparisons did not reach significance in tissue; a finding not mirrored by BAL ($P<0.001$ for all comparisons).

The strong correlations observed suggest that BAL is largely reflective of tissue eosinophilia, with the increased strength and significance of correlation for eosinophils enumerated per bronchiole inferring that BAL provides a more accurate reflection of bronchiolar as opposed to wider tissue eosinophilia. This suggests that BAL inadequately samples the peripheral lung. The dependence of tissue eosinophil numbers upon bronchiole diameter indicates the need for standardisation of the histological protocol to normalise for such confounding factors. The discrepancy in the agreement of BAL and histology between OVA and PIV-3 groups may be attributable to poor sensitivity of histology where eosinophil density is high. Likewise, it may be the case that the peripheral eosinophilia observed following OVA challenge is not accurately detected by BAL. The failure of FP to significantly inhibit tissue eosinophilia in OVA groups is likely to be due to the deposition of FP in central as opposed to peripheral airways, leading to BAL over-estimating its effects on pulmonary inflammation. In conclusion, the use of BAL as a measure of tissue eosinophilia is largely justified although protocol modification is necessary in order to improve its ability to sample peripheral tissue.

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Mechanisms of the vascular effects of amphetamines in isolated aortic rings

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Amphetamine and the trace amine β-phenylethylamine (β-PEA) are structurally related and traditionally classed as indirectly acting sympathomimetics, exerting vasoconstriction via α_1 adrenoceptors.[1] However, both have been found to stimulate the recently classified trace amine-associated receptors.[2,3] Amphetamine was found to be potent agonist of TAAR1, stimulating HEK293 cells expressing the cloned rat TAAR1 as well as cloned rhesus monkey TAARs.[3,4] Amphetamine abuse can have substantial life threatening cardiac actions.[1] It is therefore important to understand the underlying mechanisms that lead to side effects such as MI,[1] and whether they are attributed to coronary vasoconstriction. The aim of this study was to find out the mechanisms of the effects of amphetamines in isolated aortic rings.

Aortic rings were suspended in 50ml organ baths containing Krebs' bicarbonate buffer. Concentration-response curves (CRCs) for each agonist were obtained in the absence and then presence of various receptor antagonists. This was done in half logarithmic increments after the peak effect was reached for the preceding concentration. Antagonists were incubated with the tissue for 15 minutes before commencing the second CRC. Drugs used include d-amphetamine sulphate, 2-phenylethylamine hydrochloride, l-phenylephrine hydrochloride, prazosin hydrochloride and L-NG-nitro-arginine methyl ester hydrochloride (L-NAME). Contractions for phenylephrine were expressed as a percentage of maximum response obtained in the initial concentration response curve. For d-amphetamine and β-phenylethylamine contractions were expressed as a percentage of the response obtained for 60mM KCl. An N value of 4 was employed for each experiment and Student's paired t-tests were used for statistical analysis.

Phenylephrine caused concentration-related vasoconstriction of the aorta and was shown to be competitively antagonised by prazosin (α_1 –adrenoceptor antagonist) A – log K_D value of 7.47 ± 0.09 was obtained. D-amphetamine and β-PEA caused concentration-related vasoconstriction of the aorta. It was found that both d-amphetamine and β-PEA CRCs were potentiated in the presence of L-NAME (Nitric oxide synthase inhibitor). Additionally, d-amphetamine CRC were shifted upwards in the presence of prazosin. β- PEA responses however were not inhibited by prazosin, indicating that the vasoconstriction was not an indirect sympathomimetic action.

The presence of other receptors such as TAARs, may therefore mediate the vasoconstriction. The potentiation of aortic contraction in the presence of L-NAME, suggests that d-amphetamine and β- PEA responses may be modulated at least in part by an opposing vasodilator response which when blocked reveals an enhanced vasoconstriction. Since this was observed in the presence of L-NAME, it must involve a NO pathway such as, the release of NA acting directly on the endothelium causing relaxation via the release of EDRF (NO) or a novel TAAR coupled to nitric oxide and cGMP pathway through activation of guanylate cyclase. The potentiation of the response to amphetamine in the presence of prazosin may suggest that if the response to amphetamine is modulated via NO, prazosin could in fact inhibit NOS and therefore block the vasorelaxation response pathway. This would allow the potentiation and exhibition of enhanced amphetamine vasoconstrictive actions. Alternatively, an amphetamine mediated vasorelaxation may be produced by a novel receptor on which β-PEA has little or no action. Amphetamine and the trace amine β-phenylethylamine cause vasoconstriction of isolated guinea pig aortic rings via a mechanism that does not appear to be exclusively dependent on noradrenaline release via sympathomimetic action. Various studies have demonstrated actions of amphetamine and trace amines on trace amine-associated receptors, however, whether these receptors cause the vasoconstriction observed in blood vessels remains to be determined. Furthermore, the action of these amines has been found to be subject to modulation via a NO pathway, the source of which is yet to be established. This receptor may be subject to inhibition by prazosin. The key finding of this study is that contrary to treatment guidelines,[5] α-adrenoceptor antagonists should not be given to a patient with an amphetamine induced hypertensive crisis.

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An exploration of student views on feedback on assessed work

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The role of feedback on assessed work in supporting teaching and learning in higher education is a topic of continual debate between students and educators, but one that is currently underresearched. The annual National Student Survey (NSS) consistently highlights assessment and feedback as the aspects of higher education with which students are least satisfied. Results of the 2010 NSS showed that 83% of students at UK universities were satisfied with overall teaching, however only 66% of students were satisfied with assessment and feedback[1]. Feedback on assessed work is considered to be very important as it has the "capacity to turn each piece of assessed work into an instrument for the further development of each student's learning"[2] and enables students to fill the gap between current knowledge and understanding and desired learning goals[3,4]. The aim of this project was to explore the views and experiences of Pharmacy undergraduate students at the Welsh School of Pharmacy relating to provision of feedback on assessed work in order to gain an insight into the reasons behind such statistics as results of the NSS, and in order to develop a questionnaire for future distribution to a larger sample of Pharmacy undergraduate students.

Focus groups were chosen as a qualitative methodology to inform the development of a questionnaire (quantitative methodology). It was not the intention of this project to administer the questionnaire. Purposive (non-probability) sampling was used to recruit 19 students for four focus groups, which were conducted at the Welsh School of Pharmacy. Each focus group was composed of students from the same year group to promote group familiarity and homogeneity and, therefore, group discussion. Focusing exercises were used to stimulate discussion, including a ranking exercise. All focus group discussions were audiotaped and transcribed *verbatim*. Transcripts were analysed using the code and retrieve (thematic analysis) method. A questionnaire was then produced based on the themes derived from the focus group discussions and literature[5].

A number of themes were discussed during the focus group sessions, including the focus of feedback (referring to both the learning task and the student), consistency (including between different lecturers, topics and modules), clarity, modes of delivery used and the volume of feedback provided. The discussions with third and fourth year students covered a greater number of themes than those discussions with first and second years. Themes were also discussed at greater length by those in the third and fourth years. The focus of feedback and its timing were the issues that were considered by students overall to be the most important. Students expressed the opinion that feedback needs to be specific to individual students in order to be relevant and to enable students to make improvements based upon it. It was also commented that feedback needs to be provided in a timely manner so that it can be used to prepare and improve for the next relevant assessment and so that students are still mindful of the topic. The mode of delivery used and the volume of feedback provided were the issues rated least important. Students described how provision of feedback is overall more important than the particular method by which it is provided and that the quality of feedback can be more important than the quantity provided (though this quantity should be tailored to individual students' performance).

This qualitative, exploratory study at the Welsh School of Pharmacy was successful in highlighting issues that are important to students regarding provision of feedback on assessed work. This study has also revealed ways in which students consider feedback could be improved. Further studies involving a greater number of focus groups would be needed in order to reach theoretical saturation. Methodological triangulation, including interviews with staff and administration of the questionnaire to a larger sample of Pharmacy undergraduate students using probability sampling, would be needed to determine whether or not the participants' views are representative of the wider population and to improve the research design.

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The effect of magnesium sulphate on salbutamol-induced relaxation of isolated trachea

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Magnesium has been demonstrated to relax airway smooth muscle *in vitro*[1] and has the ability to cause bronchodilation in asthmatic airways *in vivo*[2]. *In vivo*, magnesium sulphate ($MgSO_4$) appears to be more effective when used in combination with β-agonist therapy compared to when used alone[2]. The mechanism of clinical improvements in asthma is most likely attributable to the influence of magnesium on calcium homeostasis[2]. It was the aim of the study to investigate the effect of $MgSO_4$ on histamine(HA)-induced contraction and to analyse whether $MgSO_4$ is able to enhance the relaxation seen to salbutamol (SB) in an *in vitro* model.

Isolated tracheal spirals were arranged in 20ml organ baths containing either normal Krebs solution (final conc. $MgSO_4$ 1.2mM) or an isotonic Krebs solution containing an additional 15mM $MgSO_4$ (final conc. $MgSO_4$ 16.2mM). The contractile tension of the tissues was measured. Two CRCs to HA were conducted in succession in the tissues (n=6), where one CRC was performed in normal Krebs solution and the other in the presence of $MgSO_4$ 16.2mM. Two cumulative CRCs to SB, following pre-contraction of the tissue with 10^{-5}M HA, were conducted in succession in the tissues (n=6), where one CRC was performed in normal Krebs solution and the other in the presence of $MgSO_4$ 16.2mM. Two tracheal spirals were set up as paired with one as a control tissue and the other in the presence of $MgSO_4$ 16.2mM. Two CRCs to SB, following pre-contraction of the tissue with 10^{-5}M HA, were conducted in succession in each tissue (n=3).

$MgSO_4$ 16.2mM failed to significantly reduce HA-induced contraction in the tissue ($P>0.05$). Additionally, $MgSO_4$ 16.2mM was unable to significantly enhance SB-induced relaxation in the tissue ($P>0.05$). There were rightward shifts ($P>0.05$) seen between the EC_{50} values of the first and second CRCs to SB, indicating possible SB-mediated desensitisation of the tissue. The degree of shift between the first and second CRCs to SB in the presence of an additional 15mM $MgSO_4$ was twice that seen in control tissues, indicating possible potentiation of SB-mediated desensitisation by $MgSO_4$ 16.2mM.

Having failed to significantly reduce HA-induced contraction of the isolated trachea, at a concentration of 16.2mM that is much greater than those used clinically, doubt has been cast over the suggestion that $MgSO_4$ directly relaxes airway smooth muscle[1]. Instead, although not investigated in this study, $MgSO_4$ is thought to inhibit bronchoconstriction *in vivo* by way of more complex mechanisms, on tissues other than smooth muscle e.g. nerves and inflammatory cells[1]. Having failed to significantly enhance SB-induced relaxation of the trachea, $MgSO_4$ 16.2M is unlikely to potentiate the effects of SB on the β_2 adrenoceptor machinery *in vitro*[2]. The SB-mediated desensitisation of the tissues observed in this study may account for the failure of $MgSO_4$ to enhance SB-induced relaxation of isolated trachea.

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Use of differentiated U937 monocyte models as targets for anti-tuberculosis drugs

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There is a need for the development of new drug delivery systems to combat tuberculosis especially in the developing world where the mortality rates are high due to an increased number of HIV patients and multidrug resistance. The aim of this study was to establish an in vitro macrophage model using the human monocytic cell line U937. The results from this study will eventually be used as a template to the development of a drug delivery system to combat TB.

U937 cells were treated with TPA (12-O-tetradecanoylphorbol-13-acetate) and incubated for 48 hours. Labelling using Dextran-TMR and Alexa488-Tf was performed on the TPA treated cells to detect the presence of endocytic apparatus , transferrin receptors and the organisation of actin filaments in the cell cytoplasm. In addition, the subcellular distribution of lysosomes and endosomes was determined by staining the cells with LAMP-2 and EEA-1 antibodies. Phagocytic activity of the differentiated cells was investigated through the use of dyna-cobalt-coated dyna beads which were immersed in the cells.

U937 cells were successfully differentiated with TPA. This was observed by the change from the rounded morphology of undifferentiated U937 cells to differentiated U937 cells that possessed a starry shape with extending pseudopodia. Cell-cell adherence was also observed Actin filaments were observed to be filamentous structures across the cytoplasm and staining with Alexa488-Tf proved the presence of transferrin receptors. The presence of endocytic organelles in the differentiated cells was confirmed through staining with LAMP-2 and EEA-1 antibodies. Phagocytic activity was illustrated by the uptake of the dyna-cobalt coated beads into the cells.

The results show that the differentiation in monocytic U937 cell lines can be induced through TPA. For more reliable differentiation results, it is necessary to determine the number of transferrin receptors on undifferentiated U937 cells also. Also, the effect of different inducers of differentiation can be examined on the U937 cells. These agents include vitamin D3 and retinoic acid.

An evaluation of mathematical prediction of transdermal permeability coefficient (kp)

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With the increasing efforts put into the employment of the transdermal route for drug delivery, the use of mathematical models to predict the transdermal permeability coefficient (kp) has become more vital. Previously developed and tested mathematical models include: empirical models (quantitative structure-permeability relationships), mechanistic models, and the Gaussian regression model (non-linear approach). The predictive efficiency of the proposed models is in no way conclusive. There has been much speculation over which of these models yields the most accurate predictions. Therefore, the aim of this study was to evaluate the efficiency of a number of mathematical models (n = 9). These models included the Potts and Guy model [1], the Gaussian regression process [2] and seven QSPR models generated in this study using different combinations of six physicochemical properties (Log P, Log S, MW, H-donor number, H-acceptor number and solubility parameters (δ)).

This evaluation was carried out by applying various statistical tests (best subset regression, multiple regression analyses and a two-way ANOVA variance test) to a wide-ranging dataset. The dataset included 121 chemical compounds. The inclusion criteria of this dataset encompassed: aqueous donor/receptor, human skin, and non-ionised drugs. Predicted permeability coefficient values were generated using the various models and compared to values measured *in vitro.*

The initial hypothesis of this study was that including more predictors to the Potts and Guy QSPR model would lead to an improvement in its predictive efficiency. Moreover, due to claims made by Moss et al [2] and Sun et al [3] it was primarily hypothesised that the non-linear Gaussian regression process would generate more accurate results than the QSPR model. However, results obtained suggested that; in terms of QSPR models, the inclusion of more physicochemical predictors into the model showed no improvement in the effectiveness of the predictive model. This was confirmed by the fact that there was no statistical significance between the differences in errors attributed to the various models used ($p > 0.05$). Furthermore, when the errors generated by QSPR models were compared with those generated by the Gaussian analysis, there was no statistical significance in the difference between them ($p > 0.05$). On the other hand, the differences between the errors attributed to the compounds tested ($n = 12$) have shown to be statistically significant ($p < 0.05$). This showed that some models are better at predicting certain compounds than others

In conclusion, this study showed that the Gaussian process failed to provide an improvement in results and that the Potts and Guy model remains the model of choice. In this study the database used was more comprehensive than the Flynn et al [4] database which was initially used to derive the initial Potts and Guy model. This lead to the derivations of an improved mathematical model that uses the Potts and Guy predictors (Log P and MW); which is:

$$\text{Log}kp = -2.30 + 0.508\ \text{Log}P - 0.00659\ MW.$$

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Developing a new performance indicator for molecularly imprinted polymers

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Molecularly imprinted polymers are polymer networks with micro-cavities of pre-determined selectivity for an analyte of interest.[1] In the process of molecular imprinting, appropriate functional monomers are introduced to interact with template molecules, and then the functional groups on the monomers are fixed with chemical cross-linkers. Extraction of the template molecule leaves a binding pocket tailored to the shape and interaction properties of the analyte molecule. MIP performance is commonly analysed through batch rebinding assay. The result of these assessments is presented in many forms, e.g. imprinting factor, K_d, and B_{max}. B_{max} and K_d are hardly ever reported in papers and the calculation of the imprinting factor is inconsistent. This makes inter-laboratory comparisons impossible. Therefore the aim of this study is to find a new performance indicator to enable MIP comparison.

Literature review of published papers on MIPs that reported imprinting factor was done. The binding data was normalised into a standard unit of nmol/mg for bound and µM for free and plotted as a bound vs. free isotherm. MIPs data was then entered into Graphpad software to estimate the B_{max} and K_d values. The B_{max} and K_d values were then used to calculate imprinting factors using different methods. The calculated imprinting factors, K_d, and B_{max} values were correlated with reported imprinting factors to find relationships in assessing MIP performance.

There was no apparent correlation between the reported imprinting factors and the ratio of both B_{max} and K_d values for MIP and NIP. The correlation between the ratio of B_{max} values and ratio of K_d values demonstrated that the inconsistency in the reported imprinting factors. The imprinting factor should be reported together with the Bmax and K_d values so as enable effective comparison of MIPs. The ratio of K_d MIP/K_d NIP should also be reported as it may provide information about selectivity.

From the extensive literature review of published papers that reported the imprinting factor, only 24 reported sufficient information to allow conversions and interpretation necessary for analysis. The major limitation for this study was the inadequate reporting of data which made the data conversions impossible. This study has demonstrated critical need for reporting of adequate and comparable data.

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Is the feedback on coursework for the Cardiff University MSc in Clinical Pharmacy appropriate and could it be improved?

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Feedback on coursework is an essential element of higher education and a current 'hot topic' within the field. Feedback is regarded by many as a vital component for academic success[1]; however, it is argued that the quality of feedback is not always to the required standard[2]. Conversely, some studies suggest students do not use feedback to benefit them in the future[3]. The aim of this study was to evaluate feedback provided on coursework for the Cardiff University MSc in Clinical Pharmacy and determine how, if deemed necessary, it can be improved. The MSc course is based at five centres, each run by an Associate Course Director (ACD) who is responsible for providing the majority of feedback on the assessments to students in their centre.

The aim was achieved by undertaking qualitative research in the form of a focus group with the ACDs (n=4) and semi-structured interviews with purposively sampled students on the course (n=10). One first and second year (third year in Oxford Centre) from each of the five centres with varying academic ability were selected for the study. Four interviews were conducted over the telephone due to the geographical location of some students. A semi-structured interview was held with one ACD as they were unable to attend the focus group. The discussions were audio recorded, transcribed *verbatim* and thematically analysed. Ethics approval was obtained prior to starting the study from the WSP Research Ethics Committee.

Eight out of ten students participated in the study. The ACDs and the majority of students commented that points for improvement and positive and negative comments are included in feedback. The feedback was provided mainly in written form and some verbal feedback was given, primarily for oral presentations. The students stated that they valued feedback and utilised it in future assignments; however, the majority of students found the feedback received on the course only partly useful. Some students believe there should be more positive and verbal feedback. A few students were also unhappy with the timeliness of feedback. The ACDs claimed the main barriers to providing better feedback were: time, location (access to students) and students' perception of feedback.

The results concur with literature that feedback is a highly subjective topic and there is no single best way to provide it[4]. This study suggests that overall the feedback provided on the course is appropriate; despite this, there have been areas for improvement identified, which need to be reported to the course Management Committee to see, if possible, these can be rectified to comply with a new draft policy on *Academic Feedback to Students* published by Cardiff University[5]. The researcher believes that it is important to continue research in this area to gain a better understanding of the views students have on the feedback they receive and hence improve the overall course.

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Is the BK channel neuroprotective in neuronal cell models of oxidative stress and hypoxia?

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The BK channel is a large conductance calcium-activated voltage dependent potassium channel, widely expressed throughout the body with a key role to play in many physiological processes from neurotransmitter release to tuning of inner ear hairs. In neurons, it has been hypothesised that activation of the BK channel, causing potassium efflux from the cell, acts to protect the cell from insult by hyperpolarising the membrane therefore dampening down cellular excitability and preventing excitotoxicity[1]. The presence of the BK channel has proved to be protective in vivo[2]. The aims of this study are to determine the presence of the BK channel in SH-SY5Y cells and determine whether activation of it protects the cell from neuronal insults, oxidative stress and ischaemia.

The human neuroblastoma cell line SH-SY5Y was cultured and was differentiated by exposure to 10µM retinoic acid for 7 days. PCR was carried out on both undifferentiated and differentiated cells to determine expression for the message of α and β subunits. Patch clamping was undertaken to examine the functionality of the BK channel in the live cell. Then H_2O_2 and $CoCl_2$ were used as cellular insults representing oxidative stress involved in Alzheimer's disease and hypoxia representative of stroke, respectively. Cell viability was measured with MTS assay. Cells were seeded and left overnight, insulted and left for 24 hours then MTS was applied. They were left for 2 hours then the plates were then read at 490nm. The next stage of the study involved BK channel modulators, openers NS1619 and isopimaric acid and blockers tetrandrine and TEA were applied to with the insult.

The major findings of this study were as follows. PCR confirmed the presence of the message for the BK channel in both undifferentiated and differentiated cells. All four types of β subunit were present; the exact proportions of each could not be ascertained. The BK channel was seen to be functional when examined with patch clamping and the characteristic BK conductance of 220 pS was seen. Differentiated cells showed increased sensitivity to both H_2O_2 and $CoCl_2$ insults compared to the undifferentiated cells. With regards to H_2O_2, differentiated cells had an ED_{50} of approximately 300 µM whereas differentiated cells had an ED_{50} of 150 µM. When channel openers NS1619 and IPA were used in conjunction with the H_2O_2, a decrease in absorbance was seen in undifferentiated cells and no change was seen in differentiated cells. $CoCl_2$ with IPA or NS1619 produced a decrease in absorbance in undifferentiated cells. When the blockers tetrandrine or TEA were used, no overall significant effect on absorbance was observed.

From this study it is clear although the BK channel is present and functional; activating it was not protective and in fact in some cases, actually decreased the cell viability. These results were not what was expected and contrary to previous work on the subject[2] It is possible that the cell experienced an overlarge potassium efflux resulting from the channel opener plus the rise in calcium produced by the insult which may have opened the channel anyway. This large K^+ movement may have also caused Cl^- and water loss from the cell, which caused cell shrinkage known as apoptotic volume decrease, driving apoptosis resulting in the decrease in absorbance seen.[1,3] β subunits play a role in calcium sensitivity and gating kinetics and different subunits produce slightly different currents[4]. The difference in sensitivity to insults between undifferentiated and differentiated cells could be attributed to the slightly different expression of β subunits however this would not be known for certain until QPCR was undertaken.

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Should viral infections such as parainfluenza be treated in asthmatics?

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Asthma involves chronic inflammation of the airways in which the airways become hypersensitive and the symptoms include coughing, wheezing, shortness of breath and chest tightness especially at night or early in the morning.[1] Inhaled corticosteroids are commonly used to keep these symptoms under control.[2] Respiratory viruses such as human parainfluenza virus type 3 (HPIV3), which is very common, cause exacerbations of asthma by prolonging the duration of infection and increasing the severity of the symptoms.[3] It is not yet fully understood whether steroids can be used to treat or prevent these asthma exacerbations caused by viral respiratory infections. Therefore, this research was carried out to investigate whether prednisolone has any anti-viral activity against HPIV3. Ribavirin, which is a well-known anti-viral drug, was used as a positive control.

Cell viability assays were performed to assess the toxicity of the drugs. Dimethyl sulfoxide (DMSO) 0.5% was used as the vehicle for prednisolone. Ribavirin was used at 4.10×10^{-8} to 2.05×10^{-4}M. The concentration of prednisolone ranged from 1×10^{-9}M to 1×10^{-3}M. The drugs were added to African green monkey kidney epithelial cells (BSC-1) and incubated for 3 days at 37ºC. After incubation, MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphphenyl)-2H-tetrazolium] solution was added to the cell monolayer and incubated for 2 hours at 37ºC. The absorbance was read and the cell viability was calculated. Plaque reduction assays were carried out to assess the anti-viral effects of the drugs on HPIV3 infection in BSC-1 cells. The drugs were added to the cells and incubated for 1 hour (ribavirin) or 24 hours (prednisolone). Then the virus was added at 3.97×10^{3} pfu/ml and the cells were overlaid with Avicel 0.6%w/v and incubated for 3 days at 37ºC. Then the cells were fixed with 2% formaldehyde, stained with crystal violet 0.1%w/v and the number of plaques was counted using a light microscope. Results were expressed as mean ± SEM and statistical analysis was performed with one-way ANOVA followed by Dunnett's post-hoc test. All the experiments were performed at least 3 times.

Ribavirin did not significantly affect cell viability and produced substantial anti-viral effects against HPIV3 at concentrations above 2.05×10^{-5}M. The IC_{50} value for Ribavirin was 4.59×10^{-5}M (95% confidence interval 1.20×10^{-5} to 1.75×10^{-4}). DMSO 0.5% caused significant cell toxicity and prednisolone reduced cell viability significantly at concentrations higher than 1×10^{-8}M. Prednisolone had significant anti-viral activity against HPIV3 only at 1×10^{-7} M and 1×10^{-6} M but, at the highest concentration, the lowest anti-viral activity was observed. This is partly due to DMSO contributing to the cell toxicity.

Ribavirin does not cause cell toxicity and has anti-viral effects against HPIV3 as expected. Prednisolone has some anti-viral activity but causes cell toxicity at high concentrations. Prednisolone could potentially be used at low doses to ameliorate virus-induced asthma exacerbations.

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Can accurate results still be obtained from ACI and NGI analysis when stages are grouped?

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Impactors such as the Andersen Mark-II Cascade Impactor and the Next Generation Pharmaceutical Impactor are crucial in the analysis of particle size and distribution for aerosols[1]. Both instruments are recommended by the European Pharmacopoeia and US Pharmacopeia[2]. Despite their common use and numerous benefits, these instruments have their drawbacks; the processes are both laborious and time consuming[2]. The aim of this project was to determine whether grouping the stages of the impactors as a method of abbreviation will still produce the same level of accuracy of aerodynamic particle size results as the full analysis, potentially saving time and money.

The Andersen Mark-II Cascade Impactor and Next Generation Pharmaceutical Impactor have 8 and 7 stages respectively[3], for this research previously obtained data was manipulated using only 3 stages of each impactor. The stages were chosen for their similarity of 50% diameter cut off to those used in current Abbreviated Impactor Measurement concepts and also their comparability to regions of the respiratory tract. Data from two different formulations types were manipulated for each impactor, nebulisers and pressurised Meter-Dose inhaler for the Andersen Mark-II Cascade Impactor; solution and suspension for the Next Generation Impactor. Once the data was manipulated the Mass Median Aerodynamic Diameter (MMAD) obtained from the abbreviated data was compared to that from the original raw data.

