# The Pharmaceutical Implications of Drug Additions to Parenteral Nutrition Admixtures

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I can't believe it! Reading and writing actually paid off!

*Homer J. Simpson*

# SUMMARY

The supply of nutrition via the parenteral route may be necessary when it is impossible for a patient to obtain sufficient nutrients via the usual, enteral, route. Many patients admitted to hospital are malnourished and this can have an adverse effect on their health and ability to recover from illness. Nutritional support has been proven to reduce morbidity and mortality.

Although it is generally recommended that drugs should not be added to parenteral nutrition (PN) due to potential incompatibilities, the possibility of doing so has been extensively discussed in the literature and several benefits proposed, including a reduction in infusion set manipulations, with a subsequent decrease in infection risk and nursing interventions, and a decrease in the number of venous access sites necessary.

The aim of the thesis was to consider the pharmaceutical problems encountered when adding drugs to PN, and to examine factors that must be investigated to enable appropriate inclusion of drugs in PN admixtures. A questionnaire was issued to pharmacists in 89 UK hospitals investigating the practice of adding drugs to PN admixtures. The replies indicated that a lack of information prevented many from adding drugs, but that there was a great deal of interest in gaining the potential advantages of doing so. Based on these results, five drugs were selected for compatibility assessment with a standard PN regimen.

PN admixtures are complex systems containing many components which may interact and the addition of a drug to the admixture may further complicate this, potentially harming the patient. For this reason, admixtures must be subjected to rigorous stability assessment, using a variety of analytical methods to thoroughly evaluate physical compatibility and chemical stability.

Following the stability analysis, a testing protocol was devised and the legal and ethical obligations of the pharmacist in the addition of drugs to PN considered.

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| | |
|---|---|
| AIO | All in One (admixture) |
| ANOVA | Analysis of Variation |
| BAPEN | British Association for Parenteral and Enteral Nutrition |
| BNF | British National Formulary |
| BP | British Pharmacopoeia |
| BPNG | British Pharmaceutical Nutrition Group |
| DI | Drug Information |
| DLVO | Derjaguin and Landau, Verwey and Overbeek |
| D[4,3] | Mean diameter of the volume distribution |
| EVA | Ethylene vinyl acetate |
| EVAOH | Ethylene vinyl alcohol |
| FDA | Food and Drug Administration |
| FK | Fresenius Kabi UK Ltd, Runcorn, UK |
| HPLC | High Pressure Liquid Chromatography |
| HSP | Hydrocortisone Sodium Phosphate |
| HSS | Hydrocortisone Sodium Succinate |
| iv | Intravenous |
| LMW | Low molecular weight (heparin) |
| mAU | milli Absorbance Units |
| MCBs | Multichambered bags |
| ML | Multilayer |
| NTU | Nephelometric turbidity units |
| Ph Eur | European Pharmacopoeia |
| PN | Parenteral nutrition |
| PVC | Poly vinyl chloride |
| QC | Quality Control |
| RSD | Relative standard deviation |
| SPC | Summary of Product Characteristics |
| SVS | Small volume sampler |
| $t_{90}$ | Time at which drug reaches 90% of original concentration |

| | |
|---|---|
| USP | United States Pharmacopeia |
| UV | Ultraviolet (light) |
| WFIs | Water for Injections |

# CHAPTER 1

# INTRODUCTION

# CHAPTER 1 - INTRODUCTION

## 1.1 INTRODUCTION TO PARENTERAL NUTRITION

Parenteral nutrition (PN) is the supply of nutrition without involving the gastrointestinal (enteral) route. This may become necessary when it is impossible or undesirable for a patient to ingest, absorb or utilise sufficient nutrients via the usual route to meet their daily nutritional requirements. All of the biochemical components of a normal diet can be supplied by a PN admixture, namely protein, carbohydrates, fats, water, electrolytes, vitamins and trace elements.

Because the normal mechanisms of digestion are not utilised when administering PN, the nutrients must be supplied in a form suitable for infusion directly into the venous circulatory system. To this end, PN manufacturers have developed sterile injectable solutions of nutrients, capable of fulfilling a patient's nutritional requirements without the involvement of the digestive system. Within the hospital setting, a multidisciplinary group of healthcare professionals is involved in prescribing, admixing and delivering the PN to the patient safely, ensuring that no problems occur and the benefits of the therapy are maximised.

When PN was introduced in the 1960s (Baumgartner 1991), a multiple bottle system was used; amino acid, glucose and lipid solutions were infused from separate bottles, each of which lasted just a few hours. Consequently the technique required several bottle changes daily in order to achieve the nutritional requirements of the patient. Other required nutrients (vitamins, trace elements and electrolytes) were added to the various infusion bottles. This complicated situation allowed ample opportunity for infection, additive and flow rate errors and suboptimal utilisation of nutrients (Pertkiewicz 1999a).

The combination of amino acids and glucose mixed in one container in the

1970s reduced the problems cited above and brought a number of other benefits, including financial and time savings (Pertkiewicz 1999a). At this time, lipid emulsions were infused through a separate line or by using a Y-connector to reduce problems associated with lipid emulsion instability.