The results for the Andersen Mark-II Cascade Impactor data show that the difference between the MMADs for nebulisers as 8% and for pressurised Meter-Dose Inhaler as 4%. The results for the Next Generation Impactor data show that the difference between the MMADs for solutions as 26% and for the suspensions 52%.

The results show that there is potential for abbreviating the Andersen Mark-II Cascade Impactor in the future, using this method, combining plates at the washing and analysis stage. However the same cannot be said for the Next Generation Pharmaceutical Impactor. Abbreviating in this manner did not produce adequate results to suggest that it may be a viable option.

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A pilot study, at a general medical practice, investigating an alternative way to identify adverse drug reactions

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Adverse drug reactions (ADRs) arise as a result of an unwanted or harmful reaction to a drug or combination of drugs which can be of a very serious nature.[1] During clinical trials many ADRs may not be identified that actually come to light when the drug is used in the general public.[2] New drugs to market are identifiable by the Black Triangle symbol (▼) which informs all healthcare professionals that the drug is still under intense surveillance and that all ADRs should be reported to the MHRA using Yellow Cards.[2] Unfortunately this scheme is not flawless and its biggest downfall is under reporting and the reasons behind this have been extensively examined in previous studies.[3,4] As a result the reporting rate for all ADRs to not only Black Triangle drugs but also for serious reactions to established drugs is greatly under estimated. The introduction of a new method for identification of ADRs could be used to complement the current methods that are in use to boost the ADR reporting rates and improve the safety of the general public. The aim of this study is to evaluate a new method of ADR identification at a GP surgery with the intention to improve ADR reporting rates.

Black Triangle drugs were identified from the January 2010 list published by the MHRA. Patients at Llanrumney Medical Centre who were prescribed these drugs were then ascertained from prescription issue data and their electronic records were examined to determine how many patients had experienced an ADR and how many had stopped treatment as a result of the ADRs they had suffered. The type and severity of ADR was then established. A comparison of the results was made with two established drugs, simvastatin and ramipril, to determine the appropriateness and effectiveness of the method with regards to the duration of data collection.

A total of 123 Black Triangle drugs were searched but only 32 (26.0%) had been prescribed to patients between January 1st 2010 and December 31st 2010 inclusive. Ten (31.3%) of those prescribed Black Triangle drugs caused at least one ADR in 31 patients and a total of 45 side effects were experienced only a few of which were serious. However 25 out of 104 side effects that occurred to the established drugs were serious. ADRs identified in this study were only added to the 'Drug Allergies and Adverse Reactions' filter that is on the computer system in a few cases for the established drugs and no evidence could be found that any Yellow Cards were sent to the MHRA for any of the ADRs.

This method has many similarities to other currently used forms of ADR detection but also has many advantages over these other forms. It ensures that all ADRs that occur within the allocated study period are identified and it does not rely on GPs to fill out Yellow Cards or Green forms that accompany Prescription Event Monitoring studies.[5] However this method does rely on the necessary information being present in the patient's records and it being of a high quality. As the records of all patients who are prescribed a drug are examined there is also a high workload so only time efficient for Black Triangle drugs and as there was only one researcher during this study, the opinion as to whether or not an ADR had occurred could not be verified. On examination of the ADRs identified by this study it was determined that 5.0% of all patients included in the study experienced at least one ADR; that equates to 7.8% of people who were prescribed Black Triangle drugs and 4.3% of patients who were prescribed an established drug experienced side effects of their medication. Furthermore, no evidence could be found that any of the required Yellow Cards had been completed. This supports the current view of the under reporting rates of ADRs and emphasises the need for a new method of ADR identification such as the one discussed in this paper.

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Design and Presentation of a Computer Assisted Learning (CAL) Package on Urinary Incontinence and its Management

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Computer Assisted Learning (CAL) is widely used as an academic learning tool in American and European universities. The key feature of CAL is that it enhances the interaction between students and the content of the package[1]. Frequently, CAL allows educators to freely utilize multimedia elements such as sound, video, pictures and animations to illustrate phenomena that are difficult to visualize, thereby ensuring that maximum impact upon learning is achieved[2]. The aims of this study were to evaluate the effectiveness and suitability of a CAL package developed for teaching purposes and also to find out the important core components of making a successful CAL package. The study consisted of developing a new core CAL package on the topic of urinary incontinence, followed by an evaluation of the package.

The package was constructed using Microsoft PowerPoint®. It covered four areas including the physiology of the bladder, types of urinary incontinence, evaluations & examinations and management. It was also associated with a self-assessment section at the end. The package was designed in a variety of forms, including text, images, graphics and animations. Content information was supported by up-to-date journals, reference books, news and online factsheets to ensure the package reflected real time conditions and patient issues. In order to facilitate student understanding of the package, tables and images were widely used in each chapter as well as for highlighting key points. The package was accessed via the internet and evaluated by MPharm II students regarding their views on computer based learning and the teaching package specifically through a questionnaire. Students were asked to rate their views on its presentation, content and relevance using a Likert scale of 1 to 5, where 1 corresponded to 'strongly agree' and 5 to 'strongly disagree.

Forty-three questionnaires were received representing a response rate of 40%. The package received an overall positive response. PACKAGE PRESENTATION: the majority of participants agreed that the package was well presented. They regarded the use of hyperlinks (93%, n=40), animation (95%, n=41), and colors (63%, n=27) in the package as appropriate. Common complaints indicated that some slides contained too much information which they found difficult to read. PACKAGE CONTENT: The feedback showed most respondents found that the package was concise and covered the topics well in each section. The downside was that some students thought the package was outside the syllabus of their current studies. It was suggested that more background information and case studies should be added. OVERALL IMPRESSION: All participants reported that they had learned something new which could be applied to their future practice after CAL package completion. It was regarded as a useful tool for external reading, but was inferior to face-to-face lecture learning. Furthermore, a considerable number of students thought the package contained too much information which was beyond the scope of their current studies.

The evaluations indicated that CAL is a useful resource for undergraduate learning. The majority of MPharm II students agreed that the package was comprehensive, informative, and concise. Several improvements were suggested, such as shortening the length of the package, adding summary slides after each section and having more self-test sections. It was concluded that the response rate would have been increased if the topic of the package was related to their current study. In conclusion, the package was a good external study material that would benefit students for their future practice.

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Patients' views of the Medicines Use Review (MUR) service in England and Wales

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Medicine Use Reviews (MURs) have been available as an advanced level service within the National Health Service (NHS) since the publication of the community pharmacy contract in 2005[1]. They comprise a private one to one consultation between a pharmacist and patient with the aim to increase patients' knowledge about their medications, ensure appropriate use of medicines and optimise adherence to medicine regimens[1]. Every pharmacy can claim up to £11,200 per year from the NHS for providing the service[2]. As such, it is important to determine whether MURs are of benefit and whether or not the service provided is of an acceptable standard from the patient's perspective. The aim of this study was to determine the views of patients, with regards to the MUR service in England and Wales, using a structured questionnaire.

Pharmacists completing the postgraduate Diploma in Pharmacy Clinical Practice at the Welsh School of Pharmacy (WSP) were approached to help recruit patients to the study. Those who agreed were sent 10 questionnaires and asked to give one out to each consecutive patient after they conducted a MUR. The questionnaire comprised 3 sections (Section A: 'The role of the pharmacist', Section B: 'The MUR consultation' and Section C: 'Written information'). Multiple choice answers on a five point Likert scale were used for the first two sections whilst Yes/No/NA answers were provided for section C. The patients were asked to complete the questionnaire and return it to the WSP using a free post envelope provided. One pharmacist known to the recruitment team also offered to participate in the study and was sent 20 questionnaires and asked to follow the same procedure. Once returned, questionnaires were analysed using the Statistical Package for the Social Sciences (SPSS©)v. 16.

A total of 160 questionnaires were sent to the pharmacists. Of these, 111 were distributed to patients (111/160=69%) and 43 returned to the WSP before 21st March 2011 yielding a response rate of 39%. Fourteen questionnaires returned as part of a pilot study were also used in the analysis. Internal consistencies of the three sections of the questionnaire were found to be high (Cronbach's alpha > 0.7). Patients' views of the role of pharmacists and of their MUR consultations were found to be generally high (N=51) with at least 92% of scores over the midpoints of the scales for each section. However, it was found that patients tended to prefer written information over verbal but most (N=28/51) either did not receive any written information or were not satisfied with it.

The results showed that patients had a positive view of both pharmacists and their MUR consultations. However, results may have been skewed as Diploma pharmacists might be expected to provide MURs to a higher standard than other pharmacists due to the additional consultation skills training provided. Results also suggested that more written information should be provided to patients. Psychometric tests of the questionnaire found it has a high internal consistency and therefore is a reliable tool for measuring patients' views of the MUR service. Initial correlation analysis indicated that the questionnaire has good construct and concurrent validity. Further research should include gaining responses across a wider population (of pharmacists providing the service and patients receiving) to allow a factor analysis of the questionnaire and enable results to be generalised further.

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Molecular modelling studies on the activation pathways of nucleoside analogues

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Human kinases are important for the phosphorylation of nucleosides analogues used in the synthesis of DNA[1]. The activities of nucleoside analogues are generally dependent upon their phosphorylation by these pathways, as the triphoshate form cannot be used as drugs themselves because they cannot cross the cell membrane. There are many first step kinases that act upon analogues, for this investigation the deoxynucleoside kinases (dNKs) and 5'nucleotidase were focused on, as these were seen to act upon the analogues. The second step is catalysed by adenylate kinases, thymidylate kinase, uridylate-cytidylate kinase and the guanylate kinase[2]. The last step is catalysed by nucleoside diphosphate kinase. Kinases catalyse the transfer of a phosphate group from a donor, usually the γ-phosphate of ATP, to an acceptor, either the 5′OH group of nucleoside, or the α- or β-phosphate group of a nucleotide. Phosphorylation usually takes place 'in-line' which requires the donor and the acceptor to be correctly positioned upon binding, but also may involve conformation changes in the enzyme, such as LID closing or hinge bending domain movements[3]. The overall aim of this investigation was to design a computer model capable of predicting the phosphorylation pathways of future nucleoside analogues and score them on well they are phosphorylated[3].

The methodology required the research into the phosphorylation pathways and binding data of a range of nucleoside analogues, and creating a database of these compounds, in MOE, used to train and test the model. The pharmacophore was developed for each kinase in MOE and then run against the database of compounds to generate hits and an RMSD value. The databases of compounds were then docked via PLANTS to generate a PLANT score for the compounds. Finally specific moieties in the crystallised substrate were selected and an SVL script was ran in MOE against the database of compounds to generate hits and RMSD distances for the compounds. All these values were then taken to design the scoring function for the model. The scoring function was then used to calculate the final scores for the compounds in the databases, to ensure it predicted scores reliably.

Altogether nine kinases were characterised. TK1, dGK, 5'nuc II, TMPK, GMPK, AMPK and NDK were all relatively simple to characterise from the literature available, as they are specific to their bases, whereas dCK and UMPCMPK were harder as they could accept a broad range of bases, both pyrimidine and purines. Binding studies was the main information used to build the scoring and pharmacophore models. The models work well for all kinases, as all the expected hits were obtained when a hit search was performed. Most had common essential features such as the aromatic base and 5'group which had to be satisfied for acceptance into the model, and partial match criteria as some of the selected features were non-essential. dCK and UMPCMPK were more complicated due to their unusual specificity and had to have multiple exclusion volumes to exclude thymidine analogues. The pharmacophore query editor in MOE allowed the specific models to be created successfully and allowed inclusion of the criteria needed. Therefore the pharmacophore models are the primary discriminator for the model. Scoring for the model however was far more difficult as the scores gathered form the docking program PLANTS did not clearly discriminate between the bases as the scores seemed close together. So a new scoring model had to be integrated with PLANTS which was an SVL script which scored the analogues via their distances from the specific atoms selected on the template in the kinase. These fragment RMSD distances gave a more clear discrimination and dismissed the unexpected analogues from the model. The moieties for the RMSD scores were the 5'group and base which were considered important for ligand interactions with the kinase and ensure correct conformation upon binding. A reference was used for all the kinases, which was the natural substrate, as it theoretically should have the best score for the kinase, and all the other compounds were compared to this to generate the final score for the compounds.

The specificity of the kinases for the analogues was found to be mainly dependent upon the base and conformation of the nucleoside in the kinase for efficient phosphorylation. The specificity also dropped through the kinase steps with NDK being the least specific and efficient phosphorylation was dependent upon the correct conformation upon binding of the analogue. The pharmacophore hits and the final model scores obtained for the compounds put through the model were as expected and show that it is reliable in predicting the scores for any future analogue put through the model.

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Design and Synthesis of Novel Inhibitors of Chikungunya Fever

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Chikungunya Fever is a viral illness classically contained within subtropical climates including regions of Africa and India.[1] Predominately presenting symptoms of fever, rash and arthritis, it has rarely been associated with fatalities.[1] However concern arose when mutation within the E1 structural protein resulted in the primary transmission vector altering from Ae. Aegypti to Ae. Albopictus;,a mosquito with wider inhabitation giving the potential for globalisation of the fever.[2,3] This mutagenic strain is associated with increased severity of symptoms and the first related fatalities arising from the mutagenic strain preferentially targeting slower replicatory tissue.[4] Due to its nature as an alphavirus, viral genomic release results in production of polyprotiens, subsequent processing generates the viral non-structural proteins that commutatively produce the apparatus for viral replication.[5] It is nsP2 protease which posses the proteolytic function that allows the generation of non-structural proteins.[5] nsP2 inhibition will theoretically prevent viral replication and further host infection. Protein crystallization of CHIKV nsP2 protease has not been achieved however previous SAR investigations and the construction of a homology model based on the crystallized VEEV nsP2[6] has generated a lead compound.[6] This investigation overcomes limitations in the lead through uses of computer aided drug design to propose new compounds for synthesis and further biological investigations.

Over 120 compounds were docked and analysed using Glide and MOE software, with four key features identified as being critical to receptor activity: ligand linearity, molecular orientation within active site, hydrogen bond formation with key receptor residues, and the high potential for receptor-ligand dissociation due to exposure of the active site. These key aspects together with ease of synthesis were used in a weighted screening model to rank each compound's suitability. Five compounds based on the N-(3-phenyl-acryloyl)-benzamide structure were thus identified as possessing good inhibitory potential. The acidic nitrogen and carbonyl oxygens were found to form critical hydrogen bonding with Asn 79, Tyr 44 and Trp 81 not only increasing receptor stability but also preferential positioning. Critical interactions also formed between Asp 243 predominately and substituted hydroxyl groups, resulting in promoted structural linearity and desired ligand orientation. Furthermore, despite environmental exposure, Methoxy and ethoxy substitution were found to promote inhibitory factors owing to their complimentary docking positions within receptor regions,

The five identified compounds were synthetically developed using traditional techniques of medicinal chemistry. Problems encountered and their proposed solutions are detailed where relevant, including the difficulty generating and maintaining anhydrous conditions required for imide generation which also resulted in high productivity of impurities. Application of an alternative synthetic method was subsequently developed where the addition of heat in solvent free conditions lead not only to decreased impurity production but also increased the ease of synthesis. Consequently four of the five compounds were successfully synthesised with one loss arising from difficulties in purification. Using relatively inexpensive starting compounds a simple synthesis route with comparatively high yields resulted, being both transferable and scalable to commercial operations. It is possible that this investigation may provide a good platform for further studies.

Further work is identified including the production of biological assays to assess activity within physiological conditions against Chikungunya viral cells and design improvements for improved yield, enhanced inhibitory effect and potential application to a pro-drug design.

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The evaluation of a pH-responsive polymer as a therapeutic for wound healing using *S. aureus* and *P. aeruginosa*

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A wound is a consequence of tissue insult.[1] The presence of colonising bacteria within a wound can affect the normal healing process and may cause substantial deterioration so that a chronic wound persists. Chronic wounds do not follow the normal trend of wound healing sequences.[2] Colonised bacteria secrete fatty acids into the wound environment, which can lead to a decrease in pH from 7.4 to as low as 5.4.[3] This study is concerned with the evaluation of poly[Ethyl Acrylate-co-Acrylic Acid] and its pH-dependent antimicrobial effects upon *S. aureus* and *P. aeruginosa*, which are two commonly colonising inhabitants of a wound. The activity of the polymer is based on its ability to create pores within the bacterial lipid membranes, thus allowing for the influx of solutes and the consequential colloid osmotic cell lysis of the bacterial cell. The synthesis and evaluation of the polymer stemmed from the studies of polymers and their effects upon eukaryotic cells by Murthy *et al.*[4] The basic concept is that at low pH ranges, the polymer undergoes conformational changes in its structure into an active form, which sequentially causes an inhibitory effect upon bacterial cell division whilst avoiding toxic consequences towards wound healing cells, such as fibroblasts and macrophages.[1]

This study involves the authentication of a previously synthesised polymer and the evaluation of its effects upon both *S. aureus* and *P. aeruginosa* at various concentrations. Authentication included NMR spectroscopy as well as pH and solubility analysis to ensure the identity and compatibility of the polymer within the assays. *S. aureus* and *P. aeruginosa* were prepared alongside the polymer in a serial diluted 96 well plate and incubated for 24 hours. Optical density readings and colony counts were measured and recorded in order to quantify the extent of bacterial growth. Results were analysed statistically to determine sound conclusions. ^{1}H-NMR and ^{13}C-NMR data confirmed that the structure of the polymer was correct. pH analysis and spectrophotometer testing concluded that the polymer did not alter the pH of BHI substantially, nor did it have difficulty in dissolving in solutions of up to 20mg/mL.

Biological evaluation results were produced from a mean of 3 repeat experiments, which suggested that both bacterial species were inhibited by the polymer at both pHs. It could also be argued that there was a pH and concentration affect upon the polymer's activity, with the polymer being most effective at pH 5.5, especially in the case of *P. aeruginosa*. *P. aeruginosa* is Gram negative (unlike *S. aureus*, which is Gram positive) and so has a lipid bilayer on the outer membrane, which could be targeted by polymers. This factor may be responsible for any observed differences in activity. Statistical analysis revealed that the concentration dependent growth of both of the organisms was not statistically significant (p value of 0.143). A p-value of 0.012 suggests that there is a significant difference in polymer activity between pH 5.5 and pH 7. The possibility of bacterial cell death occurring due to pH effect (without polymer) was ruled out via mathematical manipulation of data. However, in terms of biological analysis, growth changes greater than 3 log folds suggest significant differences: this was not achieved in these evaluations.

Nevertheless, conclusive results were encouraging in suggesting that Poly[Ethyl Acrylate-co-Acrylic Acid] has enhanced inhibitory effects at pH5.5, especially for *P. aeruginosa.* There was confidence in developing further work in an aim to create a novel therapeutic pH dependent antimicrobial polymer for wound healing therapies.

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Temporal comparison of protein tyrosine kinase activation with Zinc, EGF and IGF in tamoxifen-resistant MCF-7 cells

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Zinc is found ubiquitously in the body; it has an important role in many metabolic pathways and has been implicated in many disease states. Labile zinc located in cells is known to be important in signalling processes within a cell acting as a secondary messenger[1]. Zinc acts to inhibit protein tyrosine phosphatases (PTP) [2] which inactivate protein tyrosine kinases in cells and thus prevent prolonged stimulation of the signalling pathways that regulate gene expression and cell growth[3]. Cells treated with the antiestrogen, tamoxifen can develop resistance with prolonged treatment as the cells are able to utilise alternative signalling pathways[2]. Zinc is released from the endoplasmic reticulum into the cytosol approximately 10 minutes after exogenous zinc stimulation[1] in cells. This zinc release is known as a 'zinc wave' and it has been implemented in aggressive cancer growth through inhibiting PTP's.

The cells used were tamoxifen- resistant MCF-7 cells (TAMR). TAMR cells were treated with different agents such as zinc and sodium pyrithione, EGF and ionomycin and IGF and ionomycin for 0-20 minutes. Fluorescent microscopy was chosen as the methodology for studying the activation of EGFR and IGF1-R. TamR cells were grown on 0.17mm glass coverslips until they were of an appropriate confluence before treatments with zinc (20µM) and the zinc ionophore sodium pyrithione or with either EGF or IGF and the calcium ionophore ionomycin for 0, 2, 5, 10, 15 or 20 minutes. The cells were fixed with 3.7% formaldehyde for 15 minutes, permeabilised with 0.4% saponin in 1% bovine serum albumin (BSA) for 15 minutes and blocked with 10% normal goat serum. Cells were then incubated with the primary antibodies for 1 hour at 25°C. All cells were then incubated with Alexa Fluor 488-conjugated goat anti-rabbit and Alexa Fluor 594-conjugated goat anti-mouse secondary antibody (1/2000) for 30 minutes at 25°C. The coverslips were assembled onto slides using Vectorshield mounting medium with DAPI to counterstain the nucleus. The slides were viewed on a Lecia RPE automatic fluorescent microscope using a 63x oil immersion lens. Fluorescent superimposed images were obtained by the use of a multiple bandpass filter set appropriate for DAPI and FITC. Images were taken through one round of deconvolution before processing in the Paint Shop Pro software. Activation of the receptors was judged by relocation to the plasma membrane of the TAMR cells.

There was a noticeable increase in the activation of EGFR and IGF-1R when the cells were treated with zinc throughout the time course. There was a steady increase in the activation of EGFR and IGF-1R until 15 minutes when treated with either EGF or IGF and ionomycin. At the 20 minute time point the activation of the receptors decreased compared to the other time points. A novel discovery was the observation that, when cells were treated with zinc, cells appeared to have either activated EGFR or activated IGF1-R but not both.

As zinc has the ability to mediate the activation of downstream signalling pathways through the inhibition of protein tyrosine phosphatises [2], it can be seen to be an ideal therapeutic target. Results collated showed that there is a mechanism of 'cross talk' [4] present between IGF, IGF-1R and EGFR. Targeting zinc would be a better therapeutic target due to an increased chance of more signalling pathways being inhibited therefore preventing the likelihood of resistance.[2] However as zinc is widely spread and utilised throughout the body there could be a problem with selectivity for example using a chelator to target intracellular levels of zinc may be a useful therapeutic agent. However the risk of side effects means that using a chelator is not a feasible option for targeting anti-hormone resistance. As the release of the 'zinc wave' is controlled by a zinc transporter, ZIP7 [2], methods for the prevention of activation of this transporter should be considered as a viable therapeutic target. Recent data suggests that zinc transporters are activated by phosphorylation and that targeting the relevant kinase could be a useful means of preventing breast cancer growth. However like zinc itself, at the moment this could be the most promising route for targeting zinc in cancerous cells therapeutically. Manipulation of the trafficking systems for zinc in tamoxifen- resistant cells may prove to be the best and most realistic method of preventing resistance clinically in the future.

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Synthesis of CYP24 inhibitors for prostate cancer therapy

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Vitamin D is a fat soluble vitamin that regulates a variety of processes in the body. The active form of Vitamin D is known as calcitriol.[1] Calcitriol induces antiproliferative effects by down regulating bcl2 gene and slowing down the cell cycle at G_0 and G_1 phases. As a result, it is used in various anticancer therapies. The use of calcitriol as an anticancer therapy has been limited due to its side effects.[2] Elevated serum calcitriol induces the production of CYP24 and alternatively it reduces the benefit of calcitriol as an anticancer therapy. A raise in CYP24 levels can complicate the symptoms of cancers and some other diseases. In the past, a series of CYP24 inhibitors were synthesised and when they were given in combination with calcitriol, the effectiveness of the anticancer treatment was increased. However, most of these inhibitors were not specific to CYP24.[3] The aim of this research was to carry out a series of reactions in order to synthesise three novel compounds which could have some CYP24 inhibition and antiproliferative activities. Out of these three compounds, two contained an azole group and one contained a sulphonyl (non-azole) group.

The synthesis of these compounds began by reducing 3-(3-bromo-phenyl)propionic acid to 3-(3-bromo-phenyl)propan-1-ol. Afterwards functional groups such as, imidazole, sulfonyl and a second aromatic ring, were added to the key intermediate. The method used in this investigation was novel therefore, at the end of each experiment, the product was isolated using thin layer chromatography and characterised by ^{1}H-NMR and ^{13}C-NMR. Then the whole method was critically analysed to understand whether it was a viable method to produce the intended product.

The Molecular Operating Environment program was used to understand the interaction between the designed inhibitors and the enzyme. Investigations have shown that imidazole ring containing inhibitors have a strong interaction with the haem group of CYP24. In the first reaction scheme, a low yield was obtained and the last two experiments were unsuccessful. Therefore, the first reaction scheme was modified to obtain some invaluable results.

It was suggested that the failure of the first scheme was due to poor stability of the final product, high reactivity of the reagents and the presence of many impurities. The modifications were vital to get successful results in the second reaction scheme. Both positive and negative outcomes were identified in the overall project. One positive outcome, methanesulfonic acid 3-(3-styryl-phenyl)propyl ester was synthesised at the end of the experiment. The CYP24 inhibitory action of this compound was not identified before and it will be evaluated for its anticancer activity.

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The effect of legislation changes on over the counter sales of codeine and dihydrocodeine containing products in Wales

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Codeine and dihydrocodeine (DHC) have been available for many years in over-the-counter (OTC) products and are typically used for the management of mild to moderate pain. Following administration codeine and dihydrocodeine are metabolised to morphine in the body[1]. Morphine is a well-known drug of abuse and cause of addiction. Codeine has been shown to be the most widely abused and misused OTC medication in the UK[2]. As a consequence, voluntary and legislative changes have been implemented to ensure a tighter control on the sale of codeine and dihydrocodeine containing products. The aim of the present study was to determine if the voluntary change of 2005[3] or the legislative change of 2009[4], effectively reduced OTC sales of codeine and dihydrocodeine containing products in Wales.

Sales data were extracted from the IMS health database and transferred to an Excel spread sheet for subsequent manipulation by Public Health Wales. Monthly sales data for sequential 12 month periods from August to September each year were totalled and compared to sales in the previous 12 months. Data were inputted into SPSS (version 18) and analysed using the Wilcoxon signed-rank test.

During 2005-2006, the 12 month period after the voluntary agreement of June 2005, sales for non-effervescent codeine pack sales, (35108.00 [34097.75, 35506.00]); p=0.012, non-effervescent codeine tablet sales (733793.50 [715645.00, 748754.00]); p=0.002, non-effervescent DHC pack sales (4031.50 [3630.25, 4326.00]); p=0.002, non-effervescent DHC tablet sales (102308.00 [88480.00, 105384.00]); p=0.002 and total DHC tablet sales (107780.00 [93160.00, 110718.00]) p=0.008 all increased. In the 12 month period 2009-2010, subsequent to the legislative change in September 2009, non-effervescent codeine pack sales decreased (17177.75 [18502.00, 20700.25]); p=0.002, as did the sale of non-effervescent codeine tablets (429701.00 [412276.50, 471621.00]); p=0.005, total codeine packs (38297.50 [36399.50, 35961.00]); p=0.002, effervescent DHC pack sales (154.00 [10.00, 200.75]); p=0.008, effervescent DHC tablet sales (3696.00 [240.00, 4818.00); p=0.008, non-effervescent DHC pack sales (3368.00 [3065.00, 3495.00]); p=0.003, non-effervescent DHC tablet sales (84636.00 [78817.00, 88761.00]); p=0.003, total DHC pack sales (3368.00 [3065.00, 3495.00]); p=0.006 and total DHC tablet sales (87248.00 [80051.00, 92211.00]); p=0.003.

The sales of OTC codeine and DHC containing products, whether analysed by packs or tablets sold, generally followed an upward trend until September 2007, and thereafter the trend was downward. This suggests the voluntary change of 2005 had little effect, while the legislative change of 2009 successfully decreased sales of both codeine and DHC containing medicines from community pharmacies. It will be necessary to monitor sales for a further period to confirm the impact of the legislative changes made in 2009. Nevertheless the present study has highlighted the lack of impact of a voluntary agreement compared to legislative change.

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Investigating the anti-mycobacterial activity of *Humulus lupulus* and their active constituents against *Mycobacterium smegmatis*

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Bovine tuberculosis (bTB) is a major problem in the UK with 4000 new herd breakdowns in 2007[1]. Although it is not a major threat to human health the cost to the economy of tackling this animal infection exceeds £58 million[1]. There is considerable doubt as to the effectiveness of current control measures and it has become increasingly clear that novel, cost effective control measures are desperately needed. An approach we are pursing is the inclusion of natural products with anti-mycobacterial activity in animal feed to reduce susceptibility to bTB without affecting product and to control the spread of the disease[2]. *Humulus lupulus* (strobile hops) are readily available natural products that have been used for thousands of years in the brewing industry and as animal feed. They contain many active constituents such as alpha acids, fatty acids and polyphenols which demonstrate anti-mycobacterial activity ex vivo[3] and in vitro[4]. This study aims to investigate the anti-mycobacterial effects of strobile hops, attempt to link the activity to known active ingredients and to investigate the effects of the polyphenol EGCG on Mycobacteria in vitro.

The study tested hop extracts against *M. smegmatis*, a rapid growing and non-virulent strain of mycobacteria[2]. Hop samples were extracted by mimicking the conditions encountered during the brewing process, i.e. heating and stirring continuously over hour, samples were extracted at 25, 50, 75 and 100°C. An agar diffusion method was used to give an indication of anti-mycobacterial activity by making wells in the agar and filling with hop samples[2]. This method was used to determine effects of extraction temperature on the activity, which was assessed by measuring zones of inhibition surrounding the wells. A second method involved incorporating the hop samples into the agar in a range of concentrations across a 24 well culture plate. Following incubation with the hop / agar mixture, the results were then swabbed and incubated for a second time to assess whether the hops were bactericidal or bacteriostatic. This method allowed quantitation of anti-mycobacterial activity and calculation of MIC values[2]. The activity of EGCG were investigated using a broth dilution microtiter assay, which allowed accurate calculation of the MIC, a reference medium was necessary to account for colour density of the EGCG dilutions[2].

The results obtained from the agar diffusion assay indicated that samples extracted at 100°C demonstrated higher anti-mycobacterial activity than those extracted at 25, 50 and 75°C; the variance in activity between temperatures was found to be significant ($P \leq 0.05$). It was also found that centrifuged results were significantly more active than those that were filtered using a 0.45µm filter ($P \leq 0.05$). The 100°C samples of some hop species yielded results that were very highly significant ($P \leq 0.001$), compared with negative control. MIC values for ten hop species were calculated from the data collected in the agar incorporation method and range between ≤10% and 23% hop / agar mixture. It was found that bacteria would not grow above the MIC values following second incubation of swabs from the wells, therefore indicating bactericidal activity. The MIC values were compared with the known alpha acid concentration of the hop species. The results of the microtiter assay showed that optical density of *M. smegmatis* cultures decreases as the concentration of EGCG increased.

Hops extracted at 100°C demonstrated the highest anti-mycobacterial activity and this activity could be detected at concentrations as low as 10% v/v. We found no direct correlation between anti-mycobacterial activity and alpha acid content of individual hop samples suggesting that activity is due to other factors. Finally at the concentrations tested EGCG had direct anti-mycobacterial activity. In conclusion, hops contain multiple active constituents that enable them to inhibit the growth of *M. smegmatis* and therefore could potentially be used as cost effective, non-toxic additives to animal feeds to reduce livestock susceptibility to bTB, however further study is needed to better understand the full potential of hops as anti-mycobacterial agents.

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NHS manufactured oral liquids – investigating novel suspending agents

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Drugs in solid oral dosage forms are not appropriate for all patients. Many patients are unable to swallow capsules and tablets due to age or physical or psychological problems [1]. For these patients the availability of an oral liquid formulation of these drugs would be beneficial. Currently many drugs are unavailable in licensed liquid formulations and are therefore made up extemporaneously or as a "special" [2]. The formulation of insoluble drugs in this way is often achieved by adding a crushed or powdered form of the drug to a suspending agent [1]. This can often lead to issues surrounding dosing and bioavailability due to the unknown chemical and physical attributes of the modified active pharmaceutical ingredient in these formulations[2]. This project, conducted in conjunction with manufacturing colleagues within the NHS aimed to test and optimise one specific formulation to optimise patient care.

Hydrocortisone acetate is currently made up extemporaneously or as a pharmaceutical "special". For the purposes of this investigation the powder form was suspended in Xanthan 0.4% with and without tween, Methylcellulose 2%, Poloxamer 6% and carbomer 1% as well as combination suspending agents consisting of xanthan 0.2% with Carbomer 0.2% and Xanthan 0.2% with Methylcellulose 1%. These suspending agents were evaluated in terms of early-stage dissolution, zeta potential, particle size and resuspendability with a view to assessing suitability for use in hydrocortisone suspensions.

Results obtained indicate that suspensions containing Xanthan perform better than the other suspending agents tested, suspending agents containing Xanthan surpassed pharmacopoeial standards of 75% of drug entering solution in 45 minutes. For both samples stored at room temperature and samples stored in the fridge, a minimum of 85.95% of the hydrocortisone had gone into solution within 10 minutes, possibly indicating a good level of bioavailability. Xanthan and Carbomer combination suspension, Xanthan with tween and Xanthan without tween all contain particles with a zeta potential more negative than -30 mV, and a size distribution that remains relatively stable over two weeks suggesting a good degree of stability. All three of these suspensions produced favourable results in resuspendability testing with more hydrocortisone detected after agitation of the bottle, Xanthan without Tween produced results modelling the ideal suspension profile and showing the highest degree of resuspendability. Poloxamer and Methylcellulose suspension did not provide any data suggesting they were preferential to the other suspending agents. Throughout the investigation and testing the Carbomer suspension proved problematic due to pH sensitivity, this hindered data collection from these samples.

No firm recommendation can be made as to which of the suspensions would be most suitable for use in hydrocortisone suspensions, however from the experimental results it would appear that Xanthan with and without Tween and the Xanthan and Carbomer combination suspending agent merit further research.

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Investigation of the role for STAT3 and MAPK in the generation of intracellular zinc release

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In recent years a great deal of data has emerged implicating zinc and its transporters in breast cancer[1,2]. In particular, tamoxifen-resistant (TamR) breast cancer cells have been found to contain high intracellular levels of zinc, along with an overexpression of the zinc transporter, ZIP7[2]. It is this zinc transporter that is thought to be responsible for the increase in intracellular zinc observed in these cells, by releasing zinc from the endoplasmic reticulum stores into the cytoplasm, a phenomenon known as the zinc wave[3]. This released zinc activates various tyrosine kinases, such as epidermal growth factor receptor (EGFR), and mitogen-activated protein kinase (MAPK), proteins known to contribute to the aggressive phenotype of these TamR cells, via inhibition of protein tyrosine phosphatases[2]. Recently, a statistically significant relationship between ZIP7 and signal transducer and activator of transcription 3 (STAT3) was found in clinical breast cancer samples[1]. The aim of the study was to investigate the role of STAT3 and MAPK in the zinc wave generation, and also to discover whether EGFR, MAPK and STAT3 were activated by the zinc wave.

Fluorescent microscopy was chosen as the methodology for studying the activation of EGFR, MAPK and STAT3. MCF-7 and TamR cells were grown on 0.17mm glass coverslips until they were of an appropriate confluence. MCF-7 cells were transfected with ZIP7 prior to harvesting. MCF-7 and TamR cells were treated with zinc (20µM and 100µM respectively) and the zinc ionophore sodium pyrithione or with EGF and the calcium ionophore ionomycin for 0, 2, 5, 10, 15 or 20 minutes. The cells were fixed with 3.7% formaldehyde for 15 minutes, permeabilised with 0.4% saponin in 1% bovine serum albumin (BSA) for 15 minutes and blocked with 10% normal goat serum. MCF-7 cells were then incubated with the primary antibodies tEGFR or pMapK$^{T202/Y204}$ and TamR cells were incubated with pSTAT3^{Y705} or pSTAT3^{S727} (all 1/100) for 1 hour at 25°C. All cells were then incubated with Alexa Fluor 488-conjugated goat anti-rabbit and Alexa Fluor 594-conjugated goat anti-mouse secondary antibody (1/2000) for 30 minutes at 25°C. The coverslips were assembled onto slides using Vectorshield mounting medium with DAPI to counterstain the nucleus. The slides were viewed on a Lecia RPE automatic fluorescent microscope using a 63x oil immersion lens. Fluorescent superimposed images were obtained by the use of a multiple bandpass filter set appropriate for DAPI and FITC. Images were taken through one round of deconvolution before processing in the Paint Shop Pro software.

STAT3^{Y705}, STAT3^{S727} and MAPK$^{T202/Y204}$ were all shown to be activated by the zinc wave. After treatment with extracellular zinc, activation of these proteins was observed from 10 minutes onwards, 10 minutes being the time at which zinc is released from the endoplasmic reticulum by ZIP7[2,3]. Treatment with EGF and ionomycin, which are known to generate the zinc wave[3], also produced activation of STAT3^{S727} and MAPK$^{T202/Y204}$ downstream of the zinc wave. Another important finding was the fact that STAT3^{Y705}, STAT3^{S727} and MAPK$^{T202/Y204}$ were all activated upstream of the zinc wave following treatment with extracellular zinc. This suggests that both tyrosine 705 and serine 727 phosphorylated STAT3, and MAPK$^{T202/Y204}$ may have a role to play in the generation of the zinc wave. MAPK activation is already known to be a prerequisite for the zinc wave to occur, and so this result further confirms this. STAT3, however, has never previously been implicated in the zinc wave, therefore making this an important novel discovery.

This study provides new evidence regarding the signalling pathways that TamR cells undertake in order to continue proliferating in the presence of tamoxifen, by identifying STAT3 as a new target of the zinc wave. Constitutively active STAT3 is known to be involved in cancer progression[4] and this study has now linked it with zinc, which is also known to contribute to tumour growth. This study also gives new insights into the mechanism of the zinc wave by implicating STAT3 in its generation. These are very exciting findings and will assist researchers in the identification of potential targets to treat tamoxifen-resistant breast cancer. Due to the data being of a qualitative nature it would be beneficial to quantitate the results using fluorescent-activated cell sorting (FACS) analysis. Confirmation of the results could also be carried out by using other techniques such as Western blotting.

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A study of the effects of commensal bacteria on the contractile function of the ileum

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Trace amines are produced by many commensal bacteria including *Enterococcus, Lactobacilli, Escherichia* and *Clostridium* [1] and are also found in foods such as cheese and chocolate. These commensal bacteria have therefore been linked to many diseases including Irritable Bowel Syndrome [2], lactose intolerance, Crohn's disease [3] and colorectal cancer [4]. A commensal bacterium, *E. coli* 120, was therefore examined to see if it produced trace amines to increase the contraction of the ileum. Other aims were to determine whether the supernatant from the growth of *E. coli* 120 can affect the gut and whether any effects are due to tryptamine, whether *E. coli* could increase the synthesis of tryptamine via prior incubation with the tryptamine precursor, tryptophan, and its respective co-factor pyridoxal phosphate and to establish the stability of the ileum over the time course of bacterial incubation.

E. coli was extracted and isolated from the faeces of a healthy individual who was on a normal diet and grown on an agar plate. *E. coli* suspended in Ringer's solution (1ml) after overnight incubation (17 hours, 37º C) in tryptone soya broth and following centrifugation (2min, 20,000g) was added to the gut bath for 2 hours. The supernatant was also added for 10 minutes. Ileum (2cm) was suspended in Krebs solution (50ml, 37º C), aerated with gas (95% O_2, 5% CO_2), allowed to equilibrate with its surrounding environment and electrically stimulated to produce twitch contractions.

E. coli and the supernatant from its overnight growth both caused increases in the average twitch height of the ileum with minimal effects being observed by incubation with Ringer's solution. Tryptamine also increased the average twitch height in a concentration-dependent manner. Incubation with the tryptamine precursor, tryptophan, and its respective co-factor, pyridoxal phosphate, for two hours increased the average twitch height but to a greater extent than *E. coli*. Following washout, the twitch height at baseline was attained, attributing the effects of incubation to trace amines and other bacterial products of *E. coli*.

Thus, trace amines such as tryptamine produced by *E. coli* 120 caused a contraction on the ileum probably via binding to trace amine-associated receptors within the gut. *E. coli* produces tryptamine due to the ability of the precursor and the co-factor significantly enhancing the contraction produced. However, tryptamine was not the major agent responsible for the contraction observed due to the inability of the MAO-B inhibitor, pargyline, to significantly enhance the contraction observed. Therefore *E. coli* produces a host of agents, including trace amines to induce the contractions seen in the gut and those, which may be seen in disorders such as Irritable Bowel Syndrome and lactose intolerance.

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Comparisons of Pharmaceutical Registration to Reimbursement in the 30 European Economic Area Countries

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The inequalities presented across Europe with respects to the time delay encountered by patients receiving new medicines is underlined by the pricing and reimbursement processes that succeeds an approval of marketing authorisation. As politicians search for innovations in providing healthcare, they face the challenge of ensuring the pharmaceuticals chosen meet the needs of their healthcare system, thus providing quality and an affordable price. A greater understanding for the reasoning behind decision outcomes and the involvement of key stakeholders may facilitate the drive in reducing patient access times.

To enable systematic comparison of European regulatory to reimbursement systems, public domain data collection reflected the connections between agencies in terms of information flow and the relationship of key milestones and activities. Qualitative data was collected in the form of review literature and websites of the respective agencies to enable a schematic mapping process.

The analysis showed that the countries of Europe fell into groups of broadly similar systems with noticeable differences between the independence of the key milestones. The majority of countries demonstrated a clear decision-making path influenced by the recommending bodies within the process. The role of Health Technology Assessment (HTA) in formulating appraisals for decision-making varied not only in terms of activities undertaken but also in terms of the transparency of information available to other stakeholders in the process.

The findings indicate that the variation in the way agencies and milestones are connected can help to identify why contradictions in reimbursement outcomes occur between European countries. Inequalities in values and methodologies of appraisal reduce the consistency of providing high-quality transparent decision-making for the reimbursement of innovative pharmaceuticals. This underlines the urgency to develop a standardised HTA system across the region.

Post-Polymerisation Hydrolysis of Molecularly Imprinted Polymers

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Molecular imprinting is a technique aimed to mimic biology's natural recognition processes. The technology can be adapted to a range of sciences and has the potential to be tailored as a method of drug delivery[1]. However, there are currently many drawbacks to molecular imprinting including template extraction from polymers, imprinting in aqueous media and non-specific binding issues[2]. The aim of this study was to optimise the performance of molecularly imprinted polymers (MIPs). It was hypothesised that hydrolysis of a susceptible cross-linker would increase the flexibility of imprinted polymers, therefore increasing the availability of binding cavities and the overall maximum binding capacity of MIPs.

Post-polymerisation hydrolysis was investigated at a variety of ratios of cross-linker. Ethylene glycol dimethacrylate (EGDMA, a hydrolysable cross-linker) is liable to ester hydrolysis and was used throughout the study in combination with DVB (non-hydrolysable cross linker). Non-imprinted polymers (NIPs), and control, heated and hydrolysed variables of polymer were used within the study. Acetic acid was also used in assays in attempt to disfavour binding so that effects of hydrolysis could be better analysed.

The results of this study indicate that hydrolysis of polymers at optimum concentrations of EGDMA promotes optimum MIP performance. However, too little EGDMA has little effect and too much results in polymer breakdown[3]. Additionally, it became apparent over the course of the investigation that although hydrolysis improves MIP performance, it additionally improves NIP performance. Furthermore, the study revealed that results have been modified due to the involvement of acetic acid within binding assays. Further investigation was attempted by assessing the effects of acetic acid in comparison to hydrochloric acid.

It can be concluded that hydrolysis only impacts the non-specific binding ability of imprinted polymers. It can also be suggested that results obtained from assays involving acetic acid do not directly reflect the binding capability of polymers.

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Albumin as an enzyme – a model for investigating drug interactions

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Albumin has been reported to act as a luciferase with the luciferin coelenterazine[1].Chemiluminescence is light emission resulting from energy generated in a chemical reaction. The reaction requires oxygen, a luciferin which when oxidised emits light and a luciferase which catalyses the oxidation[1]. Albumin has the ability to bind a number of compounds, many of which are used clinically. There are two main sites of binding to albumin termed drug site 1 and drug site 2. Warfarin is known to bind to drug site 1 and diazepam and phenytoin are known to bind to drug site 2[2]. Binding to albumin will affect the pharmacokinetics of drugs often resulting in decreased free concentrations and thus reduced activity[3]. Inflammatory Bowel Disease (IBD) is an inflammatory condition of the small intestine and colon. Drugs used in this condition include the anti-inflammatory mesalazine known chemically as 5-aminosalicylic acid and the immunosuppressant 6-mercaptopurine[4]. There were a series of aims in this study. The first was to characterise the enzymatic activity of albumin by examining the effect saturation, denaturation and pH had on chemiluminescence. Once characterised the binding site of coelenterazine to albumin was also investigated. Upon establishing binding of coelenterazine the effect of drugs used in inflammatory Bowel Disease were investigated to observe their binding to albumin and thus determine whether they bound at the same site as coelenterazine.

Chemiluminescence was recorded using a luminometer with a photomultiplier to detect photons emitted in the reaction. The counts per second generated by the computer were proportional to coelenterazine oxidation and thus light emission. Each sample had a total of 8 readings taken with intervals of ten seconds between single readings (8x10s counts). Each experiment was repeated three times and the standard error of the mean was calculated. A series of steps were developed to measure luminescence. The first was to measure the machine background which indicates the level of background light throughout experiments. This consisted of placing an empty glass tube into the luminometer and taking readings. The second step was to measure the chemical background which indicates the light emission of the coelenterazine and buffer. If drugs were included the proceeding step would be to measure the drug background which highlights the amount of light emitted in the presence of the buffer, coelenterazine and drug. The final step was to measure the amount of light emitted when the reaction is catalysed by a luciferase, termed the protein reading. Once all readings were obtained graphs were prepared using the protein background minus the drug background.

The results show that albumin is a mono-oxygenase as it catalyses the addition of oxygen to coelenterazine to generate light emission. This is supported by saturation of the active site at concentrations above 20µM coelenterazine. Also, denaturation by heat caused a significant decrease in chemiluminescence. Finally, the study shows that the activity of albumin is pH dependent. These are all key criterion of an enzyme. Using pH predictions and drug studies it was determined that coelenterazine binds to drug site 1 of albumin as warfarin competitively inhibited chemiluminescence and phenytoin did not. The IBD drug 6-mercaptopurine binds to drug site 1 as it also inhibits chemiluminescence but 5-aminosalicylic acid does not. Molecular modeling proved that coelenterazine fits the pocket of drug site 1 and the interaction occurs via basic amino acids.

In conclusion this is a unique model of albumin as an enzyme which could potentially be used as a screening tool in drug design to identify compounds that may bind to drug site 1 on albumin and thus predict any alterations in their pharmacokinetics. Our model is superior to current models used in industry which rely on radiolabelling of compounds which is potentially hazardous.

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Learning and assessment of consultation skills for Non-Medical Prescribers (NMPs)

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The possession of good consultation skills enables a person to communicate information so that it is received and understood[1]. The learning and assessment of consultation skills is essential, as good communication skills are required to bridge the gap between evidence-based medicine and the individual patient to improve health outcomes[2]. Observing and analyzing consultations enables practitioners to evaluate the effectiveness of the communication process. The Welsh School of Pharmacy, at Cardiff University, has delivered the non-medical prescribing (NMP) course in collaboration with the School of Nursing and Midwifery since 2004. The aim of the study was to evaluate the inter-rater reliability of Designated Supervisor Medical Practitioners' (DSMPs') assessments of a video-taped consultation and to determine which consultation models students adopted for their new role as a prescriber. Specific research objectives were to compare DSMP ratings of a videotaped consultation and to review student essays to elucidate which consultation models were used for their reflective narratives.

This study was divided into two parts where data collected from the teaching and assessment of DSMPs and students training for the non-medical prescribing course were analysed. **Study 1** consisted of inter-rater reliability analysis of DSMP ratings of a videotaped consultation using the Medication Related Consultation Framework (MRCF) [3]. Five sections of the MRCF were rated between 1-5 and an overall score out of 25 was obtained. A database was created on SPSSv18.0® and inter-rater reliability analysis was carried out on all ratings gathered since 2005. One outlier value was removed from the analysis and Intraclass Correlation Coefficient (ICC) was calculated for all raters. **Study 2** involved a systematic qualitative analysis of the reflective assignments, to establish which theoretical models of consultation were relevant to the practice of non-medical prescribers. Content analysis of NMP student essay assignments was carried out by initially creating a coding framework containing ten consultation models and to capture the therapeutic area and care sector of each NMP.

Study 1: A total of 66 DSMP ratings, represented six groups of raters who evaluated the same consultation from 2005 to 2010. For the overall rating of the consultation the ICC score was 0.819 thus indicating 'almost perfect agreement' between raters. **Study 2:** Twenty-four essays were analysed for the 2011 student cohort and 22 were analysed for the 2010 cohort. The Calgary Cambridge model was used most frequently across all therapeutic areas (86%) followed by Neighbour (59%) and Pendleton (50%).

The results indicate a high level of reliability of assessment of consultation skills between DSMP assessors. This reliability analysis provides quality assurance for the assessment of students' skills by their supervisors in practice. The consultation models used in NMP essays showed a wide range of variability. It was evident that the frequency with which consultation models were cited was much higher in 2010/11 cohort than 2009/10. The understanding of consultation skills and the use of consultation models have therefore improved from 2010 to 2011 cohorts, since students were more likely to refer to theoretical models of consultation to apply to their practice. This shows that students apply an appropriate range of consultation models to their learning in practice and assessment of consultation skills shows good level of reliability between assessors.

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Enhancing the disinfection power of propyl paraben with essential oils: probing bacterial cell surface interactions

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Over recent years there has been concern raised as to whether resistance to biocides is occurring in the healthcare setting. This is largely due to the lower level of surveillance in comparison to antibiotics. The World Health Organisation[1] highlighted this risk earlier this year, aiming to reduce bacterial resistance to antimicrobials, globally through both government and pharmaceutical organisations taking responsibility for their practises, as isolated interventions will not be able to have an impact on the wider scale. In light of this and as a response to the global issue raised the European Commission have subjected antimicrobial agents to tighter regulation and testing. The potential to enhance the activity of existing antimicrobials to disinfectant levels is attractive and this has been described with the use of essential oils as a delivery system[2]. Propyl paraben is a preservative favoured for its broad spectrum of activity, which has been potentiated with essential oils; the precise mechanism is unknown. This project looks to explore bacterial cell surface interactions of both Gram positive and Gram negative bacteria with the antimicrobial delivery system. The methods employed here have been used in determining bactericidal concentrations of preservative alone as well as with the essential oil delivery system, characterising the surface hydrophobicity of the target organisms (*Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus*) and modifying the bacterial membrane of *Pseudomonas aeruginosa* to study surface interactions.

The Miles and Misra[3] droplet technique was adopted to assess the effect of treating bacteria with propyl paraben and essential oils alone and in combination. Samples from the bacterial suspension treated with biocide were taken at specified time periods and underwent a ten-fold dilution before being plated onto over-dried TSA plates and incubated for 24 hours at 37ºC. In addition to this microbial adherence to hydrocarbon (MATH) tests[4] were carried out to find out the bacterial adherence to essential oils. An initial optical density was determined then, three millilitres of the bacterial suspension was pipetted into a centrifuge tube, along with 800 µl of hexadecane/essential oil. This was then incubated, vortexed and centrifuged at low *g*, before recording the final OD to calculate percentage adherence.

It was found that there was no significant difference in log reduction of bacteria with treatment with essential oils alone on any of the bacteria. The bacterial inhibition exerted was modest, similar to that of bacteria treatment with propyl paraben alone. There is a correlation between the nature of the bacteria (Gram positive or Gram negative), relative hydrophobicity and the log reduction in bacteria when treated with propyl paraben in an essential oil delivery system. The level of activity is therefore assumed tobe dependent on the intrinsic antimicrobial nature of the propyl paraben as well as the bacterial interaction with essential oil. There is significant delivery of propyl paraben to the bacterial cell surface due to its inclusion in essential oils.

Escherichia coli was also found to be the most sensitive to propyl paraben concentration chosen, making the potentiation by the essential oils less clear. *Staphylococcus aureus* was more susceptible to the effects of the essential oil antimicrobial delivery system vastly due to its hydrophobic nature.