The system developed by Solassol and Joyeux in 1972 (Pertkiewicz 1999a) allowed the further benefits of the inclusion of lipid emulsion within the admixture to be realised. The addition of lipid emulsion decreases the osmolarity of a PN admixture, reducing vein irritation and phlebitis in peripheral veins and allows a more metabolically efficient use of the energy supplied (Anon. 1997). These advantages led to the "All in One" (AIO) system being adopted as the principle method for treating adult patients requiring PN support.

The development of multichambered bags (MCBs) in the late 1990s offered the advantages of the AIO system with increased ease of use and stability periods. Each bag is made up of several chambers, each containing one of the macronutrient solutions. The chambers are separated by seals that are broken to allow the solutions to mix when required for use. Micronutrient and other required additions are made possible via an inbuilt injection port. Because the solutions do not come into contact with each other prior to breaking the seals, the MCBs have long shelf-lives and do not require the rigidly controlled storage temperature conditions required by compounded AIO bags. A potential disadvantage of MCBs is the fixed composition which may make it unsuitable for some patients. However, since a considerable number of admixture formulations are available and micronutrients, and some other additional required nutrients, can be added to all of them, this disadvantage can easily be overcome for most patients. Metabolically stable patients make up 70 to 90% of those requiring nutritional support (Anon. 1999) and most can be catered for by the use of standardised regimens, either compounded in-house or by external suppliers, or with MCBs. The increasing use of these types of admixtures may free staff and resources allowing them to concentrate on

compounding tailor-made bags for those whose needs cannot be met by standardised regimens or MCBs.

## 1.1.1  INDICATIONS

According to a recent report by the Malnutrition Advisory Group of the British Association for Parenteral and Enteral Nutrition (BAPEN), up to 40% of all adult hospital patient admissions suffer from malnutrition (BAPEN 2000). These patients often continue to lose weight during their hospital stay and this has an adverse effect on their health and their ability to recover from their illnesses.

Many studies have demonstrated a relationship between malnutrition and morbidity and mortality (Allison 2000; Isabel et al. 2003; King et al. 2003). Malnourished patients suffer from an increase in wound healing time, infection risk, treatment related complications (Holmes 2004), recovery times and readmission rates, and from decreased respiratory, cardiac and immune function (Anon. 2000b) and the ability to cope with stresses such as surgery or cold. Mental wellbeing is also decreased, as is quality of life (BAPEN 2003; Holmes 2004).

Malnutrition is caused by inadequate intake, excessive losses or increased metabolic requirements, but is a preventable and treatable condition. Nutritional support can help maintain body tissues and metabolic function in patients identified and treated early enough, and rebuild tissue and restore function in those already suffering from the effects of malnutrition.

Parenteral nutrition has proven to be a safe and effective treatment, suitable when the enteral route is not fully functional or accessible (Payne-James and Khawaja 1993). It can be used to provide all of a patient's nutrients or to supplement the enteral route, which should be used whenever possible. PN may be indicated in the following groups of patients:

- Post-operatively when enteral nutrition is contraindicated for more than two to three days;
- Short bowel syndrome;
- Gastrointestinal fistulae;
- Gastrointestinal obstruction;
- Paralytic ileus;
- Acute pancreatitis;
- Multiple injuries;
- Major sepsis;
- Severe burns;
- Inflammatory bowel disease (including Crohn's disease and ulcerative colitis);
- ITU patients;
- Malabsorption states;
- Severe diarrhoea or emesis;
- Cancer patients.

## 1.1.2  COMPONENTS

A normal diet supplies all the nutrients necessary to maintain health and this should be simulated by a PN admixture. There will also be further demands made of the admixture if the patient is already malnourished or their concurrent disease state increases their requirement for some or all nutrients.

Nutrients may be divided into macro- and micronutrients. The former category consists of amino acids, fat and carbohydrates, and the latter of vitamins, trace elements and electrolytes.

### 1.1.2.1 Energy

Each of the macronutrients in PN can be used by the body as an energy source, however it is usual to calculate energy requirements in terms of non-protein energy (i.e. glucose and lipid) only, as the protein component of the admixture should be committed to maintaining or improving nitrogen balance.

## 1.1.2.2 Carbohydrates

Glucose is the most commonly used carbohydrate source in PN. It is widely available in several concentrations, is inexpensive and blood levels can be easily monitored. More importantly, glucose can be used by most cells in the body, and is the preferred carbohydrate source for many of them (Anon. 2000a).

In the past, glucose has been used as the only source of non-protein energy in PN, but this practice has fallen out of favour due to the numerous complications associated. These include:

- Hyperglycaemia leading to fatty infiltration of the liver;
- Excessive oxygen consumption and carbon dioxide production, with risk of respiratory failure;
- Hyperosmolar dehydration;
- Fluid retention due to hyperinsulinaemia;
- Decreased immune function.