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A comparative study of the delivery of Artesunate across porcine rectal and sublingual membranes, *in vitro*

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Artesunate is a member of the arteminisins family of antimalarials. It is a water-soluble semi-synthetic hemisuccinate derivative of dihydroartemisinin, indicated for the treatment of *P. falciparum* malaria. Artesunate is useful for the treatment of severe malaria due to its rapid onset of therapeutic effect and rapid elimination [1] and is predominantly prescribed as a suppository for the treatment of moderate to severe malaria especially, although there are major disadvantages. The sublingual route shares a number of advantages with the rectal route, but is less invasive, isn't affected by episodes of diarrhoea (a major symptom of malaria), more hygienic and doesn't require a trained person to administer. The sublingual route is capable of rapid absorption and onset of activity as seen with the angina treatment GTN. The aim of this study was to determine *in vitro* whether Artesunate can be delivered via the sublingual route as a viable alternative to rectal delivery.

The blunt dissection of freshly excised porcine tissue yielded sublingual and rectal epithelium membranes. Comparative membrane permeation tests were conducted using Franz diffusion cells where the receptor phase was 90% water and 10% ethanol, removed every 2 hours for 12 and again after 24 hours. The donor phase was varied starting with 1mL of a 10mg mL^{-1} Artesunate solution applied to both membrane types. Next, the in house prepared 150mg g^{-1} Artesunate suppositories were applied to both membranes. Thirdly, a 100mg Scanpharm commercial suppository was applied in a mucin matrix, in comparison to sublingual donor phase a 50µL spray of a 50mg mL^{-1} Artesunate solution. The receptor phases were assayed for Artesunate by reverse phase HPLC using method developed in-house, involving a Phenomenex Gemini NX 5m ODS column and a mobile phase comprised of 56% acetonitrile and 44% water, both of which were acidified with 0.1% trifluoroacetic acid. The flow rate was 1mL min^{-1} and detection was by UV at 210nm.

The donor phase of 1mL of Artesunate solution resulted in the permeation after 24 h of 2.45 ± 0.31 mg cm^{-2} across the rectal membrane and 1.23 ± 0.14 mg cm^{-2} across the sublingual membrane (n=7±SEM). This showed an increased permeability in the rectal membrane but no statistical difference was noted after a one way ANOVA with a post t test (*p>0.1)*. The in-house developed 150 mg g^{-1} suppositories in both membranes also resulted in no statistical difference noted between the results (*p>0.1)*. The donor phase of 100mg Scanpharm suppositories when compared to a 50 mg mL^{-1} repeat dosed sublingual spray resulted in a cumulative permeability in 1.9±0.27 mg cm^{-2} and 3.5mg±0.72 mg cm^{-2} in the sublingual and rectal membranes respectively, again no significant difference was noted between the results (*p>0.1)*

The results from the commercially available suppositories and sublingual spray were used to calculate whether a therapeutic plasma concentration could be attained. The suppositories in the rectal membrane would result in a plasma concentration of 20.68µg mL^{-1} and the sublingual spray resulted in a calculated plasma concentration of 10.65 µg mL^{-1}. Both formulations exceeded the therapeutic plasma concentration noted in the literature of 0.16 µg mL^{-1}.[2] It is important to note that over the 24 hour trial period the sublingual membrane was only dosed with a total of 17.5mg in comparison to the 100mg suppository applied to the rectal membrane, and thus the sublingual spray provided a greater percentage of Artesunate delivered 14.6% compared to the rectums 4.3%. It is very feasible that the concentration of Artesunate in the sublingual spray could be increased to provide at least bioequivalence compared to the suppository. Overall the data supported the hypothesis that sublingual delivery is a feasible alternative for the delivery of Artesunate.

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Impact of NICE CG59 on the rate of glucosamine prescribing in Welsh Local Health Boards

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Glucosamine, a nutraceutical, is a controversial intervention in the management of osteoarthritis due to the conflicting nature of the evidence for its effectiveness and cost-effectiveness. NICE therefore sought to clarify guidance on glucosamine prescribing, and dissuade prescribers from choosing glucosamine over more cost-effective alternatives, through the publication of NICE CG59, advising that 'the use of glucosamine products is not recommended for the treatment of osteoarthritis'[1]. The main objective of this study was to determine the impact that NICE CG59 has had on the rate of glucosamine prescribing. Osteoarthritis is significantly more prevalent amongst the most deprived fifth of the population of Wales compared to the least deprived fifth and for this reason the relationship between rate of glucosamine prescribing and deprivation was of interest. The final objective was to determine the effect of local prescribing guidance, which stated that practitioners should not prescribe any glucosamine preparation[2,3], on the rate of glucosamine prescribing in the Aneurin Bevan Health Board.

Glucosamine dispensing data from the CASPA database, population data from GP surgeries' registered patient lists and deprivation data from the Townsend Index were analysed. Impact of NICE CG59: Percentage change in median rate of glucosamine prescribing for the 24 months before and the 24 months after NICE CG59 was calculated for the 22 previous LHBs, the 7 current LHBs and Wales as a whole. The Wilcoxon signed rank test was used to determine statistical significance. Impact of deprivation: LHB deprivation rank and glucosamine prescribing rate were plotted on a scatter-plot and Spearman's correlation coefficient performed to determine correlation. Then the median rate of glucosamine prescribing in the 5 most deprived LHBs and the 5 least deprived LHBs was compared in the form of rate ratios and statistical significance determined using the Mann-Whitney U-test. Impact of local prescribing guidance: Percentage change in median rate of glucosamine prescribing in the Aneurin Bevan Health Board for the 6 months before and 6 months after; NICE CG59[1], the Gwent Partnership Medicines & Therapeutics Committee (GPMTC) prescribing guidance[2] and Aneurin Bevan Health Board prescribing guidance[3]. The Wilcoxon signed rank test was used to determine statistical significance.

Rate of glucosamine prescribing increased significantly after NICE CG59 for Wales as a whole ($p=0.000$), all 7 current LHBs (all p-values <0.05) and for 16 of the 22 previous LHBs. A significantly greater rate of glucosamine prescribing was found in the 5 least deprived LHBs compared to the 5 most deprived LHBs for all four 12-months periods, analysed, between February 2006 and January 2010. Rate ratios were 2.21($p=0.000$), 2.53($p=0.000$), 2.02($p=0.005$) and 1.75($p=0.002$). In the Aneurin Bevan Health Board the second local prescribing guidance produced a 78.73% ($p=0.028$) reduction in glucosamine prescribing rate. Earlier, NICE CG59 elicited a 2.35% ($p=0.0173$) decrease and the first local prescribing guidance a 2.97% ($p=0.6$) increase in rate of glucosamine prescribing.

Overall, the NICE recommendation to no longer prescribe glucosamine has not been followed and the rate of glucosamine prescribing has instead increased. This study may have been premature, not allowing sufficient time for implementation of guidance. Other factors possibly influencing adherence to recommendations are how clearly guidance is communicated to prescribers and the lack of a NICE implementation team in Wales to help facilitate effective change. Finding the rate of glucosamine prescribing to be greater amongst the least deprived LHBs was unexpected considering the increased prevalence of arthritis in the most deprived fifth of the population of Wales. This can perhaps be explained by differences in health seeking behaviour of the most and least deprived populations. Local prescribing guidance appears more effective than NICE guidance at reducing the rate of glucosamine prescribing, providing it is reiterated and the financial consequence of not implementing the guidance is explained to prescribers.

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The effect of pioglitazone and omeprazole on osteoblast growth, differentiation and mineralisation

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Pioglitazone, a thiaozlidinedione and omeprazole, a proton pump inhibitor, are dissimilar drugs but both have been linked to an increased fracture risk[1,2]. Omeprazole is a pro-drug which is converted at the acidic pH found in the parietal cells to an active sulphenamide. The bone remodelling process involves the formation of mineralised bone matrix by osteoblast cells; mesenchymal stem cells are the progenitor for osteoblast cells and several other cells types, including adipocytes. The aim of this study is thus to determine the effect of these drugs and the active form of omeprazole on osteoblast growth, differentiation and mineralisation; if the drugs affect any of these processes this could provide a mechanistic explanation for their increased fracture risk.

This study utilised two osteosarcoma cell lines: MG63 and SAOS-2 and human mesenchymal stem cells. Mineralisation assays were conducted to determine the effect of the drugs on the ability of osteoblast cells to produce a mineralised matrix. In brief, cells were cultured in media supplemented with l-ascorbic acid and β-glycerolphosphate. Once mineralisation was visible under the microscope, cells were stained for the calcium component of bone mineral using alazarin red stain; staining is visible under the microscope and the stain can be quantified. Two growth assays, namely trypan blue exclusion and MTS, were conducted to determine the effect of the drugs on osteoblast growth and viability. UV spectroscopy was conducted to determine the conversion of omeprazole to its active sulphenamide at different pHs.

In despite of a clinical picture of increased fracture risk, the results of this study were that pioglitazone and the active form of omeprazole both increased mineralisation *in vitro*. There was a trend towards increased mineralisation with normal omeprazole. All compounds tested also produced a decrease in cell growth after six days, compared with controls; this was not accompanied by any decrease in cell viability. The UV spectroscopy demonstrated that the active sulphenamide is only produced from omeprazole at the lowest pH tested: 2.3. Thus it will not be produced *in vivo* outside of the parietal cells.

In vivo studies of thiazolidinediones have shown the drugs to increase adipocyte number and reduce bone mineral density[3]. It is widely hypothesised that this reflects the drugs inhibiting differentiation of stem cells to osteoblasts whilst enhancing stem cell differentiation to adipocytes[1]; thiazolidinediones have been shown to promote formation of mesenchymal stem cells to adipocytes in adipogenic conditions[4]. The increased mineralisation seen in this study, along with decreased cell growth, suggests that pioglitazone and the active sulphenamide can increase differentiation of human mesenchymal stem cells to osteoblasts in osteogenic conditions. Thus this study demonstrates a dual role for thiazolidinediones, being able to promote both osteoblast and adipocyte differentiation in the correct environmental conditions. Thus the effect of thiazolidinediones is likely to depend on the *in vivo* environment. This may explain why increased mineralisation was seen in this *in vitro* study, whereas *in vivo* studies have shown a decrease in bone mineral density. UV spectroscopy demonstrated that the active sulphenamide is not produced outside the parietal cells and is thus not involved in the increased fracture risk. It is of interest that it increases mineralisation, as it could lead to a putative treatment for osteoporosis; especially as it has not been shown to have any other detrimental effects on bone formation like thiazolidinediones. Thus for omeprazole, the mechanism of increased fracture risk remains unknown, but the drug does not appear to have any detrimental effects on osteoblast growth, differentiation or mineralisation.

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Validation of the In-Cap Machine's capsule-filling capabilities and forecast of optimum fill weights for excipients carrier mixtures with different tapped bulk densities as a guide for hospital formulation work

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Hard capsules are one of the oldest and popular oral dosage forms which can be utilized in the new drug development or early stage of clinical trials.[1, 2] The Bonapace In-Cap is an automatic tamping capsule filling machine with an output of 3000 capsules per hour. It was designed for early stage of clinical trials and development of new drugs.[3] The objective of this study was the validation of the In-Cap, located in St. Mary's Pharmaceutical Unit, to assess its filling capabilities and to forecast optimum fill weights for excipients carrier mixtures with different flowability as a guide for hospital formulation work. Commonly used excipients, Starch 1500, Lactose monohydrate 100M, Avicel PH102 and Magnesium stearate were used in this study.

Flowability of each excipient and 4 powder carrier mixtures (A, B, C & D) was determined using a density test and the calculation of their compressibility index. The carrier formulations were tested with a powder plug test rig to investigate their plug forming properties, probable capsule fill weights and the minimum force required to make a plug. Density results showed that formulations C and D had the highest and lowest density of the 4 formulations prepared and were filled in to size 0, 1, and 2 capsules using the machine. The powder plug test results indicated that the theoretical capsule fill weights would yield plug lengths for both formulations shorter than the actual dosing disc thickness available for use. Both formulations formed loose and weak plugs at the correct fill weight in the larger capsule sizes and high rates of defective filled capsules were observed as the capsule size increased.

The machine dosing disk and its support plate have a constant thickness and the amount of tamping finger insertion into the disc is the same for all sizes. Thus as the powder column increases in length with disc thickness there is less relative pin insertion resulting in less tamping force being applied to the base of the plugs, which were consequentially weaker and crumbled when moved. This loose powder results in the capsule being damaged when they are rejoined. Another In-Cap machine limitation was the control of powder feed to the dosing hopper caused by the fixed speed of the stirrer in the feed hopper. Formulations with better flow were fed into dosing hopper at a greater rate than required. To minimise the rate of powder feed narrower feed tubes were made to restrict flow by the Clinical Engineering group at St. Mary's. A feed tube with a diameter reduced by 75% successfully slowed down the powder feed. The inner structure of the powder hopper is asymmetric and the formation of an uneven powder bed cannot be avoided because there is an accumulation of powder behind the scraper blade, which is designed to control the powder bed depth. The uneven powder bed height on longer runs will eventually affect the tamping pin settings and cause inconsistent powder feeding into the dosing disc holes affecting plug formation and the uniformity of fill weight. Suggestions were made to modify the scrapper blade to reduce this problem.

The validation exercise exposed limitations in the In-Cap machine. Modifications to the feed tube, dosing disc thickness and scraper were suggested and there were some improvements in its performance. The excipient carrier mixtures used in this study cannot be used as a gold standard, however, they can be used as potential carrier mixtures used for API test formulations without extra time being spent in performing trials.

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Activation of survival kinases Akt and ERK1/2 by BNP in ischaemia-reperfusion

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Myocardial reperfusion therapy remains the most effective intervention for infarct size reduction in patients presenting with acute myocardial infarction (AMI). However, morbidity and mortality post-AMI still remain high, which may be attributed to the paradoxical phenomenon of reperfusion injury. The detriment of reperfusion injury stresses the need for novel cardioprotective interventions. Previous research has identified the 'Reperfusion Injury Salvage Kinase' (RISK) pathway, consisting of a group of pro-survival kinases, of which the two major survival cascades are PI-3/Akt and MEK1/2/ERK1/2. When activated at the time of myocardial reperfusion, the RISK pathway confers powerful cardioprotection.[1] Several pharmacological agents have been found to activate the RISK pathway, including the natriuretic peptides. In addition to their endocrine functions, natriuretic peptides have been shown to exert pleiotropic functions on the myocardium, and confer a cytoprotective effect against reperfusion injury.[2] The present study investigated the hypothesis that B-type natriuretic peptide (BNP) mediates cardioprotection at reperfusion by elevation of phosphorylated Akt and ERK1/2, and therefore activation of the RISK pathway.

Langendorff-perfused hearts were subjected to ischaemia-reperfusion or normoxia after 20 min stabilisation in conjunction with 1 of 4 treatment protocols: time control; wortmannin 100 nM (PI-3 K inhibitor); BNP 10 nM; BNP + wortmannin. Ischaemia-reperfusion was simulated by 35 min coronary artery occlusion and 10 min reperfusion; treatment was commenced 5 min prior to reperfusion until 10 min reperfusion. Normoxic hearts received 15 min treatment following the stabilisation period. The activation of Akt and ERK1/2 was determined by analysis of their phosphorylation states. Myocardial samples were taken at completion of the assigned protocol, analysed by western blotting and quantified by optical densitometry.

All data were normalised to average control. Perfusion of BNP at reperfusion marginally elevated the level of phosphorylated Akt (1.14 ± 0.36 vs. control, not significant), whereas co-perfusion of BNP + wortmannin significantly reduced the level of phosphorylation afforded by BNP (0.20 ± 0.12 vs. 1.14 ± 0.36, $P < 0.05$). In normoxia, BNP significantly elevated the phosphorylation of Akt (2.94 ± 0.38 vs. control, $P < 0.0001$) which was significantly reduced by wortmannin in the presence of BNP (0.53 ± 0.10 vs. 2.94 ± 0.38, $P < 0.0001$). BNP resulted in a distinct rise in the level of phosphorylated ERK1/2 at reperfusion (2.45 ± 1.00 vs. control, not significant), whereas co-perfusion of BNP + wortmannin decreased the phosphorylation afforded by BNP (0.83 ± 0.61 vs. 2.45 ± 1.00, not significant). In normoxia, the phosphorylation of ERK1/2 afforded by BNP was significantly lower (0.24 ± 0.07 vs. control, $P < 0.0001$). BNP + wortmannin marginally decreased the phosphorylation of ERK1/2 in comparison to BNP (0.12 ± 0.02 vs. 0.24 ± 0.07, not significant).

BNP appears to mediate cardioprotection in ischaemia-reperfusion via activation of the RISK pathway. Previous research has demonstrated that BNP-mediated cardioprotection may involve activation of the PI-3 K/Akt pathway.[3] It is likely that the lack of activation of Akt by BNP at reperfusion seen in the present study is a reflection of the late sampling point, by which time phosphorylation levels are speculated to fall. Therefore it is likely that BNP did elevate the phosphorylation of Akt, but the effect was masked by missing the window of peak Akt phosphorylation. BNP activates Akt under normoxic conditions, but downregulates the activation of ERK1/2 under the same conditions. Therefore, BNP-mediated activation of Akt would appear to occur independently of reperfusion, whereas activation of ERK1/2 appears to occur by a reperfusion-dependent mechanism. Lastly, the wortmannin-sensitive upregulation of ERK1/2 in reperfusion by BNP indicates that this occurs downstream of Akt activation.[4] This is also supported by the late sampling point, which may have provided the optimum window in which to measure peak ERK1/2 phosphorylation. In conclusion, this research provides preliminary evidence to support the potential use of BNP as a pharmacological postconditioning agent in the prevention of reperfusion injury.

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Microneedles for siRNA therapy

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siRNA (short-interfering RNA) has received considerable attention as a possible therapeutic to correct overexpression and faulty regulation of genes in many diseases.[1,2] The aim of this study is to show that microneedles could potentially be used as a means to deliver siRNA to the epidermis for therapeutic applications. Microneedles are a minimally invasive and painless cutaneous drug delivery method. [3]

Lamin A/C was chosen as a model gene with which siRNA knockdown would be attempted in immortalized HaCaT cell line [4] and primary human keratinocytes. siRNA coated onto microneedles was recovered to determine if it was still functionally able to cause a knockdown in cells. RT-PCR (reverse transcription-polymerase chain reaction) was utilized to determine lamin mRNA expression; briefly, total RNA isolated from the cells was reverse-transcribed and cDNAs were generated. The cDNA products were then used for PCR. The PCR products were analysed on a 2% agarose gel and visualised by ethidium bromide staining. Western blot analysis was performed in order to determine knockdown of lamin at protein level and a knockdown was observed when siRNA was used against lamin in HaCaT cells. Confocal microscopy was conducted following immunofluorescence to determine if any apparent cellular morphological changes are present when siRNA was treated against lamin.

The study showed siRNA was successfully coated onto the surface of microneedles and the process of coating and drying did not appear to reduce its biological functionality. RT-PCR demonstrated a knockdown at the RNA level. Recovered siRNA from coated microneedles was shown to knockdown the target gene lamin once delivered to HaCaT cells and with knockdown more pronounced as number of coatings increased. Western blot analysis subsequently demonstrated that this knockdown also occurred at the protein level.

These results suggest that microneedle facilitated delivery of siRNA to the skin, in a therapeutic context, shows potential; however, further experiments would be required in order to confirm this, in particular experiments aimed at a real therapeutic target, for example TNF alpha.

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Regulators of G-protein Signalling (RGS) mRNA expression in a model of L-dopa induced dyskinesia

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Parkinson's disease (PD) is a progressive neurodegenerative disorder resulting from the degeneration of the dopamine (DA) containing cells of the substantia nigra, which are essential for normal basal ganglia function. This degeneration causes motor symptoms such as hypokinesia, bradykinesia, rigidity and tremor that characterise PD. The gold standard symptomatic treatment for PD is L-DOPA that was discovered in the 1960's and has been used since then. The major challenge with L-DOPA is it produces motor complications, involuntary movements called L-DOPA induced-dyskinesias (LID).[1] Several mechanisms on LID have been reported. More recently, mRNA coding for Regulators of G-protein signalling (RGS) proteins that inhibit G-protein (GP) function have been found in the brain.[2,3] Several subtypes have been reported; some RGS mRNA expression is found to be highly localised and relative to specific areas in the brain whereas others are broadly expressed and have distinct patterns of expression.[2] PD and the effect of L-DOPA can be modelled by unilateral dopamine depletion in a 6-hydroxydopamine (6-OHDA) model.[4] The aim of this experiment was to assess the role RGS proteins play in changes that occur after 6-OHDA lesioning, acute and chronic L-DOPA treatment and the involvement of LID.

From this study we found that dopamine depletion does not affect RGS4, 8 and 9 mRNA expressions, neither is there an effect of L-DOPA treatment. RGS2 was unaffected by the lesion, but increased in the lesioned striatum following acute L-DOPA treatment. RGS2 mRNA expression was still increased 24 h after the last dose of L-DOPA suggesting a therapeutic role of RGS2 that modulates effect of L-DOPA in PD. No direct correlation was found between RGS2 expression and LID. In conclusion, RGS2 may be involved in mediating the therapeutic response of L-DOPA.

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Does clathrin-dependant endocytosis have a role in Alzheimer's disease?

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Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disorder of the brain. There is neuronal loss in the hippocampus and basal forebrain resulting in memory loss, as well as a decline in cognitive and functional abilities.[1] One theory, the amyloid hypothesis, proposes that senile plaques from amyloid-beta (Aβ) accumulation are the trigger for AD.[1] APP (amyloid precursor protein) is cleaved by β- and γ-secretase to produce Aβ. There is a close link between APP endocytosis and APP processing (and hence Aβ production).[2] APP is internalised via clathrin-dependant endocytosis (CDE). A recent genome-wide association study (GWAS) suggested the involvement of PICALM variants in the pathology of AD.[3] PICALM is a clathrin assembly protein that plays an important role in the formation and maintenance of clathrin-coated vesicles.[4] It is implicated in the endocytosis of APP and consequently APP processing.[3] Clathrin and its regulatory proteins are critical in the trafficking and processing of proteins in AD.[2] Another key protein, dynamin-2, is a GTP-ase that cleaves the budding vesicle from the plasma surface. The purpose of this study was to understand if PICALM, a component of CDE, is directly involved in AD pathology. The aim was to knock down PICALM expression using siRNA and to investigate its effect on the levels of APP, Aβ40, clathrin and dynamin-2.[4]

The human neuroglioma H4 cell line was cultured using standard methods. H4 cells were transfected with PICALM siRNA and oligofectamine or green fluorescent protein (GFP) siRNA and oligofectamine, while other H4 cells were treated with oligofectamine alone. Control H4 cells did not undergo any treatment. A cell viability assay confirmed that none of these agents significantly affected the viability of the H4 cells. Western blotting and sandwich ELISA were performed to measure protein levels. Immunocytochemistry (ICC) was performed on fixed H4 cells to support the quantitative data and examine the distribution of the proteins.

Western blotting showed that PICALM siRNA had significantly down-regulated PICALM. This was confirmed by ICC which also showed perinuclear labelling and punctate staining of PICALM in the cytoplasm. Western blotting and sandwich ELISA confirmed that PICALM knockdown did not have any significant effect on the levels of clathrin, dynamin-2, APP or Aβ40. These data were supported by ICC where APP was found to be distributed along cytoskeletal elements and in the perinuclear regions while labelling for clathrin showed perinuclear, punctate and diffuse cytoplasmic staining. PICALM knockdown appeared to have affected the distribution of clathrin, however further work would need to be carried out to verify this. In western blotting, the H4 cells treated with GFP siRNA showed a significant reduction in dynamin-2. The relevance of this finding is unclear. In immunocytochemistry, labelling with the anti-dynamin-2 antibody was so faint that no meaningful comparisons could be made.

These data suggest that PICALM knockdown has no effect on APP processing and Aβ production. However, this does not rule out the involvement of endocytosis, more specifically CDE, in APP processing. PICALM is one of many clathrin adaptor proteins, thus the cells may be able to adapt to a decrease in PICALM levels. PICALM may be required in CDE but is not critical due to the presence of other regulatory proteins with similar functions. The GWAS identified PICALM as a risk gene in AD[3] and these findings suggest that this is less likely to be due to PICALM down-regulation. To assess the overall effect of PICALM on APP processing these data should be compared to PICALM over-expression studies.

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Epilepsy: development of a learning aid

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Computer Aided Learning (CAL) offers an exciting potential as a supplementary learning aid in university undergraduate curricula. Traditionally lectures are augmented with additional reading from textbooks, journal articles or the internet. CAL adds a new interactive dimension to the educational experience that is not available with other traditional information mediums. Interactivity in this CAL package was enhanced with digital animation, video and visual combinations, quizzes and case studies all integrated throughout the package to enhance understanding of difficult concepts of epilepsy[1]. The aims and objectives of this study were to evaluate the usefulness of a CAL package on Epilepsy to undergraduate MPharm III students, as a supplementary learning aid and not as a substitute to lectures. Another aim was to assess its potential as a source of learning for continuous professional development (CPD).

A background literature search was performed and the CAL package was then designed using Microsoft PowerPoint® 2007 and posted on Learning Central for MPharm III students(n = 101). The package was split into four main chapters: 'What is Epilepsy?', 'Clinical Manifestations', 'Treatment, Management and Monitoring with Anti-Epileptic Drugs (AED's)' and Future Developments and the Pharmacist's role in Epilepsy'. Online questionnaires were produced on Google Docs, with three sections and were designed with a centre-weighted, 5-point Likert Scale to obtain responses to twenty-five statements. Qualitative data was collected via the option of free text entry after each question and at the end of the package. MPharm III students were recruited by an e-mail, providing information about the study, and that all responses would be anonymous.

The response rate to questionnaires collected was 29% (n=29/101). The questionnaires had three sections: package layout, package content and the general views regarding computer aided learning. LAYOUT: the majority (90%, n=26) of students agreed hyperlinks were easy to use. The majority of students 55% (n=16) strongly disagreed or disagreed that the package was too long. CONTENT: All of the respondents agreed that the package covered a wide range of topics on epilepsy. The students (86%) also agreed that the clinical aspects of epilepsy were covered well throughout the package. A high proportion (93%; n=27) of students agreed the information on the role of the pharmacist was valuable. OVERALL IMPRESSION OF CAL: Most students agreed (78%, n=22), to the statement 'I find interactive learning through a computer package at my own time convenient'. Eighty three percent (n=24) of students agreed they would use the CAL package for CPD.

The data collected suggested that the sample of MPharm III students accepted CAL as a form of interactive learning positively. Further studies on a larger population of students would need to be carried out, for CAL to acquire a permanent place in the mainstream undergraduate course. The negative comments collected would be useful to improve the package for use in the future. The results also suggested that the use of CAL as a form of CPD learning was valuable, and that the majority of students would benefit from it.

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In vitro uptake and transepithelial transport of ipratropium across MDCK cells: differential role of OCT/OCTN transporters

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The lungs provide a suitable surface for delivery of inhaled drugs such as bronchodilators and anti-inflammatory agents. It is necessary for these drugs to be absorbed effectively across the airway epithelia in order to gain pharmacological effect. Very little is known about the effect of influx and efflux transporters on the transport of drugs within the intact lung and the impact these transporters can have upon local or systemic drug pharmacokinetics and pharmacodynamics [1]. Ipratropium, which was used as a model drug to study the effect of these transporters on its pharmacokinetics, is a positively charged anticholineregic drug used for the treatment of asthma and chronic obstructive pulmonary disease (COPD).

There are two major transporter superfamilies: ATP-Binding Cassette (ABC) transporters and Solute carrier (SLC) family. The SLC22A family is divided into two classes: the membrane potential dependent organic cation transporters, OCTs and the pH sensitive organic cation/carnitine transporters, OCTNs [2]. Various pulmonary cell lines have been used for uptake studies to assess functionality of lung transporters, but these lack highly restrictive barriers therefore transepithelial transport investigations are compromised. Madin-Darby Canine Kidney (MDCK) cells were used for transepithelial studies as they form highly restrictive monolayers and are easy to culture [3]. There are two types of MDCK cells: MDCK I (more restrictive) and MDCK II (less restrictive). The aim of this study was to determine the differential role of OCT/OCTN transporters on the uptake and transepithelial transport of ipratropium, across MDCK cells [4].

Uptake studies were carried out in both MDCK I & II cells using 6 different treatments (control, unlabeled ipratropium, L-carnitine, MPP^+, TEA and ergothioneine) to study their effect upon the uptake of [^{3}H] ipratropium. Transepithelial transport studies of [^{3}H] ipratropium were carried out only in MDCK I cells, since they are more restrictive. The experiments were performed using Transwell® inserts from apical (donor) to basolateral (receiving) and basolateral (donor) to apical (receiving) membranes using unlabeled ipratropium as the inhibitor and [^{14}C] Mannitol as a paracellular marker. Samples were collected at distinct time points from receiving chambers and radiation analysis in both studies was performed using a liquid scintillation counter.

Uptake studies in MDCK I cells showed that unlabeled ipratropium, MPP^+ and TEA significantly inhibited the uptake of [^{3}H] ipratropium. This may suggest that the uptake of ipratropium in MDCK I cells is primarily mediated by OCT transporters and not OCTN transporters, although the latter are present and functional in MDCK cells. Results in MDCK II cells showed that only unlabeled ipratropium showed a significant inhibition of [^{3}H] ipratropium. This may suggest the displacement of [^{3}H] ipratropium from non-specific binding sites or that other inhibitors weren't potent enough to inhibit uptake of [^{3}H] ipratropium by MDCK II cells. Transport studies did not show a significant difference in the transport of [^{3}H] ipratropium from both apical to basolateral sides and vice-versa. This shows that OCT and OCTN transporters have very little to no effect on the transport of [^{3}H] ipratropium across MDCK I cells.

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The effect of magnesium sulphate on the nebulisation of salbutamol sulphate

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Salbutamol sulphate (SS) was the first short acting β_2 – adrenergic receptor agonist to be marketed, in 1968. It was first sold by Allen and Hanburys under the brand name Ventolin and was used for the relief of bronchospasm in conditions such as asthma.[1] It is well known that the intravenous administration of magnesium sulphate (MS) as an appendage to conventional therapy is effective in a severe asthmatic crisis where standard treatment is unresponsive. [2] MS acts as a bronchodilator, similar to SS, making breathing easier.[3] More studies are needed to establish the effectiveness of nebulised MS.[3] Past studies have found that adding MS into the nebuliser solution improves the peak expiratory flow rates (PEFR) of the patients compared to patients receiving SS alone.[4] The aim of this project is to explore the effect of MS on the nebulisation of SS in terms of nebuliser output and particle size of both SS and MS. The aerodynamic properties of the particles were analysed through calculating the mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), fine particle fraction (FPF) and fine particle diameter (FPD).

Three different nebuliser solutions were made up, one containing SS alone at a concentration of 2.5mg/ml, one containing SS (2.5mg/ml) with MS at an isotonic concentration of 63mg/ml and the final solution of SS (2.5mg/ml) and triple strength MS at a concentration of 189mg/ml. These solutions were analysed by using a Next Generation Pharmaceutical Impactor to determine the particle size of SS and MS. For analysis of SS the nebuliser was attached to the NGI and ran for 2 minutes whereas it was ran for 6 minutes for the analysis of MS to ensure the amount of magnesium that deposited in the plate was enough to measure. The concentration of SS was then established using a HPLC instrument and the concentration of magnesium was determined by performing a complexometric titration as specified in the BP. The concentration from each plate was then entered into an Excel spreadsheet, enabling values for MMAD, GSD, FPF and FPD to be calculated. Finally, for statistical analysis, a one- way analysis of variance (ANOVA) was performed on all results using SPSS computer software.

The results indicated that as the concentration of MS was increased, the total amount of SS that was deposited on the plates of the NGI decreased, from around 700 µg for SS, to 500 µg for SS and MS, to 250µg for SS and triple strength MS. It was also found that as the concentration of MS was increased, the particle size of SS also increased. This was observed through the increase in MMAD values which increased from around 3.5µm for SS, to 5µm for SS and MS, to 6µm for SS and triple strength MS; and the decrease in the FPF values. This was further supported by the results of the ANOVA test performed, which showed that there were significant differences between the MMAD and FPF values between the groups.

In conclusion the addition of MS to nebulised SS has no real effect on nebuliser output by weight. It does however decrease the total amount of SS that is deposited on the plates of the NGI. This could be because the MS is increasing the viscosity of the nebuliser solution, thereby not letting as much SS out of the nebuliser into the airways and into the lungs. This brings me to the conclusion that MS is itself exerting an effect in the airway and in the lungs that is similar to that of SS. I believe that more studies are needed to explore this further. The most important finding of all was that the addition of MS increased the particle size of SS possibly restricting its effect in the lung. A particle generally has to be less than 5µm in diameter to get into the lung and to have an effect there, however the mean particle size of SS is increased to over 5µm by the addition of triple strength MS. Therefore the improved peak flow of patients receiving nebulised SS with MS may be explained again by the fact that MS has its own effect in the airways and in the lungs. This is also supported by the results show that the MMAD value for magnesium was calculated at 3.5µm, which is small enough for the particles to reach the lungs and therefore have an effect there.

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Comparing the aerosol performance of salmeterol and fluticasone combination inhalers

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The inhaler drug market was valued at $18.5 billion in 2006, this value is increasing as number of people suffering from asthma and COPD are globally rising. [1] Seretide was the first salmeterol & fluticasone inhaler released by GSK in 1999. Salmeterol is a long acting beta-2-adrenoceptor agonist causing bronchodilatation. Fluticasone propionate is a glucocorticoid steroid that has anti-inflammatory action. Seretide has become known as an efficient and patient compliant product which achieved sales of £4,977 million in 2009 demonstrating its popularity.[2] This has led to the production of generic products (ForAir and Seroflo) which are available in the Indian markets at affordable prices. The aim of the project is to compare the aerodynamic drug profiles of five salmeterol/fluticasone combination inhalers, Seretide 50 inhaler, two Seretide 125 inhalers, Seroflo 125 autoinhaler and ForAir MDI. Each inhaler contains 25µg of salmeterol base and 125µg fluticasone propionate except Seretide 50 which contains 50µg fluticasone propionate. Similarities found in aerosol performance of the generic inhalers can be serious competition for Seretide.

Firstly, an appropriate high performance liquid chromatography (HPLC) assay was established after studying previous researches [3] for salmeterol xinafoate (SX), fluticasone propionate (FP) and beclomethasone dipropionate (BDP) which was the internal standard. Linear regression calibration curves were developed from constituting a range of suitable concentrations of SX and FP against a constant BDP concentration. The inhalers were tested on a next generation impactor (NGI) at a flow rate of 30L min^{-1} which generates particle distribution of the drugs over nine stages. Samples were collected from each stage and then analysed on the HPLC where the area of the peaks and calibration curves were used to calculate the total amount of drug present at each NGI stage. This data was used in an excel spreadsheet to calculate four important parameters, the emitted dose of salmeterol base and FP in one actuation, the MMAD, GSD and FPF below 5µm. These results were used to do statistical analysis using a one-way analysis of the variance test and a post- hoc test (Bonferroni's) on the SPSS database.

The results showed that the Seretide inhalers and ForAir inhaler had reasonable average drug recovery data per actuation. However, Seroflo displayed the most inconsistent data which was demonstrated by the low p-values from the statistical tests. The highest significant difference was with Seretide 3, where the p-value was 0.0001 for salmeterol base and 0.0004 for FP. Through investigation, it was found that the valve was malfunctioning and delivered a low average dose of 15.61µg for salmeterol base and 79µg for FP. All inhalers displayed similar particle distribution by the NGI except with the first and second experiment of Seretide 1 (125) inhaler which greatly influenced its aerodynamic parameters. There were issues of drug blockage in the NGI preventing flow and therefore affecting precise drug deposition. Apart from Seretide 1's inconsistent data, all other MMAD, GSD and FPF data from each inhaler was very similar and therefore it indicates that there are possible resemblances in aerosol performances between inhalers.

In conclusion, this is the beginning of a new area of research into comparing the efficiency of the generic products from developing countries with an international product, Seretide. More research is required to prove the generic standard is the same as Seretide or better. Due to current financial issues in the health sector, a lower cost inhaler would be an attractive alternative. Currently these generic products need to pass the legal regulations and technical criteria before we can see them in the European markets.[4]

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The clinical and non-clinical factors influencing discharge decisions in dermatology

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The most critical clinical decision considered at the end of most consultation is whether or not to discharge a patient. In the secondary care setting many specialist services, such as dermatology, are delivered through outpatient services; where resources are finite, and the clinician's time is split between new referrals and follow-up patients. The time taken to see new referrals is directly dependent on the number and frequency of follow-up patient visits. In many clinical services the waiting time for referral patients could be dramatically reduced if the number of patients being followed up was reduced. Hence the most critical clinical decisions influencing outpatient waiting lists is the decision of whether or not to discharge a patient. Previous research has been conducted to assess factors influencing clinical management decisions[1]; but to date no such study has been conducted to identify the influential factors of the discharge decision. The aim of this study is to identify the clinical and non-clinical factors influencing the decisions to discharge dermatology outpatients. Ethical approval was granted by the Local Research Ethics Committee in February 2011. The Welsh School of Pharmacy Ethics Committee and Research & Development also granted their approval to conduct the study.

As there is limited literature available surrounding this topic it was deuced that this study is hypothesis generating rather than hypothesis testing[2]. Semi-structured interviews was the data collection method of choice as it was deemed the most appropriate method of obtaining rich data within the limited time scale that was available to conduct the study[3]. Clinicians were recruited from four dermatology outpatients' departments across South Wales. All participants provided written consent before interviews commenced. Demographic information was also collected at the beginning of each interview to allow for the identification of any differences emerging between different demographic categories at a later stage. Interviews were timed, audio-recorded and transcribed verbatim to a written format using NVivo computer software; with three interviews being conducted beyond the point where no new themes where emerging, i.e. saturation point.

Twenty-two clinicians participated in the study (92% response rate), 12 of which were male, clinicians median age=41.5, age range=30-64. Five key themes were identified as the main themes influencing dermatology outpatients' discharge decisions; 'Patient Related' had 13 subthemes linked with it including 'patients wishes'; the most frequently reported 'most important non-clinical factor to consider before discharge'(n=4).'Clinical setting Related' had 5 subthemes linked with it including 'condition at primary care management level'. 'Condition Related' had 5 subthemes linked with it and included 'improvement in condition' and 'diagnosis'; the two most frequently reported 'most important clinical factor to consider before discharge' (n=4 and n=3 respectively).'Policy Related' had 4 subthemes linked with it including waiting list pressures, which were mentioned by 18 clinicians. 'Clinician Related' was linked with 10 subthemes including 'fear of litigation'.

Hundreds of thousands of dermatology discharge decisions are taken daily with virtually no research conducted up until now to explore what factors actually influence these decisions. The decision for or against discharge is ultimately one of immense importance both socially and financially. Although all clinicians share a number of factors that influence their discharge decisions there are a substantial number of additional factors influencing such decisions to varying extents; which are dependent on foundations such as clinician's level of experience, clinical post and personal approach. The range of supporting and opposing opinions of different clinicians emphasises the wide range of factors considered by clinicians when considering the appropriateness of discharge. There is no 'standard strategy for determining a patient's suitability for discharge'; and such decisions are made by clinicians based on their personal judgements. Hence decisions are not made in a uniform manner and can vary from clinician to clinician considerably.

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Difference between uptake and transepithelial transport of L-Carnitine across an *in-vitro* MDCK model: role of OCT/OCTN transporters

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The lung has become a very popular drug delivery route for local and systemically acting drugs in the 20th century. The attractive properties of the lung as a drug delivery route include its non-invasive nature, large absorptive surface area and avoidance of hepatic first-pass metabolism. Despite the common usage today as a route of drug delivery, very little is known about pharmacokinetics of pulmonary drug delivery. The lung is a host to a wide range of drug transporters, which include members of both ATP-Binding Cassette (ABC) and Solute Carrier (SLC) Transporter superfamilies. There is a significant gap of knowledge in the role that SLC22 family transporters play in the uptake and transepithelial transport of cationic substances across the lung and elsewhere. SLC22 family contains Organic Cation Transporters (OCTs), the ones of interest to the current study are OCT1, OCT2, OCT3, OCTN1 and OCTN2 transporters. The model used in this study was an *in-vitro* Madin Darby Canine Kidney (MDCK) cell line, which expresses OCT/OCTN transporters[1]. The substance used was L-Carnitine, which is a well-known substrate for OCTN2[2]. The aim of this study is to investigate the effect of OCT/OCTN transporters on cellular uptake and transepithelial transport of L-Carnitine across an epithelial monolayer of MDCK cells.

MDCKI and MDCKII cell lines were grown for 4 day until they reached confluency. Uptake studies were conducted on 24-well plates with MDCKI and MDCKII cell lines. The plates were pre-treated with L-Carnitine 100µM, Ipratropium 500µM, MPP^+ 500µM, TEA 5mM and Ergothoneine 500µM solutions, pre-incubated for 15min, and the radiolabelled [^{3}H]-L-Carnitine was applied to each well. The plate was incubated for 60min, and the uptake measured using the scintillation counter. Transport studies were conducted using Transwell® semi permeable inserts. MDCKI cells were pre-treated with 100µM L-Carnitine, and pre-incubated for 15min. Following this, [^{3}H]-L-Carnitine was added to the apical or basolateral side and samples were taken from the receiving chamber at time-points of 15, 30, 45, 60 and 90min for measurement of cumulative transport of the radiolabelled probe.

The results showed that it was possible to significantly ($p<0.05$) inhibit uptake of [^{3}H]-L-Carnitine in MDCKI and MDCKII by all the used inhibitors except Ergothioneine 500µM in MDCKI which didn't show any inhibition of [3H]-L-Carnintine in this cell line. In contrast Transport studies showed that transcellular transport of [^{3}H]-L-Carnitine was not affected in the presence of the competitive inhibitor L-Carnitine 100µM.

It was concluded that although OCT/OCTN transporters play a significant role in the active uptake of cationic substances like L-Carnitine, they do not necessarily contribute to the total transcellular transport of OCTN substrates.

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The efficacy of biocide, Peracetic acid, against *Clostridium difficile* spores

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Clostridium difficile is a major cause of hospital acquired infection, not just in the UK but in many countries of the world.[1] The infection can vary in severity, but the leading symptom is diarrhoea with pseudomembranous colitis being the more severe effect. Infection is transferred by the ingestion of bacterial spores existing in the hospital environment. Spores are consequently passed out of the patient's body together with organic matter. The main issue with *C. difficile* Infection (CDI) is the ability of these spores to adhere to surfaces such as stainless steel; even with the recommended disinfectants being used persistence is still a problem.[2] Chlorine-releasing disinfectants, such as sodium hypochlorite and sodium dichloroisocyanurate (NaDCC), are the current biocides of choice. However, these substances carry occupational health hazards and so, are less than ideal. It is therefore, important to ensure there is not a safer or more effective biocide option that could be utilised for the purpose of dealing with *C. difficile.* Peracetic acid (PAA) is a well-known, highly-oxidising biocide active against a wide range of microorganisms including viruses, fungi and spores.[3] It has numerous advantages over the currently used disinfectants including less harmful degradation products. However, its reported efficacy against *C. difficile* spores is extremely contentious especially in comparison to NaDCC. The aim of this investigation was to evaluate the efficacy of PAA on *C. difficile* spores of various strains and ribotypes. The second aim of this study was to attempt to replicate the readings observed in a previous investigation that reported a phenomenon of spore aggregation on exposure to sporicidal agents.[4]

Mean diameter of spore stocks were recorded as a means of investigating aggregation properties. 3.5mL spore samples were inserted into a Submicron Particle Analyser (N4 Plus Beckman Coulter) with three repeats performed for each strain and generating results for the mean diameter of particles as well as standard deviation. To assess the biocidal efficacy of PAA, 50µL of spore stocks for each strain were exposed to an equal volume of PAA (0.1% v/v) for various exposure times. The exposure times used were 30 seconds, 1 minute, 5 minutes and 10 minutes. The subsequent mix was then neutralised by the addition of 400µL sodium thiosulphate (0.1% v/v). Serial dilutions were made and plated onto Brain and Heart Infusion medium (BHI) and anaerobically incubated at 37℃ for 48 hours before enumeration was completed.

Results of the particle size analysis for all 19 strains showed very similar readings giving an average diameter of approximately 1150nm. Following statistical analysis (ANOVA) it can be seen that in general there is no statistical difference between the mean diameter of *C. difficile* spores and the individual strains ($P>0.05$). Following exposure of microorganisms to PAA, enumeration of the plated samples and $\log_{10}$ reductions were calculated. The mean $\log_{10}$ reductions of 4 assays were plotted. A wide variation in susceptibility of spores to biocide exposure was observed. Noticeably, on statistical comparison (ANOVA) it can be seen that there is not a significant difference between the majority of the strains tested ($P>0.05$), after a 10 minute exposure time. However, strains DS1724, DS1747, DS1813, DS1801, and DS1807 all produced very significant $\log_{10}$ reductions ($P<0.001$) when compared to the $\log_{10}$ reductions of R20291, R8652, DS1750, DS1752, CD630, DS1684, R10459, and DS1721. These results suggest the variation is due to the intrinsic differences between the strains and is not reliant on the experimental procedure.

From the results, it has been seen that susceptibility of *C. difficile* spores to PAA varies greatly between strains. There also seems to be no significant differences between strains of the epidemic ribotype 027. It is also clear that most strains would need an exposure far exceeding the trialled 10 minutes to cause significant reductions in spores. PAA has many features that add to its value in comparison to the recommended biocides. However, a major disadvantage, although not proved for peracetic acid, is the fact that non-chlorine-releasing agent are thought to increase sporulation. Further investigation into the effects of PAA on clumping and sporulation rates is clearly required. It would be beneficial to test efficacy in conditions more like the actual setting of a hospital e.g. presence of organic matter and accurate exposure times. Only once the evidence has accumulated considerably more than what is currently available can a well-informed recommendation be made. This study, however, has shown that there are important properties of PAA that if effectively utilised could result in a revolution of the current guidelines.

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Use of group contributions to energy of vaporisation and molar volume in calculating Hildebrand solubility parameters

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Topical preparations are widely used for systemic drug delivery. The outermost skin layer, stratum corneum (SC), is the effective barrier that must be passed. Drugs must dissolve in and diffuse thought the lipid phase that surrounds the corneocytes. Hildebrand introduced the concept of Solubility Parameter (δ) to describe the average cohesive properties within a molecular species. Two molecules with the same δ will be freely miscible[1] - a quantification of the "like dissolves like"[2] adage that is widely used. In principle if a drug has a δ close to that of the SC it should enter easily; similarly if the vehicle has a similar δ it should enter and disrupt the barrier function. The reverse reasoning applies if absorption is undesirable as would be the case for sunscreens, pesticide/herbicide sprays and cosmetics. The use of sunscreens, in particular, is encouraged on a regular daily basis and are found in a large proportion of cosmetic products, although the long term side effects are not fully known.[3] Fedors proposed a method of estimating δ from group contributions for Energy and Molar Volume terms for low molecular weight liquids[4] that has been widely applied in formulation development. Fedors paper is lacking in description of how these group contributions were calculated and Fedors' estimate of reliability is that the Energy and Molar Volume values generally give results within 10% of the experimental values. This project aimed to estimate group contribution terms from regression analyses and compare the results with those of Fedors.

A database was compiled for a wide range of chemicals for Standard Energy of Vaporisation (E) and Molar Volume (MV). δ was calculated from $\delta = \sqrt{\frac{E}{MV}}$. Regression analysis was carried out, using Minitab 16^{TM}, taking p-value <0.05 as statistically significant. Stevenson group contribution values (ΔE, Δ MV and $\Delta\delta$) were given by the coefficients in the regression equations. Differences between the experimental values of E, MV and δ, and those estimated from Stevenson and Fedors group contributions for the compounds in the database, and the success of the Stevenson and Fedors values compared by a paired t-test.

Regression analyses showed that MV was closely related to Experimental. The relationship for E (and hence δ) was weak. These results are not surprising since intermolecular interactions would be expected to influence E, whilst MV is more simply a sum of volume terms. Two component groups for Stevenson's (Carbon (tetra-substituted) and Conjugation in ring of each double bond) coefficients were found not be statistically significant for E and three for MV (Carbon (tetra-substituted), Nitrogen (tri-substituted) and ring closure 3-4 and 5+). Differences were found between Stevenson coefficients and those of Fedors. Confidence intervals could be calculated for the Stevenson coefficients. Accuracy was assessed by residual differences between Experimental and Stevenson's and Fedors' data. The Stevenson and Fedors accuracies were compared by a paired t-test. Stevenson's E and MV were found to have a smaller residual difference than Fedors' E and MV. Two methods were used to calculate Stevenson's δ. The first method (direct) used the group contributions for Stevenson's δ coefficients, while the second method (indirect) used Stevenson's coefficients for E and MV. Indirect Stevenson's δ was different from Fedors' δ. Fedors' Energy had 43 compounds that had a difference of 10% from experimental, while Stevenson's had 27. Fedors' MV had 11 compounds with a difference greater than 10%, while Stevenson's MV had 2 compounds.

173 chemical compounds were studied and it was found that δ should be calculated out using E and MV rather than δ coefficients. Stevenson's predictions were more accurate than Fedors.

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Delineating the role of the actin cytoskeleton in fluid-phase endocytosis

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Drug delivery systems are involved in the internalisation of drugs using mechanisms like endocytic pathways. Analysis of a single endocytic pathway is at present limited by the lack of specific chemical inhibitors.[1] Chemical inhibitors, fluorescent dyes, stains and endocytic probes need to be applied to cells to identify the roles of cellular components in endocytosis. The actin cytoskeleton has been suggested to play various roles at different stages of endocytosis[2], but there is a general lack of agreement between researchers. Research conducted in skin fibroblast (A431) cells indicated that neither actin assembly nor actin filament organisation played an obligatory role in endocytic coated vesicle formation in the clathrin-mediated endocytic pathway.[3] The aims of this study were ;(1) to investigate the role of the actin cytoskeleton in fluid phase endocytosis in cells, using different types of fluorescent dextrans and (2) to investigate the effects of the drug, cytochalasin D (cytD) on dextran uptake in cells.

HeLa cells (cervical cancer cells) were used as the cell model in all the endocytic experiments. The uptake of the fluorescently labelled fluid-phase endocytic probe, dextran, was examined after incubation on cells. Cells were incubated with varying fluorescently conjugated dextrans of a range of molecular weights to determine the baseline standard controls, for later comparisons with cytD treated cells. Rhodamine phalloidin was used to stain the actin filaments and to observe its disruption when the cells were incubated with cytD. Cells were fixed using paraformaldehyde and viewed on a microscope. Flow cytometry (FACS) was used to determine whether molecular weight altered the extent of fluorescein isothiocyanate (FITC) conjugated dextran uptake in cytD treated cells.

Uptake of dextran and distribution appeared similar irrespective of the dextran used. Rhodamine phalloidin labelling confirmed that the concentration of cytD used in the experiments was sufficient to disrupt the actin to a suitable degree. Dextran uptake in cytD treated cells appeared more evenly distributed around the plasma membrane and less concentrated in the perinuclear region. CytD treated cells viewed on the microscope, showed severe actin disruption with the cells appearing rounder and smaller, and aggregations of disrupted actin forming bridges between cells. The aggregations have been studied and are unable to be removed by increasing the concentration of cytD or by adopting a longer incubation period so this was not attempted in this research.[4] The dextran uptake of TMR neutral dextran, 10kDa did not visually display any alteration between the untreated cells and the cytD treated cells. However, the FITC conjugated dextrans visually displayed a significant decrease in uptake in the cytD treated cells. Preliminary flow cytometry data displayed variations in dextran uptake based on molecular weight, but this will need to be corroborated by further research.

In conclusion, dextran uptake and its distribution are similar in HeLa cells no matter what the conjugate or molecular weight. FITC dextran uptake in cytD treated cells is decreased in HeLa cells, and the extent of the decrease is dependent on the molecular weight of the FITC conjugated dextrans, as highlighted by the preliminary FACS data. Actin does play a role in fluid-phase endocytosis as dextran uptake is significantly decreased when the actin cytoskeleton is disrupted. However, the extent of involvement or exact role of actin in this endocytic pathway is still unclear and requires further research. The collated data suggests that actin is not solely involved in the uptake of dextran or that dextran is entering cells via an actin-independent pathway. Further quantitative data in HeLa cells is required for confirmation and clarification of the main findings. Experimentation and collation of dextran uptake data in different cell lines, using the same experimental method would also be of interest to this field of research.

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Validation of the T-QoL - a novel Health Related Quality of Life Instrument for adolescents with skin disease.