Although excessive quantities of glucose can cause complications, it is nonetheless important to supply some of the daily energy requirements as carbohydrate. It is recommended that a minimum of 100 to 150 g of glucose is given daily (Anon. 2000a), because a carbohydrate-free nutritional supply will not stimulate the release of insulin and can lead to ketosis and a negative nitrogen balance (Sauerwein and Romjin 1995) because amino acids are used as an energy source (Milla 1995).

## 1.1.2.3 Lipids

Lipid emulsions provide a large amount of energy in a relatively small volume, yielding approximately 9 kilocalories per gram of lipid (Szczygiel 1999). In PN, since the advent of the dual energy technique (using both glucose and lipid), it is common to use lipid emulsions to supply between 30 and 60% of the daily energy requirements (Anon. 1997). This method avoids the metabolic

problems associated with using glucose as the only energy source. Other advantages of including lipid emulsions in PN admixtures are that administration via the peripheral route is possible due to the isotonicity and veno-protective effect of the lipid emulsion, and that the emulsion may contain fat-soluble vitamins and essential fatty acids (Anon. 1997).

## 1.1.2.4 Amino Acids

Amino acids are given in PN in order to maintain nitrogen balance. Protein is constantly synthesised and broken down within the body, but in a healthy adult the amount in the body remains constant (Matthews and Fong 1993). When body protein is lost due to malnourishment, fever, burns or trauma (Fürst 1999), or when a patient is in a catabolic state, as is commonly the case following major surgery, trauma or infection (Taylor 1994), nitrogen is lost and levels of protein in the body will decrease with subsequent loss of tissue function. These losses must be regained to allow tissue healing and rebuilding.

The twenty amino acids needed by the human body can be broadly classified as essential or non-essential. Those that are classed as essential cannot be manufactured by the body and must be ingested, whereas the nine designated as non-essential can be produced *in vivo* using dietary precursors, which include the essential amino acids. In addition to these categories, some amino acids can be defined as being conditionally essential. These can usually be produced by the body, but in certain circumstances, such as stress or some disease states, the body's ability to produce them may be reduced or lost. This change makes it necessary for the body to acquire the amino acid from an external source. Examples of conditionally essential amino acids important in PN patients are glutamine (important for the gut mucosa), histidine (particularly in patients with renal failure), and tyrosine and cysteine (in patients with liver failure).

## 1.1.2.5 Vitamins

Vitamins are essential in maintaining normal body function; each has an

important role in various metabolic pathways and a dietary deficiency of any one may lead to adverse consequences such as poor wound healing and sepsis (see Tables 1.1 and 1.2). However, it is difficult to recognise the early stages of deficiency and, as such, appropriate quantities of vitamins (both water and fat soluble) should be included in the PN admixture daily. In addition to this, concurrent illness and increased protein and energy metabolism can increase vitamin requirements (Anon. 2000a).

Table 1.1 Daily Requirements, Function and Effects of Deficiency of the Water Soluble Vitamins

| Vitamin | Daily requirements oral / iv | Functions | Effects of deficiency |
|---|---|---|---|
| $B_1$ | 0.4 mg/1000 kcal / 3 mg | Carbohydrate, fat and alcohol metabolism | Beriberi; Wernicke-Korsakov syndrome |
| $B_2$ | 1.1-1.3 mg$^A$ / 3.6 mg | Oxidative metabolism | Lesions of lips, tongue and skin |
| $B_6$ | 15 µg/g protein / 4 mg | Transamination of amino acids | Anaemia; skin and lip lesions. |
| Niacin | 6.6 mg/1000kcal / 40 mg | Role in oxidative metabolism | Pellagra; rash, weakness, diarrhoea |
| $B_{12}$ | 1.5 µg / 5 µg | Recycling coenzymes; valine metabolism | Megaloblastic anaemia; neurone demyelination |
| Folate | 200 µg / 400 µg | Purine/pyrimidine metabolism | Megaloblastic anaemia; growth retardation |
| Biotin | 10-200 µg / 60 µg | Lipogenesis; gluconeogenesis | Dermatitis; hair loss |
| C | 40 mg / 100 mg | Antioxidant; iron absorption | Scurvy; impaired wound healing |

Adapted from Shenkin (1995)

Table 1.2 Daily Requirements, Function and Effects of Deficiency of the Fat Soluble Vitamins

| Vitamin | Daily requirements oral / iv | Functions | Effects of deficiency |
|---|---|---|---|
| A | 200-700 µg$^A$ / 1000 µg | Growth and development; tissue differentiation | Impaired dark adaptation |

| D | 0-10 µg[B] / 5 µg | Calcium absorption; differentiation of macrophages | Osteomalacia; rickets |
| E | >3 mg[A] / 10 mg | Antioxidant | Neuropathy; myopathy |
| K | 1 µg/kg / 150 µg | Role in blood clotting | Bleeding disorders |

Adapted from Shenkin (1995)

Notes:

[A] varies with sex  [B] varies with age

## 1.1.2.6 Trace Elements

As with vitamins, trace elements are essential to the normal functioning of the body, but less is known about daily requirements and deficiency states. Their functions are many and varied, and include catalysing or acting as cofactors in enzyme activity and involvement in utilisation of nutrients (Anon. 1997) (see Table 1.3). To maintain the health of patients receiving PN, an adequate supply of trace elements must be provided daily in the PN admixture (Jeejeebhoy 1982).