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Skin diseases commonly occur in adolescence. Depending on the severity of the disease, some may find having a skin condition more difficult to deal with than others. Skin disease such as acne, eczema and psoriasis can have a significant physical, social and psychological impact on adolescent health related quality of life (HRQoL). Adolescence is a unique period of life where the individual undergoes various physical, biological and psychological changes. Adolescents differ from both children and adults and should be in their own right a specific category. If adolescents are viewed as children there is an increased likelihood of neglecting certain aspects such as their increasing developing needs for intimate relations and becoming increasingly autonomous towards parents. While if viewed as adults; different sort of problems are faced in terms of the importance of peers, striving to be independent and developmental aspects on intimacy and sexuality[1]. Currently there is no thoroughly validated instrument for measuring the impact of skin disease on adolescent HRQoL[2]. The aim of this study was to determine the psychometric properties of the Teenagers Quality of Life instrument (T-QoL) including validity, reliability and sensitivity to change.

Ninety-six adolescents between the ages of 12–19 years, suffering from acute or chronic skin disease were recruited into the study from the Dermatology out-patient clinic at UHW, Cardiff. Once written informed consent was obtained, study participants completed questionnaire packs consisting of T-QoL, Skindex-Teen, Dermatology Life Quality Index (DLQI) or Children's Dermatology Life Quality Index (CDLQI) and self-assessed disease severity Global Question (GQ) on three different occasions over a period of one month. Stage one assessed the validity of the T-QoL and involved the study participants completing the questionnaires for the first time following recruitment. Stage two examined the reliability of the T-QoL requiring study participants to complete questionnaires for the second time (retest with 3-7days interval) and the final stage, to assess the sensitivity to change, the questionnaires were to be completed a month after stage one.

Findings of this study confirmed that the most common skin conditions in the adolescent population are acne, eczema and psoriasis. Psoriasis had the highest mean score hence had the greatest impact on adolescent QoL. Mean item scores were higher for females however; the differences were not statistically significant except for the item which focussed on the need to cover up affected areas of the skin. Female participants scored significantly higher in the self-image domain, meaning they have greater sensitivity to the issues relating to self-image such as feeling self-conscious or upset about their skin condition. The completion of the T-QoL was found to be half to that of Skindex-Teen, (79, 139 seconds, respectively). Psychometric evaluation was conducted to determine the validity (n=96), the reliability (n=22) and the sensitivity to change over time (n=11). The strongest correlation was seen between the T-QoL scores and the Skindex-Teen scores, (r_s=0.75, $p<0.001$) mostly likely due to both instrument targeting the adolescent population. The T-QoL demonstrated high internal consistency (Cronbach's alpha coefficient = 0.89) and test-retest reliability (ICC value of 0.89). The ICC values for the three domains within T-QoL ranged from 0.66 – 0.93 demonstrating moderate to high reproducibility. The T-QoL proved to be sensitive to change in patient disease severity over time as shown by the level of significance (p-value of 0.04).

This study confirmed that the patient's perception may not only depend on the severity of the disease[3] but patient demographics such as gender can also contribute to the overall HRQoL. Despite a small sample size, the findings of the psychometric evaluation of the T-QoL demonstrated the validity, reliability and sensitivity to change over time. Once a larger sample size is obtained and full validation is complete, the T-QoL can be used in clinical practice to aid treatment decision making in adolescents and be used as an additional outcome measure in clinical research. A validated instrument such as this can provide additional information for health economists and be utilised when arguing for important resources, especially as the general public or non-dermatologists may not realise the extent to which skin diseases can affect adolescent QoL[4].

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Fragment based approach to the design and synthesis of novel hymenialdisine analogues as CDK inhibitors

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Uncontrolled cell growth and proliferation is a hallmark of all cancers[1]. This uncontrolled cell growth can result from mutations in genes encoding proteins that are involved in regulation of cell growth and proliferation. Proteins that are involved in this regulation are CDKs (Cyclin Dependent Kinases) and these are a family of serine/threonine kinases[2]. Cyclins are regulatory subunits that bind to the CDKs to activate them. The cell cycle is driven and co-ordinated by CDKs and Cyclins[2]. CDK-inhibiting proteins are also involved in regulation of the cell cycle. If a defect is detected, these inhibitors, such as the INK family or Cip/Kip family[3], are activated and inhibit the CDK leading to cell cycle arrest. Sometimes this precise regulation of the cell cycle does not occur resulting in uncontrolled cell growth and proliferation, which can lead to cancer. As a result, inhibition of CDKs is an interesting target for cancer therapy. A compound isolated from marine sponges, hymenialdisine, has been found to be a very potent inhibitor of CDKs (CDK2 IC_{50}= 40-70 nM)[4]. However, it is difficult to synthesise and has poor aqueous solubility. By retaining the pharmacophore of hymenialdisine, three inhibitors were designed which showed excellent docking interactions to the target enzyme (CDK2). Interactions with Glu81 and Leu83 were retained, which is important for inhibitory activity, and other interactions were also formed, for example, an interaction with the Phe80 residue.

A four step procedure was proposed for the synthesis - the first step – amide formation - worked very efficiently yielding approximately 54% of product to be used in step 2. The second step – nucleophilic aromatic substitution reaction (SNAr) - was the step that differed for each of the three compounds designed as different nucleophiles were used. All the nucleophiles were alcohols. A variety of conditions were used. Various bases were used to deprotonate the nucleophile. Different solvents were used to investigate whether this had an effect on yield, and different catalysts were also used. The third step – catalytic hydrogenation - would be to use hydrogen and a palladium catalyst to convert the nitro group to an amine, and the final step – nucleophilic substitution and reduction - would use benzaldehyde and sodium borohydride to yield the final compounds.

The synthesis of the compounds proved to be challenging. 5-Nitro-2-phenethyloxy-benzamide was synthesised using two different methods. This compound is a product of step 2 and used the nucleophile 2-phenylethanol. It could not undergo further reactions to make the final compound due to the low yield, which only left enough compound to undergo an NMR scan. Most of the work focussed on making the compound using 2-phenylethanol as it was this compound that exhibited the best molecular modelling. The conditions that resulted in the production of 5-nitro-2-phenethyloxy-benzamide were firstly, 2-phenylethanol as the solvent at 150°C and secondly; DMF as the solvent, sodium hydride as the base, copper powder as the catalyst, and heat (80°).

Based on the molecular modelling of the three compounds designed, biological activity would be expected to be the same or even better than hymenialdisine. The general conclusion for these experiments is that step 2 of the synthesis needs to be optimised before the synthesis can progress to making the final compounds which could potentially be inhibitors of CDKs. The conditions need to be optimised to result in a greater yield, so more investigation needs to take place into this step of the synthesis.

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Development of chemical fluoridation methods of nucleosides for potential application in Positron Emission Tomography

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Positron emission tomography (PET) is a form of nuclear medicine which uses short lived radionuclides attached to tracers, allowing metabolic processes to be studied. It is used to aid detection and treatment in neurology, cardiology but mainly in oncology.[1] It is now widely used in combination with CT or MRI scanners to provide greater sensitivity and specificity. ^{18}F is the most commonly used radioisotope as it has a half life of 110 minutes, an optimum time for preparation but not too long to cause serious harm.[2] There is a requirement for rapid synthesis methods for specific drug molecules to be labelled with a suitably short lived radioisotope. It is known that fluoride is a poor nucleophile therefore it is essential to devise suitable methods for its successful incorporation. One fluorinated nucleoside that is already in use in PET is 3-[^{18}F]-fluoro-3-deoxy-thymidine. The aim of this project was to develop chemical methods for the synthesis of fluorinated nucleosides that can later be applied to radiolabelled analogues for the use in positron emission tomography. The main objectives were to synthesise both 2' and 3' fluorinated nucleosides using efficient and high yield methods.

The synthesis methods involved the formation of cyclic anhydro intermediates that are predisposed to react with fluorine to give the desired fluorinated nucleoside with fixed stereochemistry. The three nucleosides that were explored in this research were uridine, 2-deoxyuridine and cytidine. In the first method each of the nucleosides first underwent a Mitsunobu reaction to form the anhydro intermediate.[3] A protecting group of dimethoxytrityl was then added to ensure the fluoride was added in the desired position, and then later removed to leave the final product. The second method involved the stereoselective hydrolysis of the cyclic ring followed by the addition of a Nos group, 4-nitrobenzenesulfonyl, which acted as a good leaving group to aid in the fluorine addition.[4]

The Mitsunobu reaction was successful in producing 3-anhydro-2-deoxyuridine from the starting nucleoside 2-deoxyuridine. However when the reaction was attempted with uridine and cytidine the final cyclic intermediate was not obtained. As a result 3-anhydro-2-deoxyuridine was taken further and used for all remaining steps. The addition of a protecting group in the 5' position was successful but the following fluoridation step did not render a 3' fluorinated nucleoside as anticipated. A second alternative method was then explored using an additional step to aid in the fluorine incorporation. The hydrolysis of the protected anhydro intermediate achieved the required product with the correct fixed stereochemistry. The next step was the addition of a Nos leaving group in the 3' position. During crude product purification the DMTr protecting group was lost, which then resulted in the failure of the final fluoridation attempt.

It can be concluded that the successful synthesis of a fluorinated nucleoside was not fully achieved. However many of the key intermediate steps were highly successful, which will significantly help for future work in this area. The use of 2-deoxyuridine proved to be the most successful nucleoside examined. The second method, using a Nos precursor, showed more promise than the first. With the use of milder conditions, which are favoured for fast efficient synthesis, this method would show easier translation to the radioactive analogues. More work is required to perfect the fluoridation step, however in this research the failure of the second fluoridation was probably due to the loss of the DMTr group. So with the required modification to this step the fluoridation should work. The failure of the uridine Mitsunobu reaction appeared to be caused by an unsuitable purification method as the crude product NMR analysis showed promise. The failure to produce an anhydro intermediate from cytidine could have been due to the presence of the amino group. Protection of this group could result in the successful completion of this step. There is still huge potential in this area of research as PET technology can be used to trace any biological process in humans, providing associated compounds can be radiolabelled.

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The identification of T-type calcium channels in osteoblast-like cells and their putative role in proliferation and mineralisation

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Osteoporosis, a type of mineralisation-resorption disorder, affects 1 in 2 women and 1 in 5 men over 50 years old in England and Wales, and its treatment and management cost the NHS£1.7 billion per year[1]. Tissues, like bone, in which cellular turnover occurs, must regulate processes like proliferation, mineralisation and apoptosis very carefully[2]. To date, a number of different receptors and ion channels have been identified in bone forming cells (osteoblasts).Given the role of osteoblasts, it is presumed that these ion channels and receptors are involved in many processes which are critical to bone remodelling. There has been a lot of interest surrounding T-type calcium channels in the past decade as they are involved in many cellular processes through which calcium signalling is crucial[3]. Recently, T-type calcium channels have been identified in osteoblasts, but it is not clear what their role is[4]. The main aims of this project were to elucidate the role of T-type calcium channels in the regulation of bone remodelling in osteoblasts, and to also investigate whether there was a basis for the claim that Zinc is good for bone remodelling, by presuming this is modulated through the T-type calcium channels.

To investigate these project aims, MG-63 and SaOS2 cells were used as osteoblast models, along with Human mesenchymal stem cells (HMSc). Four methods were utilised. RT-PCR was carried out using primer pairs for CACNA1G, CACNA1H and CACNA1I subunits for the three different T-type calcium channels ($Ca_V3.1$-3.3 respectively), in undifferentiated HMSc, MG-63 and SaOS2 cell lines. The products were visualised on 2% agarose gel. Mineralisation was performed in SaOS2 and HMSc cells and incubated for 2-4 weeks respectively. Cells were stained with Alazarin Red S for Ca^{2+} deposits (mineralised bone nodules). The colour was extracted with 10% Hexadecylpyridinium and absorbances were measured using UV Spectrophotometry (560nm). Trypan Blue Exclusion testing and MTS absorbance measurements were performed in SaOS2 and MG-63 to test the effects of T-type blockers and modulators on cell number and cell death. The cells were treated with the test compounds and incubated for 72-144 hours. A one-way ANOVA was used to analyse the results.

Expression of CACNA1G and CACNA1I T-type calcium channel subunits at the mRNA level was shown in MG-63 and SaOS2, but not in undifferentiated HMSc. The message for the CACNA1H subunit was present in all cell lines. Zinc caused a significant ($P<0.05$) dose-dependent increase in proliferation (n=6) and mineralisation of SaOS2 cells (n=8) since it can act as a $Ca_V3.3$ gating modulator[5].Zinc caused a significant dose-dependent increase in mineralisation in HMSc (n=4). Mibefradil stimulated a significant dose-dependent decrease in proliferation (n=6) and mineralisation (n=6 for SaOS2 and n=4 for HMSc) and also increased the percentage cell death in all cell lines by blocking $Ca_V3.1$ and 3.2. Zinc appears to demonstrate a 'rescue response' in the presence of high Mibefradil concentrations in MG-63 cells (n=4). This suggests that $Ca_V3.3$ currents modulated by Zinc override the blockade of $Ca_V3.2$ and 3.1 with Mibefradil. However, its lack of specificity makes this precise mode of action uncertain[6]. Nickel caused a dose-dependent decrease in mineralisation (n=8) and caused a dose-dependent increase in proliferation (n=6) in SaOS2 cells but the viability also decreased. Nickel selectively blocks $Ca_V3.2$. Verapamil had no effect on proliferation (n=6) or mineralisation (n=8) in SaOS2 cells, indicating that L-type calcium channels have little role in osteoblasts.

From the overall pharmacological picture we suggest that T-type calcium channels may play a role in osteoblast function, but their precise role is still emerging. Inquiries for now must focus on elucidating these roles and understanding the cellular mechanisms and signalling cascades. A putative role in proliferation and mineralisation opens the door to a possible new therapeutic target for mineralisation-resorption disorders. In this investigation Zinc has been shown to have a positive impact on osteoblast-like cells and it appears that the effects are mediated through T-type calcium channels. Further research into T-type calcium channels in osteoblasts is needed involving techniques such as electrophysiology (Patch-clamping), Western blotting, Q-PCR and Fluorescence imaging.

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Kinase profiling in triple negative breast cancer using novel acquired resistance *in vitro* models

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Triple negative (TN) breast cancers lack steroid hormone receptors and HER2 and therefore cannot be treated with endocrine or anti-HER2 therapies and have poor clinical outcome[1]. The TN phenotype may be intrinsic or acquired following prolonged endocrine treatment[2]. Traditional chemotherapy, which is associated with toxicity and eventual relapse is the only systemic therapeutic option and is nonspecific to TN disease[3]. Available targeted therapies including PARP inhibitors, EGFR inhibitors and anti-angiogenics have yielded little activity in TN disease. Thus identification of innovative targets for further drug development is required for TN disease. A significant barrier to identifying such targets is the scarcity of representative TN models. However, two novel *in vitro* human breast cancer models, EGFR positive 22.2 and EGFR negative 1.2, which acquired a TN phenotype after prolonged endocrine treatment, have been successfully cloned in the Welsh School of Pharmacy. Kinases are the most frequently mutated or overexpressed proteins in human cancers and represent potentially powerful anti-cancer targets[4]. The aim of this project was to profile 6 kinases deregulated in preliminary studies of the novel TN cell lines and to begin evaluating their potential as future therapeutic targets.

mRNA expression profiles of the kinases GNE, FGFR4, CDK2, MAPK6, VRK1 and c-Met were generated for the EGFR positive 22.2 versus EGFR negative 1.2 TN clones using Genesifter microarray analysis software and semi-quantitative RT-PCR. Web-based ontological tools (Medline, Genecards, Genedecks) were used to accumulate information regarding their potential role with reference to cancer-related functions that could feasibly contribute to TN breast cancer. The impact of their deregulation at the mRNA level on relapse-free survival, distant metastasis-free survival and overall survival was explored in a publically available ER-negative patient dataset using online Kaplan-Meier Survival Plotter software.

Significant GNE and FGFR4 upregulation in EGFR positive 22.2 clone and CDK2, MAPK6, VRK1 and c-Met upregulation in EGFR negative 1.2 clone was demonstrated using Genesifter and successfully confirmed by RT-PCR. Ontological interrogation revealed links between the kinases and regulation of proliferation or invasiveness. Online clinical analysis of the 6 upregulated kinases also revealed associations with poorer prognosis in the ER negative patient dataset.

The project in total has successfully revealed particular kinases worthy of further investigation as potential future targets for EGFR positive/negative TN breast cancer. Furthermore, it has demonstrated the novel TN cell lines are useful research tools capable of discriminating clinically relevant deregulated pathway elements in TN disease.

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Design and synthesis of novel CYP26A1 inhibitors for potential use in therapeutics

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All *trans* retinoic acid (at-RA) is the active form of vitamin A within the body. It is an endogenous compound with roles in regulation of cell apoptosis, differentiation and proliferation and thus alteration of its levels can effect signalling in these pathways.[1] Currently, tretinoin (retinoic acid) is used in the therapy of hyperkeratinisation disorders, neuroblastoma and acute promyelocytic leukaemia, although studies suggest it has potential for use in other areas of oncology as well as in neurodegenerative disorders.[1] However, tretinoin use is associated with complex and serious side-effects which can limit its use. Tretinoin also causes auto-induction of its metabolising enzyme, CYP26A1, which can lead to a failure to respond to therapy.[1] Several agents which inhibit CYP26A1, known as retinoic acid metabolism blocking agents (RAMBAs) have been synthesised and studied.[1] The two most successful, liarozole and talarozole, are currently in trials for use in dermatology.[1] SAR studies on RAMBAs have identified 3-imidazol-1-yl-2-methyl-3-[4-napthalen-2-ylamino)-phenyl]-propanoate as a promising lead compound, however it has problems with selectivity and solubility.[2] In this study we attempted to design and synthesise compounds based on the lead, but with a lower logP value with the aim of improving solubility.

Eight compounds were designed which all had a lower logP than the lead, due to the inclusion of polar substituents in the place of the naphthalene group. LogP was calculated using ChemDraw software. Using MOE software, both the 'R' and 'S' enantiomers were docked in a homology model[3] and the distance between the N-3 atom and the haem of the enzyme measured and interactions with the amino acids in the active site examined. Synthesis of the designed compounds was then attempted using previously documented methods.

Docking showed that in general there was little difference in the binding of the different enantiomers with the ligand interacting with similar amino acids and the distance between the N-3 atom and the haem being comparable. 3-Imidazol-1-yl-2,2-dimethyl-3-[4-(pyridin-3-ylamino)-phenyl]-propanoate however was much closer to the haem when in the R conformation than the S. Conversely, 3-(1*H*-benzimidazol-5-yl)-2,2-dimethyl-3-[1,2,4]triazol-1-yl-propanoate showed the closest binding in the S configuration, with the distance between the N-3 and the haem being the lowest observed at 1.98Å. Of the eight designed compounds, only two, 3-(4-(6-bromopyridin-3ylamino)phenyl)-2,2-dimethyl-3-(1*H*-1,2,4-triazol-1-yl)propanoate and 3-(4-(6-bromopyridin-3ylamino)phenyl)-3-(1*H*-imidazol-1-yl)-2,2-dimethylpropanoate were successfully synthesised. Both were identified by high resolution mass spectrometry, however only 3-(4-(6-bromopyridin-3ylamino)phenyl)-2,2-dimethyl-3-(1*H*-1,2,4-triazol-1-yl)propanoate was identified using ^{1}H-NMR and ^{13}C-NMR as the spectrum for the other compound was inconclusive. Both reactions gave good yields, were fairly quick and the final compound easily isolated. Additionally, it appeared from TLC that 3-hydroxy-2,2-dimethyl-3-[4-(pyridine-3-ylamino)-phenyl]-propanoate had been synthesised at a very low yield, however ^{1}H-NMR and ^{13}C-NMR concluded that a mixture had been synthesised and it was not possible to isolate the compound.

Eight new potential RAMBAs, with a logP lower than that of the lead compound, have been designed, synthesised and successfully docked in the homology model of CYP26A1. Synthetic aspects of this study were partially successful, with two novel compounds being designed and identified. The failure to react occurred mainly at the last stage of the reaction pathway, which is a Suzuki coupling. The failure is probably due to the presence of an electron donating group at the para position on the pyridinyl boronic acid causing the carbon to be unreactive. It is possible that with a higher molar equivalence of the boronic acid, or a higher temperature, it would be possible to synthesise and isolate 3-hydroxy-2,2-dimethyl-3-[4-(pyridine-3-yl-amino)-phenyl]propanoate. The two synthesised compounds have been sent to the Northern Cancer Research Institute, Newcastle University for *in vitro* studies.

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An exploration of the views of pharmacy undergraduate students on their approach to, and styles of learning

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Students take in and process information in different ways and preference to a particular learning strategy, illustrates the existence of a personal learning style which is distinct to that individual[1]. Learning can be improved by instruction that is matched in some way to an individual's determined learning style[2]. It is unrealistic however, to expect lecturers to accommodate the learning styles of each individual student in every class taught. The realistic approach would be to encourage students to adapt their learning habit based on their own distinctive learning styles. If styles of students can be understood staff could potentially take advantage of their strengths and correct their weaknesses[1]. The aim of this study was to explore the views of undergraduate pharmacy students on their approach to and styles of learning.

Due to the exploratory nature of this project, a qualitative method of data collection was used. A series of focus groups were conducted with Welsh School of Pharmacy undergraduate students. School ethics approval was granted by the Research Ethics Committee. Purposive sampling was used to recruit participants, with the researcher briefly attending appropriate teaching sessions to explain the project to students. Potential participants were asked to contact the researcher if they were interested in taking part in the study. It was ensured that written informed consent was obtained from all participants. A focus group schedule was designed as a data collection tool, concentrating the group's attention and interaction on the topic. The schedule combined the topics of two project researchers, reducing the burden on potential participants. Each focus group was audio taped with consent, then transcribed 'ad verbatim' before being analysed using the code and retrieve method, producing a thematic framework. The data generated from the focus groups was then used to inform the development of a questionnaire designed for use in a larger sample of pharmacy undergraduate students. It was not the intention of this current project to administer the questionnaire.

Four focus groups (one from each year of study) were conducted using a total of 19 participants. Participants identified their learning style using VARK show cards[3]. Students involved in this research described how they were capable of using different approaches to learning when necessary. Participants also realised the importance of reading around topics in order to aid their learning, however at times had difficulty in doing so. The project researcher wanted to investigate student opinion on the usefulness of teaching learning styles and approaches to learning at university level. Students were willing to suggest how staff could improve their learning by introducing a lecture or workshop based on learning styles, incorporating the use of learning style inventories. Some were opposed to the ideas raised, with the majority of students then concluding that being taught learning styles and approaches would be '*boring*', '*patronising*' and '*a waste of time*'. Factors affecting students' approach to learning were discussed by participants and these could be taken into account by lecturers in order to produce a more effective learning experience, potentially improving cognitive outcomes. Factors impacting on learning included workload, life outside university and handout design.

This small study was successful in exploring the views of pharmacy undergraduate students on the topics of learning styles and approach to learning. The researcher believes that the attitudes of current MPharm students regarding this topic need to be addressed in order to induce effective learning strategies. If we ensure that students become more aware of their own approaches and the implications of adopting them, it may be possible to improve the quality of learning outcomes[1]. This study however, only uses a small sample size and the findings cannot be said to be generalisable to the whole student popualtion. A much wider population can be assessed through the use of a quantitative data collection tool, allowing the collection of numerical data furthering the work of this project. The questionnaire has the potential to be distributed to all current undergraduate students studying at the Welsh School of Pharmacy and if considered successful could then be distributed among other schools of Pharmacy in the UK. Staff interviews should also be conducted as strategies to improve learning will need to be aimed both at students and staff[4].

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Studies towards the synthesis of carbonate co-drugs for the treatment of psoriasis

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Psoriasis is a chronic inflammatory skin condition, affecting 2% of the UK population. There is currently no cure and combination therapy is often the first-line treatment. One common chemical enhancement strategy is the co-drug approach, which can improve skin permeation and allows for the synergistic treatment of the disease[1]. A novel ester co-drug of dithranol and naproxen has recently been developed, illustrating several advantages over its individual components[2]. Having established the first in-class example, this concept was expanded further to investigate the synthesis of the carbonate group and its potential as an effective co-drug linker. A co-drug incorporating the JAK3 inhibitor, 4-[(6,7-dimethoxyquinazolin-4-yl)amino]phenol was the primary target as it was found to be an effective immunosuppressant in T-cell mediated disorders such as psoriasis[3]. The second drug was to be a model representative of dithranol, to simplify the chemistry[2].

Several methodologies were researched and tested in an attempt to synthesise the carbonate co-drug. The JAK3 inhibitor was treated with either 4-nitrophenyl chloroformate or 1,1'-carbonyldiimidazole, followed by equal equivalents of the dithranol drug model. These are reagents used elsewhere for the preparation of organic carbonates[4].

Two novel methodologies for synthesising asymmetrical carbonate co-drugs were established. These methods successfully produced a carbonate co-drug of the JAK3 inhibitor and the dithranol drug model. However, the yield generated was relatively low as only 20mg of the co-drug was synthesised. Furthermore, a novel carbonate dimer of the JAK3 inhibitor was developed which may demonstrate improved physicochemical properties and be better suited to topical delivery[1].

In summary, our findings indicate that asymmetrical carbonates are synthetically viable; however their validity as an effective co-drug linker has yet to be determined. The results emphasise the need for further research into the stability of the carbonate bond. Furthermore, the number of potential drugs that can be used to form carbonate co-drugs is limited, as few compounds contain the required hydroxyl group. This study successfully determined two methodologies for synthesising asymmetrical carbonate co-drugs. These methods were non-hazardous and easy to perform. However the yield generated was insufficient and their reproducibility was uncertain. This could reflect the small quantities of starting materials used or experimental error may have been a causative factor. What is more, the drug models did not truly represent the active drug and therefore it is uncertain whether these methods would succeed if the models were substituted for actual therapeutic agents like dithranol. To conclude, there are still some limitations associated with these methods and further improvement is still required before they can be used to generate novel carbonate co-drugs for the treatment of psoriasis.

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Antibiotic prescribing practice: an evaluation of junior doctor and medical students' perceptions of their educational needs in comparison with their measured actual needs

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Increasing antimicrobial resistance as a result of unnecessary or inappropriate antibiotic use is a globally recognised problem.[1] Although historically an area poorly understood by medical professionals, the prescribing of antibiotics is often a duty fulfilled by junior doctors.[2] It is essential therefore, that adequate antibiotic prescribing training is given at both undergraduate and postgraduate level. Current education seems deficient in preparing junior doctors for their role as prescribers in general.[3] However, little work has looked specifically at educational needs with regard to antibiotic prescribing. The aim of this study therefore was to identify what the educational needs of junior doctors and medical students are with regard to antibiotic prescribing and compare their needs at these different career stages. The study was conducted in a District General Hospital in North Wales. It was intended that study findings would inform future developments in the hospital's antimicrobial teaching programme.

Ethical approval from the WSP REC was obtained. Mixed methods were utilised to generate quantitative and qualitative data. Semi-structured interviews with key informants were conducted to gather background information. Researchers designed a questionnaire for medical students and junior doctors to document what education was currently received, educational formats preferred and perceptions of respondents' need for further antibiotic education. This was combined with a pre-prepared confidence based prescribing assessment to measure prescribing ability and confidence. The measurement tool was distributed to 5th year medical undergraduates and FY1 and FY2 doctors attending a scheduled education session who agreed to take part. Volunteers were supervised to ensure test conditions. Data was coded and entered into SPSS and analysed using descriptive statistics.

Five interviews were conducted and information obtained used to inform questionnaire design. A response rate of 98% was obtained (n=45) for combined questionnaires/assessments. Lectures were most widely received: 96% received undergraduate teaching in this format. Educational formats preferred were on-ward teaching (86% agree/strongly agree), informal training from seniors (89%), and undergraduate teaching (83%). Respondents expressed agreement (92%) that there should be an antibiotic teaching session given in each hospital as well as an increase in antibiotic teaching in the medical degree (87%). Prescribing assessment scores ranged from -26 to 21 (possible range -66 to 33) and confidence scores ranged from 13 to 33 (Possible range 11 to 33). The means were 7 and 26 respectively. Junior doctors were significantly more likely to say they were confident to prescribe in a range of situations when compared to medical students' responses, while medical students were significantly less likely to agree that their previous teaching had adequately prepared them for such roles (22% vs. 46%). However there were no statistically significant differences between the groups regarding prescribing ability or desire for more teaching.

Respondents' antibiotic educational needs are not currently being met according to their own perceptions and by measurement of prescribing ability; regardless of career stage. The majority expressed their desire for more education and for it to be of a practical nature rather than lecture format which is dominant currently; a finding echoed in the literature.[4] Further work is needed to establish what education will meet educational needs. Discrepancies between prescribing ability and prescribing confidence were seen, with junior doctors unjustified in their confidence on occasions and medical students were able but unconfident. Findings have direct relevance to the hospital in question but also have wider implications in terms of highlighting deficiencies in undergraduate teaching although this study cannot claim to be generalisable due to the small sample size and conductance in only one hospital.

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The following abstracts have been withheld as they have been or will be published elsewhere, and/or due to intellectual property or confidentiality issues:

Do growth conditions of *Pseudomonas aeruginosa* affect its susceptibility to biocides and antibiotics?

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Structure-based design and synthesis of novel inhibitors of hepatitis C virus NS3 helicase

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Investigation to see if the Growth Conditions of *Staphylococcus aureus* affect its susceptibility to Biocides and Antimicrobials

Purvi Hiteshkumar Dodhia and J-Y Maillard
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Application of the ProTide approach to analogues of 6-methoxy-2'-C-methylguanosine: synthesis, characterisation and bioassay versus Hepatitis C Virus

Helen Jarvis, K Madela, C Bourdin and C McGuigan
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Targeting Focal Adhesion Kinase (FAK) in multiple breast cancer subtypes

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Effect of growth conditions on susceptibility of *Salmonella* to selected biocides and antibiotics

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Probing the chelating properties of ionic zinc and iron with extract of *Punica Granatum* rind

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Skin Penetration Enhancement of Caffeine by Ethanol and Propylene Glycol: Probing the Co-Permeation Effect

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Investigation of FAK as a therapeutic target in HER2+ breast cancer

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FAK inhibition as a therapeutic strategy in ER+, endocrine-sensitive breast cancer

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Intracellular delivery of microparticles using cell-penetrating peptides

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Development and application of a measurement tool for the training of commercial clinical researchers – the SPITFIRE

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Clinical trials are essential for the development of medicines and treatments for patients. Effective training is fundamental for ensuring the safety of trial subjects, obtaining accurate and credible data and reducing errors in clinical research. The aims of this study was to comprehensively evaluate a standardised approach to protocol training for clinical research staff working on multiple, and often complex clinical trials.

The attitudes and opinions towards this training approach will be explored in three areas: i) General feedback from all staff ii) Study Coordinator feedback iii) Feedback from staff who were closely involved in a complex study using this approach. In the absence of a measurement tool to pursue the aims of this study, an electronic questionnaire (SPITFIRE) was developed to obtain feedback on a variety of characteristics of this approach, specifically, the perceived improvement in the following five key areas: Safety; Quality; Knowledge; Skills; Confidence.

The findings demonstrate very strong positive opinions towards the standardised approach to protocol training, with over two thirds of study participants (n=42) rating Strongly Agree in all of the five key areas. The most compelling feedback was with regard to the preparation and delivery of the training by the Study Coordinators in which 75% strongly agreed this approach helped them gain a greater understanding of the protocol and disease area/drug being studied. The most important aspects of dry run training on the complex trial by were performing the task 'hands on' (94%) and being able to educate and train subjects (88%). Ongoing support, training and communication were the highest rated features in the lengthy and complex trial for maintaining safety, quality and consistently high standards of clinical conduct throughout.

The implementation of this questionnaire has successfully obtained valuable feedback which clearly highlights the importance of a standardised approach to protocol training for helping to improve the safety and quality of clinical trials and the knowledge, skills and confidence of research staff.

A cross sectional study exploring the views of the associate course directors and students on the reflective assignment for module PHT727 of the MSc in Clinical Pharmacy

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The importance of reflective practice within healthcare professionals is well recognised and it is considered an essential characteristic of professional competence. Reflection has therefore become a key component of the healthcare professions' educational programmes and one of the core aims of the MSc in Clinical Pharmacy is to help students develop as reflective practitioners. Many of the course's assessments have reflective elements; one such assessment is the module PHT727 Reflective Assignment which requires students to reflect on an event which has challenged them through the course of their practice and identify an action plan detailing how they plan to improve their practice. This assignment is assessed by their Associate Course Director (ACD) who acts as the students' academic supervisor. As this was a new assignment it was decided to undertake an exploratory study to ascertain how students approach this piece of work and also establish the perspectives of the ACDs and students towards the assignment and identify whether they felt it had impacted on their professional practice.

Due to the exploratory nature of the research, focus groups were selected to yield qualitative data and capture a range of themes. For the ACDs, one focus group (n=4) and one semi structured interview was undertaken. Student focus groups were held in each of the five MSc Centres. All focus groups and the semi-structured interview were transcribed verbatim and transcripts were used to identify themes and allow construction of a thematic framework for analysis. The learning styles of all ACDs and students were determined and all students' assignments were categorised according to themes; these activities were conducted in order to establish whether there was an association between learning style, assignment topic and perceptions.

ACDs understood the purpose of the assignment but identified a number of challenges with its assessment. Students indicated that critical reflection is a new concept to them and the content of this assignment is different to the academic work with which they are familiar, therefore students find the assignment challenging. Some students gain from the assignment and can identify specific ways their practice has changed as a result of its completion. Other students have more negative views. Many students do not understand the purpose of the assignment and demonstrated poor understanding of the purpose of critical reflection. There seem to be a number of factors which influence student opinion towards reflection such as gender, learning style, workplace culture, experience and whether their motivators for learning are internal or external. The misconceptions about critical reflection need to be addressed by the MSc Course Team as the potential benefits of the assignment will only be realised if students understand its intended outcomes. Student engagement is essential for these outcomes to be realised, therefore ACDs must adopt an individualised approach to guiding students with the task, taking into account their gender, learning style and motivators in order to ensure students select topics which they believe will be beneficial to their practice in order to encourage them to maximise the learning opportunity.

The effect of haemoglobin level at darbepoetin alfa initiation on transfusion reduction and the cost savings impact of this reduction on chemotherapy induced anaemia treatment

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The aim of this study was to investigate the impact of hemoglobin (Hb) level at the start of darbepoetin alfa (DA) treatment on transfusion reduction and to identify the cost- saving impact of this reduction on chemotherapy-induced anaemia (CIA) treatment costs. Patients with cancer who receive chemotherapy may develop CIA. According to current guidelines, treatment with erythropoiesis-stimulating agents (ESA) should be initiated as the Hb level approaches either 10gIdL [1] or between 9-1 1g/dL in patients with anaemia-related symptoms[2]. A systematic review was performed of the clinical literature in PubMed and selected conference abstract databases between 2006 and 2010. Another systematic review of economic studies on cost-of-transfusion was performed using PubMed for the period 2000-20 10. Reference lists of retrieved studies from this review were also scanned. Eight studies were retrieved from the clinical review. Despite the differences in baseline patient characteristics, the length of the studies, and analytical techniques, the need for transfusions decreased across all studies when DA initiation occurred at higher Hb levels. Twenty-one studies met the inclusion criteria for the economic review. Cost of transfusion studies were grouped according to regions to identify range of cost of transfusing one unit red blood cell (RBC) in the US, Europe, UK, Canada, and Australia. Cost of transfusion was found to vary from country to country and depended on which cost driving items were included. To identify the cost savings in CIA treatment, the difference in transfusion rates was multiplied by the region-weighted midpoint of the transfusion cost. These findings suggest the resulting cost savings depends on the number of RBC units transfused and the cost of transfusion. Initiation of DA according to the guidelines is important in terms of reducing the number of transfusions as well as the cost saving impact on CIA treatment.

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An Assessment of the Reasons Why Patients are Admitted to Hospital with a Simple Exacerbation of Chronic Obstructive Pulmonary Disease (COPD)

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Chronic Obstructive Pulmonary Disease (COPD) is a major burden on the National Health Service and is the second largest cause of hospital admissions in the UK. With the number of COPD patients in South Wales among the highest in the UK this study was undertaken to ascertain if those patients coming into hospital with an exacerbation of their COPD could have been treated at home (with a self management plan) and whether or not the treatments they had received prior to admission were sub-optimal, therefore resulting in a hospital admission.

Data was collected on admission rates and treatments provided, both prior to and during their admission. Appropriate patients were then interviewed to establish their thoughts and opinions regarding self management plans and exacerbation treatment at home with rescue medications.

The study showed that around 50% of the patients admitted to the Royal Gwent Hospital with an exacerbation of COPD were classified with a simple exacerbation that could have been managed in primary care. Only 2% of these patients had used a rescue pack prior to their admission.

A major limitation to the study was a lack of interviews. There were insufficient patients interviewed in order to fully ascertain all themes relating to barriers and facilitators for self management plan use. It is advised that subsequent interviews be undertaken post study in order to complete the data.

There were many issues identified as a result of this study that require further work, including research in to additional literature for patients and also educational needs of both patients and general practitioners.

The Aneurin Bevan Health Board is currently working on a COPD pathway that aims to improve the links between primary and secondary care. The results of this study can be feed directly into this pathway development, allowing for real changes to the management of COPD in primary care.

Measuring preferences for temporary health states

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The creation of NICE'S Diagnostics Assessment Programme (DAP) has raised the awareness of temporary health states amongst practitioners of economic evaluations. These states are short in duration (less than 1 year) and are transient in nature, such that, they are followed by another health state other than death. In contrast, the elicitation of utilities has largely focussed, for theoretical reasons, on chronic health states. These states last for a long duration (usually years) and are permanent from age of onset, such that, death follows the chronic health state. The EQ-5D is recommended by the DAP for the measurement of preferences for health states. Whilst consistent with methodology across other NICE programmes, little is known of the ability of the EQ- 5D to estimate preferences for temporary health states. This study investigated how accurately the long duration chronic health state description used in the EQ-5D instrument's TTO value set was likely to estimate preferences for temporary health states. The individual components of temporary health states (short duration and transient nature) were also assessed independently of one another. Feasibility of the new lead time TTO method for measuring preferences for temporary health states was also explored. 28 participants valued five scenarios for two EQ-5D states using the lead time TTO. Scenarios were constructed by combining multiple durations with either permanent or transient health state descriptions. It is concluded that preferences for temporary health states are unlikely to be accurately estimated by the EQ-5D. The short duration and transient nature of temporary health states contributed equally to this inaccuracy. Ultimately, imprecision of the preferences for temporary health states has the ability to impact the adoption of technologies within the healthcare system. The lead time TTO method was found to be suitable for use with temporary health states. Theoretical and empirical conclusions can be drawn.

Evaluating the future of hepatitis C treatment: a cost-effectiveness analysis of response guided therapy and telaprevir using a dynamic modelling approach

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Hepatitis C poses a severe burden on healthcare systems around the world but little is currently understood about the exact incidence and prevalence of the disease. CHC presents an even more substantial problem in the UK, which is the only developed country in which liver disease continues to rise (The Hepatitis C Trust 2009). Through the development of new, more potent treatments and the creation of patient specific treatment regimes it may be possible to increase the success of treatment regimes while at the same time ensuring minimal impact to the budget. This thesis used the dynamic MONARCH model to investigate the cost-effectiveness of response guided therapy and the incorporation of a newly emerging antiviral drug, Telaprevir, and proposed an optimal treatment regime that would optimise treatment outcomes while minimising the impact on healthcare budgets. The model was used to evaluate the cost-effectiveness of response guided therapy and the inclusion of Telaprevir using clinical data from the recent CHARIOT (Cheng et al. 2010), ADVANCE (Jacobson et al. 2010) and PROVE 3 (McHutchison et al. 2010) clinical trials. The results demonstrate response guided therapy to provide cost savings of up to £1,534 when compared to the current standard of care and suggest that commencing treatment in the earlier stages of the disease is more cost-effective. The addition of Telaprevir in treatment-naive patients leads to a maximum cost-effective price of £78,617 per patient per course of Telaprevir, while in previously treated patients the maximum cost-effective price is revealed to be £38,796 in previous non-responders and £99,933 in patients who relapsed following previous treatment. The results of this research demonstrate the potential for further improvements to the way in which hepatitis C treatment, which remains largely unchanged in recent years, may be approached on an individual patient-specific basis; it is an exciting starting point from which to continue economic investigations into the numerous newly emerging treatments for hepatitis C currently undergoing clinical trial.

Evaluation of the National Institute for Health Research Clinical Research Network's capacity to improve the delivery of commercial research in the NHS

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In recent years the government has sought to address the issues associated with conducting commercial research in the UK. Problems such as long approval times, unsuccessful recruitment and expense have been detrimental to the industry conducing research in the UK. Best Research for Best Health targeted NHS collaborations with the industry and has attempted to improve the ability and capacity of the NHS to meet the needs of commercial sponsors. The implementation of this strategy was rolled out by the National Institute of Health Research (NIHR) who were charged with addressing the requirements of the industry, and via the newly established Clinical Research Networks (CRN), set about enhancing the overall delivery of commercial trials in the NHS. The aim of this study was therefore to evaluate if the NIHR CRNs have been successful in improving the delivery of commercial research in the UK.

To reveal if NIHR adopted portfolio studies were delivered more successfully than non adopted studies, the five Acute Trusts in the Trent CLRN area were approached to provide data on non adopted commercial studies that were approved between November 2008 and November 2010. Study set up metrics were complied in a database for both adopted studies and non adopted studies within the specified time frame, and the time taken to gain NHS permissions at each Trust were compared. Key performance indicators were measured for adopted studies across all of the CRNs in correlation with the NW Exemplar Programme. The following assessments were made: relationship to the NW Exemplar Programme, and contrast in commercial research activity/performance between Networks where full coverage was not present in the Trent area. To evaluate how the CRNs support the industry via methods which are not captured in key performance indicators, data available locally was analysed to demonstrate the provision of infrastructure, and to show how commercial sponsors engage and use the resource available.

The NHS permissions for adopted studies are not achieved faster than approval times for non adopted studies. Inconsistency in approval processes and the start point of local governance checks affects the measurements; however the NIHR CRNs do have the potential to make improvements as demonstrated by the results of the NW Exemplar Programme. Commercial trial activity tends to be greater where there is coverage and support of a CRNs, and the successful placement of clinical studies could be maximised by the industry if they used to the full potential local intelligence and knowledge of research capacity within the CLRNs.

These findings show that while the study set up metrics does not reveal adopted studies to be at an advantage over non adopted studies, there is great potential to improve these times across all commercial trials if the systems and tools introduced by the NIHR to make efficiencies were fully embraced and implemented consistently across all NHS organisations. The underpinning infrastructure provided by the NIHR CRNs is key to delivering commercial research in the UK, and the industry must fully engage with the CRNs to maximise on the potential to deliver studies in the UK successfully via the NHS. Collaborative approaches are essential; as disjointed and isolated improvements by individual bodies will do little to advance the UK nationally towards the aspired status of achieving excellence.

Investigation into incidence, types and causes of prescribing errors on handwritten and electronic discharge prescriptions

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Traditional handwritten discharge communication (to take home, TTH) has been shown to be unsatisfactory and cause medication errors; and it is thought that computerised prescriptions reduce such risks. The study hospitals implemented an electronic discharge prescription (electronic transfer of care, eTOC) to improve communication to GPs and improve patient safety by reducing errors. The study aimed to compare the error rate between TTH and eTOC, and identify causes of errors on electronic prescriptions and potential solutions. The methods used included retrospective data collection from prescriptions and interviews with prescribers.

There was no difference in overall intervention (error and clarification) rate, but there were significantly more errors on eTOCs than TTHs when looking at all error types. This is due to the increased level of detail required on eTOCs. This was further demonstrated when only like-for-like error types were compared, where there were significantly more errors and interventions on TTHs than eTOCs. The eTOC system has reduced the rate of some types of error due to careful design (e.g. duration) but has not reduced all types (e.g. omission). The errors on eTOCs tended to be classified less severe than those from TTHs. This could mean that if an error was to slip through the pharmacist safety net the chance of a patient being harmed is lower for eTOCs than TTHs. Analysis of interviews with prescribers highlighted many causes for prescribing errors, such as time pressure, poor documentation and distractions on the ward. Several suggestions to reduce error rate have been proposed. The eTOC system was introduced to improve patient safety by increasing the amount and timeliness of communication to the GP and the patient about medicines. Whilst this aim for eTOC has been achieved and some prescribing errors at discharge have reduced and seem to be less severe, more work is required to further reduce the prescribing error rate.

The impact of the European Union Clinical Trial Directive on multinational non-commercial clinical trials – a UK perspective

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The European Union Clinical Trials Directive (2001/20/EC) was aimed at harmonising the many divergent national regulations that governed the conduct of clinical trials however, after its entry into force in May 2004, it was apparent that differences in interpretation had led to further disparity in national requirements. The aim of this research project was to identify key differences in implementation of the Directive and to assess its impact on the conduct of multinational trials carried out by non-commercial sponsors; the group believed to have been the most affected by the Directive.

Following an extensive literature review, a web-based questionnaire was distributed to 48 UK Clinical Research Collaboration Registered Clinical Trails Units to establish their experience of conducting multinational clinical trials and their opinions on the success of the Clinical Trials Directive. 20 questionnaires were returned and from this it was derived that 16% of all the trials being conducted by respondents were being carried out in the EU, across 14 of the 27 Member States. Respondents reported that the time, cost and number of employees required to conduct a clinical trial had increased since the introduction of the Directive and that overall the number of multinational non-commercial clinical trials conducted had fallen. 67% of respondents gave a verdict of an overall negative impact of the Directive on multinational, non-commercial trials.

This project has identified some of the key areas where the aims of the Clinical Trial Directive have been unsuccessful. The perceived negative impact of the Directive on the non-commercial research community is also supported by the results of this research. In order to facilitate the conduct of non-commercial research, a key factor for continuing medical advancement, it will be essential for the EU to address the barriers and divergence of national legislation associated with the Clinical Trials Directive.

An evaluation of the orthopaedic surgical thromboprophylaxis guidelines at Airedale NHS Trust

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Venous thromboembolism (VTE) is a major killer of hospitalised patients with many risk factors contributing to developing a VTE with surgery adding to the risks. NICE produced thromboprophylaxis guidelines to advise hospital Trusts on appropriate treatment and eliminate the risk in patients undergoing surgery. Within Airedale guidelines were developed however there has been no review as to the actual benefits to patients and if more are now experiencing adverse effects and also how patients feel about using an injection for a considerable length of time on discharge.

The objectives were to assess if the orthopaedic surgeons at Airedale are following the current thromboprophylaxis guidelines; to identify the number of patients who experience side-effects and also if there is a reduction in the occurrence of DVT/PE, to discover if patients would prefer an oral agent; to determine the number of patients who self injected their Fragmin post surgery.

Forty patients' notes and treatment charts were reviewed, twenty before guidelines (June 2007) and twenty after (June 2009). Details were obtained on the type of thromboprophylaxis used, any adverse effects documented and the occurrence of a DVT or PE. One hundred and fifty questionnaires were sent to patients to gauge their experiences of using Fragmin injections.

Prior to the guidelines being introduced only six patients (30%) had the correct dose of Fragmin prescribed if comparing prescribing practice to the current guidelines. Once guidelines had been introduced the prescribing of Fragmin was correct in fifteen (75%) patients. Only one patient experienced a DVT (0.6%) and one suffered from bleeding. The questionnaire had a response rate of 94%. 88% of patients reported they self injected or had help from family members. 91% managed to complete the full course of treatment. 56% of patients stated they would prefer an oral medication instead of an injectable.

Auditing systems shows how any changes have improved practice and is the best way to prove that guidelines are working. When reviewing the types of thromboprophylaxis used within Airedale there was 100% compliance with the use of graduate elastic compression stockings before and after the guidelines were implemented. Implementation of the guidelines improved Fragmin prescribing from 30% of patients receiving correct dose of Fragmin pre-guidelines to 75% of patients post guidelines. It is important to take into account patient beliefs and understanding to improve compliance with treatment.

Systematic review and meta-analysis of adverse events of special interest in ankylosing spondylitis patients treated with tumor necrosis factor (TNF) antagonists

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TNF antagonists provide an important treatment option for chronic inflammatory disorders such as ankylosing spondylitis (AS). However adverse events of special interest (AESI) such as serious infections, neurologic disorders and malignancies have been demonstrated to be a risk in rheumatoid arthritis (RA) treated with TNF antagonists. Few studies have studied incidence or prevalence of AESI in AS patients treated with TNF antagoists.

The objective of this study was to perform a systematic review and meta-analyses to assess the risk of AESI (serious and opportunistic infections, malignancies, neurologic disorders such as demyelinating disease and progressive multifocal leukoencephalopathy) in AS patients treated with TNF antagonists compared to control conditions (e.g. NSAIDs nbDMARDs).

A study protocol and data eligibility forms were prospectively created and a systematic literature search using DataStar, PubMed, The Cochrane Library and Web of Science was performed between November 2010 and January 2011. All randomised controlled trials (RCTs), open label extensions (OLEs) and controlled observational studies monitoring AESI in patients diagnosed by the modified New York criteria and treated with TNF antagonists were assessed. The second person (VI) independently extracted and assessed the methodological quality of 20% of the RCT data.

Twelve RCTs and ten OLEs were retrieved (2821 patients). The methodological quality of the RCTs were measured using the Downs and Black Checklist and Jadad Scale. Meta-analysis of risk differences (RD) were performed on the RCT data of SAEs [RD=0.01, 95%, CI: -0.01,0.02] and serious infections [RD=0.01, 95% CI: -0.00,0.02] but not on other AESI because the overall number of patients retrieved was too small to make robust conclusions.

There was no evidence that the risk of serious adverse events (SAEs) and serious infections in AS patients treated with TNF antagonists was different from placebo, however, the overall number of patients was low and the calculations may have lacked power, therefore continued monitoring is necessary.

Antibiotic intravenous to oral switch guidelines: barriers to adherence and solutions to poor adherence

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Antimicrobial stewardship is becoming increasingly important as the incidence of resistance and healthcare associated infections rise due to the overuse of antibiotics. The timely switch of intravenous to oral antibiotics has been identified as one target for improving antibiotic prescribing. The early switch of intravenous antibiotics has been shown to reduce medication costs, improve patient experience and facilitate earlier discharge from hospital. Hospitals have employed antibiotic intravenous to oral switch guidelines in line with department of health recommendations to empower doctors to review intravenous therapy appropriately. Local audit has highlighted repeated poor adherence to the guideline. This study aims to explore the barriers to guideline adherence and solutions to poor adherence as perceived by a multi-disciplinary team. A Delphi study has been employed to identify these themes and then obtain consensus on the importance of each. The expert panel of 29 consulted for the Delphi study consisted of doctors, nurses and pharmacists with experience of the local intravenous to oral switch guideline. The Delphi study was supported by semi-structured interviews as it should not be used as a standalone method. Consensus was achieved for 26 out of 35 statements with the most important barrier being that of inappropriate antibiotic review at the weekend and the most important solution to raise guideline awareness. No nurses responded to the Delphi study bringing into doubt their perceived role in the switch process. The interviews largely supported the findings of the Delphi study but offered some more specific solutions. The combined use of quantitative and qualitative methods has identified several barriers to explore further and offered many solutions to improve practice. A multi-disciplinary approach must be utilised to fully understand the barriers to adherence and clinical guidelines must be well publicised and well written to prevent a feeling of guideline-saturation in the healthcare populous.

Evaluation of patient compliance with renal replacement therapy and its impact on patient-reported outcomes

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The ultimate goal of medical care is to improve patients' functional behaviour, both physical and psychological. In recent years, the value of quality of life information in clinical trials has been widely demonstrated. However, whilst it is generally agreed that it is an important area of research, it still remains a difficult concept to translate into practice. The aims of the study were to determine the reliability, validity and interpretability of the Renal Quality of Life Profile (RQLP). In addition, to determine if RQLP scores can predict compliance and non-compliance with dietary/fluid restrictions and immunosuppressant drugs.

Patients were recruited into the study from the renal outpatients clinics of the University Hospital of Wales (UHW) and Royal Gwent Hospital. Patients' HRQoL, compliance and symptoms were measured using the RQLP, the Morisky Compliance Scale (MCS) and Leicester Uraemic Symptom Scale (LUSS), respectively. IN addition, several biomedical markers were collected for triangulation of patients' compliance with treatment. An anchor based technique was used to develop a banding system for the RQLP scores using a Global Question (GQ). The GQ scores (i.e. no effect to large effect) were mapped against the RQLP scores and intraclass correlation coefficient (ICC) was used to test their agreement.