*Table 1.3 Daily Requirements, Function and Effects of Deficiency of the Trace Elements*

| Trace Element | Daily requirements oral / iv | Functions | Effects of deficiency |
|---|---|---|---|
| Chromium | >25 µg / 10-20 µg | Insulin activity; lipoprotein metabolism; gene expression | Glucose intolerance; peripheral neuropathy |
| Copper | 1.2 mg / 0.3-1.3 mg | Cytochrome oxidase; Neuroactive amines | Anaemia; neutropenia; bleeding; arrhythmia |
| Fluoride | 0.05 mg/kg / 0.95 mg | Bone mineralisation | Dental caries |
| Iodine | 140 µg / 131 µg | Role in thyroid system | Hypothyroidism; goitre |
| Iron | 4.7-14.8 mg[A] / 1.2 mg | Haemoglobin system | Anaemia |

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| Manganese | >1.4 mg / 0.3 mg | Enzyme cofactor | Lipid abnormalities; anaemia |
|-----------|------------------|-----------------|------------------------------|
| Molybdenum | 50-400 µg / 19 µg | Role in DNA metabolism | Tachycardia; visual problems |
| Selenium | 40-75 µg[A] / 30-60 µg | Enzyme cofactor; protects against oxidative damage | Myopathy; cardiomyopathy; macrocytosis |
| Zinc | 4.0-9.5 mg[A]/ 3.2-6.5 mg | Involved in metabolism, protein synthesis, gene transcription | Growth retardation; diarrhoea; immune deficiency |

Adapted from Shenkin (1995)

Note:

[A] varies with sex

## 1.1.2.7 Electrolytes

Electrolytes are another group of micronutrients with many functions. The body maintains levels within precise ranges in order to allow proper functioning (see Table 1.4). Addition of sodium, potassium, magnesium, phosphate and calcium to PN is necessary to allow this. Electrolyte levels can vary widely from patient to patient and day to day, and must be monitored and the PN adjusted, to maintain the balance within the body.

*Table 1.4 Reference Ranges, Function and Effects of Deficiency of Electrolytes*

| Trace Element | Plasma Reference Ranges / mmol/l | Functions | Effects of deficiency |
|---------------|----------------------------------|-----------|-----------------------|
| Calcium | 2.3-2.7 | Bone structure; muscle contraction; blood clotting | Osteoporosis; tetany |
| Magnesium | 0.7-1.1 | Energy metabolism; muscle contraction | Tetany; arrhythmia; depression |
| Phosphate | 1.0-1.3 | Energy transport; absorption and transport of nutrients | Altered oxygen dissociation curve; muscle weakness |
| Potassium | 3.5-5.0 | Maintenance of cellular and metabolic functions | Vomiting; muscle weakness; paralysis; arrhythmias. |

| Sodium | 135-145 | Maintenance of water distribution, blood volume and acid-base balance | Weakness; seizures; cramps; hypotension; death |
| --- | --- | --- | --- |
| Bicarbonate | 22-30 | Maintenance of acid-base balance | Acidosis |
| Chloride | 95-105 | Maintenance of acid-base balance | Acidosis |

Adapted from Marshall (2000)

## 1.1.2.8 Water

Over half of the body weight of a healthy adult is attributable to water (Albina and Melnik 1990). The volume is kept constant by mechanisms which regulate input and output, to maintain the fluid-electrolyte balance. In PN patients, the monitoring of output and clinical signs is required in order to input the appropriate volume, as thirst, the usual regulatory mechanism for intake, is bypassed. Water and electrolytes work together to maintain the optimum inter- and intracellular functioning conditions.

## 1.2 PARENTERAL NUTRITION STABILITY

Parenteral nutrition admixtures are complex systems containing upwards of fifty chemical entities, each with different physical and chemical properties. As with any combination of chemicals there is a possibility that the components may interact and this may be to the detriment of the overall physicochemical stability of the system. External factors can also influence the stability profile and must be considered when investigating PN stability.

Properly designed and compounded admixtures can be stable for several months, if stored under appropriate conditions, but even apparently minor changes in the formulation may completely alter the nature of the admixture and cause unpredictable changes.

### 1.2.1 IMPLICATIONS OF INSTABILITY

Incompatibilities in PN admixtures may lead to physical or chemical

instability, but either is unacceptable. Physicochemical instability can cause changes in the aqueous or lipid phase of an admixture. Insoluble precipitates can form in an unstable aqueous phase and destabilised lipid emulsions are prone to coalescing or "cracking", leading to the production of oversized globules.