The RQLP demonstrated good test-retest reliability ($r_s>0.7$, n=29, males=19, females=10; mean age 70.5, median 77, range 24-90) and construct validity (n=291, $p\leq0.01$) of the overall and domain scores. The strong Cronbach's α value of 0.9 (n=308, males=194, females=114; mean age 59.6, median 61, range 21-90) indicated that the items were internally consistent and measured the same construct. A total of 260 patients completed the RQLP and GQ (males=165, females=95; mean age 58.4, median 59, range 21-90). The mean, median and mode of the GQ scores for each RQLP score were sued to devise several sets of bands of RQLP scores (ICC=0.80) to aid its interpretability in practice. Applicability and practicality of the RQLP banding system was examined in 46 outpatient consultations where 74% of the clinicians found the banding system helpful in interpreting the RQLP scores and predominantly used for patients with poor QoL.

In this study (n=262, males=168, females=94, mean age 58, median 59, range 21-90) non-compliance was reported to be between 4 to 17% measured by MCS and 3 to 39% using the CKD biomarkers. Non-compliance was associated with poor quality of life and was higher among those receiving dialysis than transplant patients. Symptom severity was a predicator of poor quality of life and non-compliance.

An evaluation of the regulatory review processes, the quality of decision-making and strategic planning in the Gulf Cooperation Council (GCC) States

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Problems related to the safety and quality of medicines exists globally, in developing and developed countries alike. The responsibility of all regulatory authorities to safeguard the public health is common; however the structures, strategies, practices and processes vary considerably around the world.

The aim of this study was to examine and document the experience of the seven GCC regulatory authorities and identify their strengths and weaknesses and the reasons for them. The seven Gulf States are: Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, United Arab Emirates (UAE) and Yemen. It describes and evaluates the regulatory environment and the strategic planning processes, their impacts on patients' access to medicines and the degree of harmonization between the seven authorities. In addition, it was hoped that this study would make recommendations for improved harmonization strategies for the benefit of efficient regulatory review practices in the region.

Differences in the review processes in the milestones and target approval times have been identified. While an efficient review system is important to enable the timely access of new medicines to patients, quality is essential. The quality measures used in each GCC authorities to assure this were evaluated and revealed further disparities across the Gulf region. The Kuwaiti review procedure and decision-making process were assessed in detail to have a deep insight into a model review system in the GCC region. Differences were revealed in the patients' access time for new active substances (NASs) and existing active substances (EASs) in the government and private sectors. These differences were related to the quality control (QC) process which is carried out on the EASs. NASs are not analysed in the GCC QC laboratories as their registration in reference authority is sufficient to ensure their quality. Products in the private sector generally take longer to be made available to patients as they go through a pricing step which government health supply (GHS) medicines do not encounter.

In general, all pharmaceutical products must be approved in competent authorities to be registered in Kuwait as well as the other GCC States.

Furthermore, GCC authorities made considerable efforts over the last decade to harmonize their regulatory requirements. However, these efforts have been hampered by the differences in their strategic planning processes. The nature of the individual authorities makes the design of the harmonization strategy rather difficult before a full evaluation of each of the seven regulatory systems is carried out. Therefore, this study focuses on evaluating the strategic planning process in each GCC State by comparing their visions, missions, values, goals, objectives, priorities and action plans. The resulting similarities are enhanced and the differences are minimized by placing the research findings into context using the balanced-scorecard model.

This research project has highlighted a number of variables in particular areas of the GCC regulatory field that could be standardized to create a more harmonized GCC regulatory systems, thereby minimizing delays to patients' access to new medicines.

It is hoped that this research project increases harmonization and produces striking results in terms of reducing approval timelines for the registration of new medicines through the GCC central registration system (GCC-DR) by standardizing and optimizing the quality of the review practices in each member states. These improvements will further encourage companies to use the GCC centralized system, which is a step closer to an Arab and then a global central registration system.

Evaluation of healthcare professionals' attitudes towards non-compliance with drug treatments and impact of their behaviour on patient-reported outcomes.

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The Medication Non-Compliance profile (MNCP) is a self-administered instrument consisting of ninety items grouped into seven categories. A total of 3563 (10% of the sampling frame-British Medical Association) general practitioners from England, 503 (30% of the sampling frame – GP register in Wales), 1011 nurses, 411 from Wales (50% of sampling frame - general practice in Wales; 100 %, 600 Dermatology nurses – Nurse Specialist data base-UK), 703 pharmacists (100% sampling frame-Wales) and 1800 geriatricians (100% sampling frame-UK) randomly selected were recruited into the study.

The results showed that poor communication with patients as a healthcare professional behaviour was reported as one of the most important reasons for patients non-compliance with medications and healthcare professionals' poor communication skills was one of the main constraints that hindered communication with patients. In addition the findings of this study revealed that there was some weakness in communication between healthcare professionals' and patients in two dimensions of concordance model negotiating with patients and supporting them in medicine taking. A communication skills training programme for healthcare professionals aimed to improve healthcare professionals communication skills in general and in concordance approach in particular; and evaluate its impact on patients' reported outcomes such as patients' compliance with medications, blood pressure level, and patients' health-related quality of life (HRQoL). The communication skills training programme for healthcare professionals resulted in significant improvements in both hypertensive patients' compliance with medications and their blood pressure level. This study proved that good communication with patients had positively influenced patients' reported HRQoL.

Novel pDNA particles for pulmonary administration

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Pressurised metered dose inhalers (pMDIs) offer a potentially efficacious means for pDNA delivery. The aim of this thesis was to investigate the potential of a novel low-energy nanotechnology process, to prepare surfactant-coated pDNA particles for pulmonary gene delivery via pMDI technology.

Optimisation of water-in-oil microemulsions containing pEGFP-N1 reporter plasmid was investigated using carbohydrate lyoprotectants in aqueous solution, lectithin:propan-2-ol (surfactant) and iso-octane (organic phase). Resultant microemulsions were snap-frozen in liquid nitrogen and lyophilised. pDNA integrity was retained after lyophilisation and reduction of excess surfactant by organic solvent. pDNA particles were incorporated into pMDI formulations containing hydrofluoroalkane 134a (HFA134a) propellant and ethanol as co-solvent. A549 human lung epithelial cells were exposed to pMDI aerosolised pDNA particles with dioleoyl-trimethylammonium propane (DOTAP) in the culture medium. Cellular gene expression studies demonstrated that pDNA biological functionality was maintained whilst an in vitro toxicity (MTT) assay showed no significant loss of cell viability following pMDI aerosolised pDNA treatment. Subsequent investigations incorporating DOTAP transfection agent into pDNA pMDI formulations demonstrated a proof-of-concept that aerosolised pMDI DOTAP-pDNA formulations can confer significant cellular gene expression with the potential for pulmonary gene delivery.

Methods have been successfully developed and evaluated to aerosolise surfactant-coated pDNA particles and maintain pDNA viability. Furthermore, investigations have demonstrated that the pDNA particles and a transfection agent (DOTAP) can be incorporated into a generic HFA134a formulation and successfully aerosolised using a standard valve and actuator. Further studies demonstrated the performance of these formulations after four week accelerated stability conditions.

Pharmacy education and training in the hospital service in Wales: Identifying demand and developing capacity

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This exploratory study investigated NHS hospital pharmacy training workload and capacity in Wales using an unfolding research approach. Preliminary interviews with key stakeholders were used to inform the development of the main study. The study then aimed to estimate workload and capacity for work-based pharmacy training, explore reasons for variations in training workload between sites and develop recommendations for practice that would optimise NHS hospital preregistration pharmacist training capacity in Wales.

A multi-method study design using interviews, questionnaires and a multiple-site case study was employed. All NHS hospital pharmacy training sites in Wales (n=17) were included in the study and a 100% response rate was achieved. Estimates of training workload for diploma pharmacists, preregistration training pharmacists and student pharmacy technicians were obtained. The workload involved in preregistration pharmacist training was 6.5 hours per week per trainee (range 3.0 – 14.9), which was higher than for any other staff group and resulted in this area being identified as the priority for further study.

Subsequent research into training practices identified that some preregistration trainee pharmacists were not given enough responsibility and did not always make an appropriate contribution to service delivery; the content and level of some training was not appropriate for those approaching registration and there was a lack of common understanding amongst some tutors and trainers about the purpose of some elements of the training programme.

Strategies and recommendations to address these issues were developed and agreed with lead tutors. Implementation of these recommendations should optimise use of available training capacity which could lessen the impact of an increase in training demand on NHS services. A significant amount of time and money is dedicated to work-based training of NHS professionals; strategies like those described here, that aim to make effective use of existing capacity, are essential in a resource-constrained NHS.

The action of atypical antipsychotics on body weight and associated metabolic factors

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Despite having revolutionized the treatment of psychiatric illnesses, atypical antipsychotic (APSs) agents raise an increasing medical concern regarding the association between APS therapy and prominent body weight gain and metabolic abnormalities resulting from the treatment. As a consequence, the use of APSs medication has been linked to a substantial increase in the development of obesity, type 2 diabetes and cardiovascular diseases (CVDs) in patients under APS therapy.

In this study we aimed to develop a mouse model of APSs-induced body weight gain and adiposity. Moreover, the chosen agents clozapine and olanzapine were investigated in a fibroblastic-like cell line model (7-F2) and in primary bone marrow cells, in order to test a possible direct contribution of these agents in causing adipogenesis and alteration of lipid metabolism in peripheral tissues.

The inability to reproduce a reliable and consistent mouse model that mimics the clinical situation suggest caution in the interpretation of the reproducibility of previous models and, in general, question the predictive validity of them.

Furthermore, although morphological study on 7-F2 cells shows that clozapine and olanzapine do not enhance the differentiation of fibroblastic cells into adipocytes, mRNA over-expression of genes involved in adipocyte formation and metabolism suggest new formation of fat cells in the bone marrow. Further investigations are needed to confirm whether clozapine and olanzapine might directly cause an increase in adipocyte number or alter adipocyte size.

The Post-WTO Sustainability of the Pharmaceutical Industry in Iran

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The outcome of joining the WTO and implementing TRIPS agreement has caused a never-ending dispute in terms of both positive and negative perspectives. Access to the essential medicines and sustainability of the domestic pharmaceutical industry are to important relevant topics in the developing countries. Two major consequences are trade liberalisation and enforcing an international intellectual property rights. The immediate impact will be free flow of the medicines from developed countries in case of innovative drugs and more recently from India and China for cheap commodity medicines that are surely supported by the internationally enforceable intellectual property protections.

To evaluate the sustainability of the Iranian pharmaceutical industry and in the absence of previously developed instruments, two study tools were developed and validated in the course of this study namely WTO pharmaceutical Industry (PI) Impact Rating Scale and Pharmaceutical Industry Transition Instrument (PITI).

The study organised deep discussions with expert panel including members from academia, industry and regulatory authority using Delphi technique in order to identify the study parameters. Importation tariff, management knowledge, training, R&D, customer satisfaction and patent review were at the top of the 29 recognised parameters.

The study was then carried out throughout the industry to evaluate the current situation and future importance of the identified parameters that were restructured in 66 statements of the PITI. The outcomes of the studies reported in this thesis suggests that with the current situation in the pharmaceutical industry in Iran, it is unlikely that the industry will be able to cope with the post-WTO challenges to deliver the growth that guarantee the long term profitability of the industry. The compliance of the industry with the requirements of the WTO at the current situation was concluded as “unsatisfactory” for a majority of the PITI statements using binomial test ($p<0.05$). The future importance of all of the PITI statements were confirmed with binomial test statistical significance ($p<0.05$). The analysis was also made on the basis of the gap analysis and measuring the readiness using Pharmaceutical Industry Readiness (PIR) index. Measuring the readiness of the industry using the PIR index resulted in the overall value of 57% that varied between different companies. The findings of this study include the priorities given by the respondents to the identified parameters and related categories. The study ended with analysing the causal effect between the categories of the parameters and proposing a strategy map for the industry to reach the post-WTO sustainability.

Qualitative evaluation of clinical and non clinical influences on decision making in dermatology

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The process of clinical decision making is the essence of everyday clinical practice. This process involves an interaction of application of clinical and biomedical knowledge, problem solving, weighing of probabilities and various outcomes, and balancing benefit-risk. Although most clinical decisions are based on these clinical criteria, they are also influenced by a wide range of non-clinical factors.

The research in this thesis aimed at exploring factors influencing clinical decision making in dermatology. Chapter one is general introduction which reviews the medical and surgical literature regarding this topic. The first part of chapter one reviews the process of clinical decision making and the concept of "shared decision making", while the second part reviews various clinical and non clinical influences on clinical decision making.

Chapter two describes methodological framework and reasons behind selecting qualitative research methods. The research in this thesis is divided into 3 studies; study one involved observation of consultations, study two involved observation of consultations followed by patient interview, and study three involved interviews with clinicians working in dermatology in Wales.

Results of the three studies are presented in three results chapters; chapter three, chapter four and chapter five. We identified various types of management decisions made during the dermatology outpatient consultations. In addition, we found that there were several clinical and non-clinical influences on patient management decisions in dermatology. For example; ineffectiveness of previous therapy, clinician adherence to clinical guidelines, and side effects of medications were the most common clinical influences. We have also identified various non-clinical influences related to patient, physician and practice setting and explored this extensively during clinicians' interviews'. Chapter six provides general discussion as well as policy implication, future recommendation and conclusion of the study.

It is hoped that the results of this research would stimulate future researchers in undertaking further work to investigate in more details one or more of the factors identified in this study. It is also hoped that policy makers and administrators in the healthcare services consider various non-clinical factors identified in this study in order to improve the quality of patient care.

Investigation of the role of adenosine in the growth of human breast cancer cells

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Breast cancer is the second leading cause of cancer death in women in the UK and so there is a need to develop new treatments for this. The aims of these studies were to investigate the effects of adenosine receptor agonists and antagonists, the endogenous compounds adenosine and 2'-deoxyadenosine and the clinically used purine nucleoside analogues on the viability of breast cancer cells *in vitro.*

Initial studies identified the expression of all four adenosine receptors in the MCF-7 (ER-positive) and MDAMB231 (ER-negative) breast cancer cell lines but these receptors were not involved *per se* in maintaining cell viability. Adenosine and 2'-deoxyadenosine were able to reduce cell viability by mechanisms involving adenosine receptor activation in the MCF-7 cells, a reduction in extracellular regulated kinase 1/2 (ERK 1/2) phosphorylation in the MDAMB231 cells and intracellular phosphorylation by adenosine kinase in both cell lines.

Further studies identified that the clinically used 2'-deoxyadenosine analogue, clofarabine, had equal potency in the ER-negative cell lines compared to a leukaemia cell line *in vitro*. The ER-positive cells were 8-fold more resistant to clofarabine, however. Inhibition of ribonucleotide reductase appeared to be the primary mechanism of action of clofarabine and cladribine, but not fludarabine in the MCF-7 and MDAMB231 cell lines. ERK 1/2 signalling was also partly required for the action of clofarabine in the MDAMB231, but not the MCF-7 cells.

Studies on the resistance of clofarabine in ER-positive cells identified that increased basal expression of the metabolising enzyme, cytostolic 5'-nucleotidase II and the ribonucleotide reductase subunit, p53R2, were correlated with decreased sensitivity to clofarabine. Drug-induced p53R2 expression in p53 wild-type MCF-7 cells also contributed to resistance.

The present study highlights the potential for clofarabine as a treatment for breast cancers, particularly ER-negative cancers where patients have a very poor prognosis.

Development of functional plastibodies

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For many years, molecularly imprinted polymers (MIPs) have been described as "plastic antibodies", yet even some modern examples cannot approach the true binding affinity and specificity of monoclonal antibodies. This project sought to include within the imprinted site a short peptide sequence isolated from a phage display library with high affinity and specificity. A system is hypothesised in which synergism between the robust nature of the polymer and the binding affinity and specificity of the peptide may be exploited.

Peptide phage display is a technique that can rapidly enrich binding peptides from a combinatorial library of over 109 unique moieties. Initial studies attempted to isolate peptides with high affinity and specificity to propranolol from this library. However, when several methodologies failed to demonstrate any binding effect, a peptide was selected from the literature that had been found to bind the fluorophore Texas Red. The peptide was immobilised to a Merrifield Resin support, its binding properties thoroughly assessed, and a polymerisation protocol was developed using living radical polymerisation.

Preliminary studies suggested that when peptide-functionalised resin was washed in ethanol, no binding to Texas Red was evident, whereas once a protective polymer shell was formed, the peptide retained a binding conformation and affinity for Texas Red was slightly increased. This was, however, at the expense of binding capacity, which fell dramatically. Whilst the evidence presented here is by no means complete, it provides proof-of-principle for a functional peptide- molecularly-imprinted polymer. Further work in this area may lead to the development of a truly biomimetic artificial antibody: the plastibody.

The design, synthesis and biological evaluation of novel antitumour compounds

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Cancer is a leading cause of death worldwide. Chemotherapy is the main approach used currently for the treatment and management of this disease especially disseminated malignant tumours. Traditional anti-cancer drugs have limited selectivity as they target mainly DNA synthesis or cell proliferation leading to severe side effects. However, the newer anti-cancer drugs are more selective in targeting certain aberrant signalling pathways in cancer cells.

In the first part of this work, a series of benzimidazole and indole analogues of a potent antitumour benzothiazole lead compound were synthesised to enhance its pharmaceutical properties. Their antitumour activity was tested against different human cancer cell lines. In most cases, these analogues did not display any significant activity except for some of the N-substituted ones with *p*-toluenesulfonyl group, which yielded submicromolar GI_{50} values.

The second part of the work focuses on the design and synthesis of antitumour agents targeting the Wnt signalling pathway. The aberrant activation of this signalling pathway has been implicated in a wide spectrum of cancers. Disheveled PDZ domain is an essential protein-protein interaction mediator in the Wnt signalling pathway. Novel antitumour agents were designed using molecular modelling approaches to selectively inhibit the PDZ domain and hence abolish tumourogenic effects associated with the aberrant activation of Wnt signalling. Biochemical binding assays showed that one of the hits obtained through high-throughput docking binds specifically to the Disheveled PDZ domain. Structural optimisation studies of this hit were carried out in order to improve activity.

Computer-aided drug design and synthesis of novel antivirals

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The Flaviviridae is a family of 66 viruses of which almost half have been associated with human disease. The most well-known members are: Hepatitis C virus (HCV), Dengue virus (DV), and West Nile virus (WNV). Diseases caused by these viruses are global health problem that put an estimated 2.5 billion people at risk. At present, there are neither vaccines nor other treatments available to prevent or cure these diseases. Potential targets for the development of therapeutics against the virus are the viral protease and polymerase.

The aims of this project are to design and synthesize compounds that can be used as inhibitors for these two key enzymes for Dengue. Structure-based drug design methods utilize knowledge of a three dimensional structure of an enzyme/receptor to develop small molecules able to bind to the desired target, generating a specific biological response. These computer-based methodologies are now becoming an integral part of the drug discovery process and, although the principles of molecular recognition are far from being completely understood, some marketed compounds (i.e. Zanamivir, Lopinavir) have been developed with the help the successful application of structure-based design techniques.

Different structure-based drug design approaches have been used to identify putative new inhibitors for the Dengue protease and polymerase. A pharmacophore query has been built based on the active site of the Dengue protease enzyme and then used for screening different databases for identification of potential inhibitors. For the polymerase fragment-based approach has been used to find the fragments that would interact more efficiently with a specific binding pocket on the enzyme. The virtual library obtained by linking the best scored fragment was then docked to identify the most promising structures to be synthesized. The identification of potent small molecules that bind to receptor and enzymes is one of the major goals of chemical and biological research.

Isolation and chemical and pharmacological characterization of potential trace amine receptor antagonists from plant sources

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The present study describes the preliminary evaluation of Philippine medicinal plants *Artemisia vulgaris, Chrysanthemum coronarium, Moringa oleifera, Sesbania grandiflora and Vitex negundo* for their antagonistic activity at selected biogenic amine receptors on smooth muscle of the airways, gastrointestinal tract and vascular system.

The antagonistic activity of these plants were studied against dose-response curves for contractions of the ileum, trachea and aorta to 5-hydroxytryptamine (5-HT_2 receptors), methacholine (M_3 muscarinic receptors), histamine (H_1 receptors), phenylephrine (α_1-adrenoceptors) and β-phenylethylamine (trace amine-associated receptors, $TAAR_1$).

The methanolic extracts of *S. grandiflora* (flowers and leaves) revealed the presence of histamine H_1 receptor and muscarinic M_3 receptor antagonist in the ileum. The *A. vulgaris* chloroform (AV-$CHCl_3$) and methanol (AV-MeOH) extracts, and the acid-base extract of *V. negundo* (VN-E) showed histamine H_1 antagonism in the ileum and trachea. Further analysis of AV-$CHCl_3$ isolated two major components yomogin and 1,2,3,4-diepoxy-11(13)-eudesmen-12,8-olide. Yomogin a sesquiterpene lactone exhibited a novel histamine H_1 receptor antagonism in the ileum. Repeated exposure of aortic rings to phenylephrine and β-PEA CRCs produced significant increases in maximum vascular tension due to enhanced intracellular Ca^{2+} mobilization. Both the AV-$CHCl_3$ and VN-E inhibited this enhanced response. Further analysis of AV-$CHCl_3$ revealed that it is probably inhibiting the increase of vascular tone mediated via intracellular Ca^{2+} release regulated by ryanodine.

This study further validates the traditional use of *S. grandiflora, A. vulgaris* and *V. negundo* in the treatment of hyperactive gut, asthma and hypertension

PGSE-NMR and SANS studies of the interaction of model polymer therapeutics with mucin

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The effective uptake/absorption of macromolecules through the body involves several processes: one of these is the transport of the molecule through the mucus layer. The mucus layer is a complex mixture of biological components. Among them, mucin is the molecule that mainly contributes to the gel properties of the layer. Therefore, an *in-vitro* mucin gel can mimic, to a first approximation, the *in-vivo* physico-chemical properties of the mucus.

Therapeutic agents are often conjugated to polymers which behave as drug carriers to improve the tissue targeting specificity of the therapeutic molecule. Therefore, the understanding of the permeability of polymers through mucin solutions is fundamental in the construction of polymerbased drug delivery systems for therapeutics which can be adsorbed through the airways and the gastrointestinal tract. Furthermore, in the case of respiratory disorders such as cystic fibrosis (CF), the mucus can represent a real barrier for the therapeutics' access. The mobility through, and interactions with, mucin solutions of non-ionic/cationic/anionic polymers with different structures (from linear to dendritic) were studied by pulsed-gradient spin-echo nuclear magnetic resonance (PGSE-NMR) and small-angle neutron scattering (SANS). The interaction of non-ionic polymers were limited and related to the steric hindrance of the mucin networks. On the contrary, charged polymers such as polyamidoamines (PAMAM) dendrimers exhibited a pH-dependent interaction with the mucin molecules. At physiological pH, strong binding with mucin molecules was observed for positively charged polymers.

PEGylation is a widely used modification of molecules, proteins and drug delivery systems by covalently attaching one or more polyethylene glycol (PEG) chains: in fact, PEG-modification can reduce the toxicity, increase the half-life of drug delivery systems by enhancing their body resistance and reducing the plasma clearance. PEGylation of positively charged PAMAM dendrimers reduced their adhesive interaction with the mucin molecules, improving greatly the diffusion of these polymers in mucin solutions. After being PEGylated, PEG-PAMAM conjugates can be positively considered as model drug carriers.

Although mucin is the main component in mucus, a more complex and realistic mucus system was studied by SANS. Mucin solutions were enriched with extra components present in mucus, such as phospholipids and serum albumin. Hydrophobic lipid-mucin and protein-mucin interactions were observed. However, the adhesion of mucus components with mucin should be positively considered for the understanding of the mucus as a protective barrier and in the improvement of any treatment for the reinforcement of the mucosal barrier.

New methods for synthesis of substituted 2-phenylbenzothiazoles

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In recent years, substituted 2-arylbenzothiazoles have emerged as an important pharmacophore with a number of possible diagnostic and therapeutic applications. An example of this is provided by the simple 2-(4-aminophenyl)benzothiazole series which has shown both exquisite antitumour activity and potential as a PET tracer in non-invasive imaging of Alzheimer's disease. Although there are documented procedures for their synthesis, the majority refer to those benzothiazoles unsubstituted in the benzothiazole ring and involve the use of harsh reaction conditions, and chromatographic purification.

In this work a simple method for the rapid access to a range of 2-phenylbenzothiazoles both substituted and unsubstituted in the benzothiazole ring is reported, importantly the method described requires no chromatographic purification.

A simple one-step synthesis to 2-phenylbenzothiazoles unsubstituted in the benzothiazole ring is described whereby the desired product was made in high yield and with a short reaction time under either thermal or microwave irradiation of a variety of benzaldehydes and 2-aminothiophenol using sodium metabisulfite as a mild oxidant in DMSO.

The methodology was extended to the synthesis of biologically relevant 2-phenylbenzothiazoles substituted on the benzothiazole ring, starting from the appropriately substituted 2-aminophenyldisulfide. Under thermal conditions, a small diverse library of compounds was obtained in short reaction times with no chromatographic purification necessary.

The synthesis of a number of 2-phenylbenzothiazoles either substituted or unsubstituted in the benzothiazole ring is also reported by polymer-supported synthesis, utilising polymer-supported triphenylphosphine and *p*-toluenesulfonic acid as catalysts for the reaction.

Biological evaluation was undertaken on four cancer cell lines, namely, A549, LoVo, MCF-7, and PC3, with A549 and MCF-7 the most active. Although no exquisite activity was found, as these compounds contain either or both a carbon and fluorine atom, they have the possibility to be labelled with either a ^{11}C or ^{18}F label and therefore have potential use in PET imaging.

The following abstracts have been withheld as they have been or will be published elsewhere, and/or due to intellectual property or confidentiality issues:

Combination of post-transcriptional and post-translational down-regulation of the oestrogen reception in breast cancer

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Analysis of the uptake, subcellular distribution and bioactivity of cell penetrating peptides and associated cargo

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Design, synthesis and biological evaluation of novel pro-apoptotic antitumour agents

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