The presence of a significant number of particles greater than 5 to 10 $\mu$m in diameter poses a threat to the patient, as these particles are capable of blocking small capillaries after infusion through a large central vein (Driscoll et al. 1995). A blockage within the capillary beds will lead to a reduction in blood flow to the tissue which, in the lungs, can cause pulmonary embolism (Barber 1993), which may ultimately cause death. An alert issued by the Food and Drug Administration in 1994 (Lumpkin and Burlington) recorded the deaths of two patients, and respiratory distress in two more, after receiving PN as an AIO admixture. It was proposed that the deaths were attributable to the presence of calcium phosphate precipitation within the admixture. Pharmacists were reminded of the importance of a number of factors, namely the nature of the amino acid solution, the chemical form of the calcium and phosphate additives and the mixing process and order, in maximising the safety of admixtures.

In addition to the above, a peripheral infusion containing a significant number of particles can cause thrombophlebitis, damaging the vein. Enlarged particles are also potentially capable of blocking catheters, a significant problem faced in the administration of PN.

Chemical instability can result in loss of activity of components, for example vitamin C (Allwood and Kearney 1998) and $B_1$ (Dickerson et al. 1993), or the production of potentially toxic breakdown products. Knowledge of the stability of each PN component is therefore necessary, as stated by Allwood and Kearney (1998) and Manning and Washington (1992).

## 1.2.2 FACTORS AFFECTING STABILITY - NUTRIENTS

### 1.2.2.1 Glucose

The most important stability factor involving glucose is its ability to disrupt lipid emulsions by virtue of its low pH in solution. The pHs of glucose solutions range from 3.5 to 4.5, which is outside the stability range for lipid emulsions (see Section 1.2.2.2).

The presence of concentrated glucose in an admixture has been reported to increase the rate of creaming by allowing more interparticulate reactions (Barat et al. 1987). However, Driscoll (1995) and Washington et al. (1990) reported a decreased rate due to the physical resistance caused by the increased viscosity in the solution and subsequent reduced particle mobility.

Glucose is known to react with amino acid solutions, via the Maillard reaction which can result in a darkening of the solution and the formation of complex polymers. However, there is no loss of nitrogen content (Allwood 1984) and this reaction is not a major stability concern.

Glucose is detrimental to the stability of the antioxidant sodium metabisulphite in aqueous solution (Akers 2002). Although some amino acid solutions contain antioxidants to protect oxygen labile amino acids, this is not generally the case in the UK (see Sections 1.2.2.4 and 1.2.3.2).

### 1.2.2.2 Lipid Emulsions

Lipid emulsions for use in PN admixtures contain discrete lipid globules dispersed in an aqueous phase. The lipid phase consists of triglycerides with long, medium or mixed chain lengths. In order to ensure that the globules within the lipid emulsion are taken up and used by the body in the same way as the naturally synthesised lipid globules (chylomicrons), they are manufactured to have approximately the same mean globule size, metabolism and elimination profile as the chylomicrons (Pharmacia 1994).

The lipid globules remain dispersed throughout the water phase by the mechanical and electrostatic action of a layer of emulsifying agent surrounding the globules. The emulsifier is typically an egg or soya lecithin phospholipid which prevents coalescence of the lipid droplets by providing both a mechanical barrier and a negative surface charge ("zeta potential") to the globule, leading to a mutual repulsion between adjacent globules. This negative charge exists between pH 5.5 and 9.0 (Driscoll et al. 1995), when the phosphate group of the emulsifier is ionised.

The destabilisation of an emulsion is a sequence of events. Initially, globules aggregate as they come together and as the repulsive forces between them decrease, but they remain as distinct globules. Next, the aggregates, which have a lower density than the emulsion, float to the surface, forming a cream layer. Both of these stages are reversible upon agitation. When the repulsive and mechanical barriers between the lipid globules decrease to the extent that globules can merge, forming larger globules, the emulsion is said to have cracked or coalesced. This may ultimately be seen as the presence of free oil as droplets or as a layer on the surface of the emulsion. This is an irreversible change in the nature of the emulsion and renders the product unsuitable for infusion.

The repulsive forces between the lipid globules are highly reliant on the maintenance of the negative charge surrounding the particles. A decrease in environmental pH will decrease the repulsive forces, thus decreasing the emulsion stability. Zeringue et al. (1964) showed that instability was evident in lecithin stabilised emulsions when the pH of the environment fell below 5.0. The addition of an acidic solution, such as glucose, will cause the pH of the emulsion to fall, leading to the loss of emulsion stability. However, the addition of a pre-mixed amino acid and glucose solution should not initiate this destabilisation process, due to the buffering capacity of the amino acids neutralising the acidity of the glucose solution. This is one of the reasons why it is recommended that the lipid component of an admixture be added last.

Mechanical forces can also play a role in determining the stability of an emulsion. Decreased viscosity, associated with the addition of large volumes of water or low strength glucose solution to an admixture, may reduce physical resistance to coalescence (Driscoll 1995). This factor is considered less important than maintaining zeta potential, but more work must be done to confirm this.

A component of an admixture can diffuse into the outer layer of a globule, changing its surface charge (Pertkiewicz 1999b). The addition of cations, whether as nutritional components or drug additives, will reduce the repulsive forces between the lipid globules and can result in aggregation and the formation of large particles. This is a particular problem with di- and trivalent cations as discussed by Washington et al. (1990). The interaction between emulsion globules and electrolytes can be described using the DLVO theory, which explains that ions bind to lipid globules using electrostatic forces. As the concentration of ions increases, more ion-globule interactions occur, changing the surface charge of the globule and thus reducing the repulsive forces between globules and allowing flocculation. DLVO theory and the Schultze-Hardy rule describe the relationship between the valency of the ions and the concentration required to cause flocculation, showing that ions with higher charges are potentially more destabilising. The complex nature of PN admixtures means that these theories are not completely accurate in their description of the interactions occurring within PN admixtures but do show the increased potential for instability when adding high valency ions to an admixture. Significant quantities of trivalent cations are less commonly required in PN, but in the case of divalent cations, such as calcium and magnesium, large doses are often required by patients on a daily basis and so present more of a challenge for the compounding pharmacist. Again, by adding the lipid emulsion as the last component, emulsion stability is likely to be maintained due to maximum dilution of the cations in the PN admixture and by allowing complexation of these destabilising ions with other PN components to occur, thus minimising the concentration available for destabilisation of the lipid emulsion (Barnett et al. 1990).

## 1.2.2.3 Amino Acids

The inclusion of amino acids in an admixture normally exerts a protective effect upon the lipid emulsion (Barat et al. 1987) in two ways. Firstly, the buffering capacity of the amino acid solution prevents the pH decrease caused by the addition of glucose solutions. Secondly, at pHs greater than their isoelectric point the amino acids have a negative charge, which repels the like-charged lipid particles, thus decreasing the likelihood of coalescence. However, the nature of the amino acid formulation must be considered, as an increased relative concentration of acidic to basic amino acids may lead to decreased stability in an admixture (Hardy 1989).

## 1.2.2.4 Vitamins

Vitamins are a chemically diverse group of compounds, with a similarly diverse collection of factors influencing their stability. Admixture pH, composition, temperature, storage time, light and oxygen exposure and the infusion device may all impact on the stability of one or more of the vitamins.

Vitamins A, D and E are known to adsorb onto PVC bags and infusion devices (Allwood 1984), so may not reach the patient at the prescribed level. However, in the case of vitamin A this only occurs when it is given as its acetate ester. This vitamin is also subject to photodegradation by ultraviolet (UV) light which is also damaging to vitamins $B_2$ and $B_6$. The presence of UV light is also an important environmental factor necessary for the photooxidation of vitamin E.

Vitamin C is the least stable vitamin added to PN (Allwood and Kearney 1998), being oxidised to an inactive compound, which not only results in a reduction of active vitamin reaching the patient but can, in the presence of calcium, lead to the formation of potentially harmful calcium oxalate crystals. Storage of the admixture in gas impermeable multilayer (ML) infusion bags prevents oxygen ingress during storage and delivery, reducing the amount available to degrade susceptible compounds; the impermeability does however,

prevent oxygen introduced during compounding from leaving the bag. The presence of certain trace elements (especially copper) can catalyse the oxidation reaction, whilst increased temperatures also increase the rate of degradation (Allwood and Kearney 1998), thus limiting the stability period.

Some amino acid preparations contain sodium metabisulphite as an antioxidant which, while providing protection for oxygen labile compounds, leads to the reduction, and consequent clinical loss, of vitamin $B_1$. This is not usually a problem in the UK as most, if not all, commercially available amino acid solutions are free of this antioxidant (see Section 1.2.3.2).

It has been recommended that vitamins should be added to the admixture shortly before administration and protected from light during infusion to minimise the losses described above (Dickerson et al. 1993).

1.2.2.5 Trace Elements

Trace elements, as well as catalysing biochemical reactions, can act as catalysts for a number of reactions within a PN admixture. As mentioned above (Section 1.2.2.4) copper catalyses the oxidation of vitamin C. Other trace elements, namely iron, zinc and manganese, can also do this.

Physical incompatibility, in the form of precipitate formation, is possible when trace elements are added to PN, depending on the type of amino acid solution used. Both copper and iron have been shown to form insoluble complexes (Allwood et al. 1998) which, if large enough, can block capillaries as described in Section 1.2.1.

Although, theoretically, the inclusion of trace elements could reduce surface charge on lipid globules and damage emulsion stability, the addition of the recommended daily amounts has not been seen to cause this in the short term (Wells 1992). However after longer storage periods (over 7 days), this phenomenon has been reported (Cosslett 2004).

## 1.2.2.6 Electrolytes

In addition to the destabilisation of lipid emulsion associated with the inclusion of multivalent cations discussed above (Section 1.2.2.2), the inclusion of electrolytes in a PN admixture can also cause physicochemical instability in the form of precipitation. Calcium phosphate is the most common cause of precipitation in PN (Lee and Allwood 2001) and many interrelated factors can influence it, of which pH is the most important (Allwood and Kearney 1998). Calcium phosphate typically exists in PN in one of two forms; at low pH the soluble monobasic form ($Ca(H_2PO_4)_2$) predominates, but as pH increases, the less soluble dibasic calcium phosphate ($CaHPO_4$) salt forms and may precipitate (Wells 1992). The pH of the PN admixture will be influenced predominantly by the pH of the amino acid solution and its buffering capacity, and the concentration and volume of glucose in the final formulation.

Consideration must also be given to both concentration and chemical form of the calcium and phosphate salts used; increased concentrations of either will increase the likelihood of detrimental interactions occurring. Calcium is available as inorganic and organic compounds and the increased dissociation seen with the use of inorganic salts increases the amount of free calcium available to interact with phosphate. The choice of an inorganic phosphate additive will influence the pH of the solution, affecting the solubility of calcium phosphate as detailed above. The use of organic phosphates has been reported to cause fewer precipitation problems (Eggert et al. 1982) but with this comes increased financial outlay.

Other factors have a less important but nonetheless valuable role to play in influencing calcium phosphate precipitation. The presence of magnesium can improve calcium phosphate solubility, by forming soluble salts with phosphate (Allwood and Kearney 1998), whilst a rise in temperature increases the likelihood of precipitation as calcium dissociation from organic salts is increased and the reaction equilibrium favours production of the less soluble dibasic salt (Henry et al. 1980).

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Once again, the practice of adding the lipid emulsion as the last component to the admixture must be recommended to ensure that any precipitation formed in the aqueous phase during admixing is visible and not masked by the opacity of the lipid emulsion.

## 1.2.3 FACTORS AFFECTING STABILITY - NON-NUTRIENT

### 1.2.3.1 pH

The influence of pH on many factors in PN physical stability has been detailed in the appropriate sections above. In summary, a relatively high pH is beneficial for lipid emulsion stability, but a relatively low pH is preferable to ensure calcium phosphate solubility. The main factors dictating the pH of an admixture are the glucose concentration and the amino acid concentration and profile (and hence potential buffering capacity), and in practice a balance must be struck in the admixture formulation to maximise stability.

### 1.2.3.2 Oxygen

Many PN components interact with oxygen but for most the implications of this have not been determined. Oxidative losses may not always have significant nutritional implications, but some relatively insoluble amino acid breakdown products are formed and the characteristics and potential toxicity of many of the byproducts are unknown (Allwood et al. 1996).

All of the macronutrients can be affected by the presence of oxygen, but problems have only been reported for the lipid. Lipid emulsions contain a large amount of polyunsaturated fatty acids (Silva et al. 1998), which can peroxidise to form potentially harmful compounds (Steger and Mühlebach 1997). Amino acids and glucose can also be oxidised, but this process also requires the presence of light, and in the case of glucose, a sensitising compound such as vitamin $B_2$. Unlike the photodegradation discussed in Section 1.2.3.3 the process of photooxidation can be mediated by light of visible wavelengths (Silva et al. 1999).

Of the micronutrients, only the degradation of the vitamins is likely to have any clinical or stability implications. Several vitamins are known to be degraded by oxygen. Vitamin C is the most susceptible to damage and the rate is influenced by the presence of trace elements as catalysts and by environmental variables such as ambient temperature. Vitamin E is oxidised only in the presence of UV light.

Removing oxygen, or preventing its ingress, will prevent oxidative damage of PN components. In the past, amino acid manufacturers added chemical antioxidants such as sodium metabisulphite to their solutions but, since sodium metabisulphite has been reported to cause release of toxic oxidants from amino acid solutions exposed to light (Martindale 1999) and cause the loss of vitamin $B_1$, many manufacturers now prefer to purge the solution with an inert gas, for example nitrogen or helium (Allwood et al. 1996) in accordance with British Pharmacopoeia (BP) recommendations (2003). Unfortunately, the benefits of this process can be lost when the PN admixture is compounded and although the use of ML bags reduces oxygen ingress compared to EVA bags (Allwood and Kearney 1998), it does not prevent the introduction of oxygen during compounding, which may be even more an important factor in oxygen mediated damage than oxygen ingress (Ball and Barnett 1996).

### 1.2.3.3 Light Exposure

Several components of PN are light sensitive and consequently light exposure can lead to instability within the admixture. In order for a chemical to be degraded by light, it must be able to absorb the light, which supplies the activation energy for the degradation reaction. However, the absorption of light by a compound does not necessarily mean that the compound will be degraded (Allwood 2000a). In order for a light source to mediate photodegradation within PN, it must contain light from within the UV spectrum (Allwood 2000a). Artificial light sources do not emit a significant amount of UV light and consequently do not cause photodegradation. Daylight, however, does contain light of these wavelengths and therefore

enables light mediated degradation (Allwood 2000a). Within the hospital setting, infusion solutions may conceivably be exposed to light for extended periods if, for example, they are positioned close to a window or administered to a patient undergoing phototherapy. This may lead to the degradation of light sensitive compounds and the administration of different quantities from those required and from those delivered from infusion bags subjected to no light, or artificial light only.

Some compounds, including Vitamin $B_2$ and the related compound flavin mononucleotide, can catalyse photooxidation of other PN components when exposed to light from the visible part of the spectrum (Silva et al. 1999) (see Section 1.2.3.2).

Vitamins A, $B_2$, $B_6$ and E are all damaged by light exposure. The mechanisms are complex and proceed through several steps, ultimately resulting in the production of inactive compounds. Vitamin A and the two B vitamins mentioned are subject to photolysis, while Vitamin E is damaged by the process of photooxidation which requires the presence of both UV light and oxygen (Allwood 2000a).

The peroxidisation of lipid emulsions (see Section 1.2.3.2) is increased in the presence of light and prevented by the use of light protection methods (Steger and Mühlebach 1997).

Despite the evidence, not all hospitals routinely light protect PN admixtures, even though light sensitive compounds can be protected by using simple methods such as storage and use away from light sources or by covering the infusion bag with coloured overwraps. However, even if the bag is protected from light, considerable losses of light sensitive compounds can occur within the administration set (Allwood 1982). It has been suggested that lipid emulsion provides a degree of protection (Dahl et al. 1986), but the evidence is not conclusive (Allwood and Martin 2000) and any protection given is possibly at the expense of lipid stability. As stated above, the absorption of light by a

compound will not necessarily damage it. Therefore, absorption of UV light by some amino acids can protect sensitive compounds, without themselves incurring damage (Allwood 2000a).

## 1.2.3.4 Ambient Temperature

The shelf-lives of compounded admixtures can be increased by the use of refrigerated storage, but not by a predictable amount. These conditions can slow microbial growth, decrease the likelihood of interparticulate interactions, reduce lipid emulsion peroxidation (Steger and Mühlebach 1997) and increase calcium phosphate solubility, depending on the salts used, as described in Section 1.2.2.6. Increased creaming has been noted during experiments involving refrigerated storage but, as admixtures warm to room temperature, this aggregation redisperses with no apparent detrimental effect upon admixture stability.

Admixtures may be administered at temperatures of up to 37°C, and this may decrease admixture stability by encouraging the formation of calcium phosphate crystals (see Section 1.2.2.6).

## 1.2.3.5 Infusion Bags

Infusion bags were originally made of plasticised PVC (Allwood 2000b) but lipid emulsions stored in these bags leached the phthalate plasticiser into the admixture, with potentially harmful results, and some additives, including the fat-soluble vitamins A, D and E, were adsorbed by the material thus reducing the dose to the patient (Allwood 1984). These incompatibilities brought about the search for alternative materials. Silicone containers were chosen by Solassol and Joyeux for storing the first AIO admixtures in the 1970s (Pertkiewicz 1999a), but these were prohibitively expensive, leading to the development of EVA bags (Pertkiewicz 1999a), which are still used today, but these are not without disadvantages. EVA bags are gas permeable, permitting the passage of oxygen into the admixture, thus allowing oxidative degradation to occur (see Section 1.2.3.2). The introduction of ML bags, usually

constructed of alternating layers of an inert plastic such as EVA (suitable to be in contact with the infusion solution) and plastics such as EVAOH, has allowed extended storage of PN admixtures due to their reduced gas permeability, but at an increased cost. All of these bags admit light, but this can be overcome by the use of coloured overwraps.

### 1.2.3.6 Admixing Process

Due to the myriad potential interactions between PN admixture components, orders of mixing have been proposed which separate incompatible compounds as far as possible, for example, calcium and phosphate preparations should be separated by the addition of other components and the contents of the bag should be thoroughly yet gently mixed after each addition, to prevent formation of localised areas containing a high concentration of components, and visually examined for signs of gross incompatibility. It is recommended that lipid emulsions are the last addition to be made to a bag, to allow visual inspection of the admixture for the maximum time. Literature is available from PN manufacturers detailing appropriate protocols.

### 1.2.4    ASSESSING AND ASSURING STABILITY AND COMPATIBILITY

The assurance of stability, clinical suitability and sterility are all important when assessing the safety of PN admixtures. Only admixture stability is studied in this work but all three factors must be assured to produce a safe admixture.

For a PN admixture to be classed as stable there should be no significant changes in its characteristics over time. Stability assessment of an admixture should include determination of lipid globule size distribution, the presence and size of insoluble particles and changes in pH, any of which, if adverse, can have detrimental effects on the patient. These in-depth investigations cannot be routinely carried out within the hospital setting, but manufacturers are able to provide examples of stability-assured PN regimens, which must nonetheless be prepared following strict compounding guidelines with recommendations

that the admixtures undergo visual examination as part of a routine quality control check prior to use.

## 1.3    DRUGS IN PARENTERAL NUTRITION

It is generally recommended that drugs should not be added to PN unless absolutely necessary (Lee and Allwood 2001) due to the complex nature of PN admixtures and the subsequent compatibility and stability issues detailed above.    However, many authors have addressed the issue because, from a clinical practice viewpoint, it may be beneficial where relevant validated evidence of compatibility and stability is available.    Several advantages have been proposed by Driscoll et al. (1991), Trissel et al. (1999) and Niemiec and Vanderveen (1984).    By adding the drug to the PN, fewer administration set and catheter manipulations are necessary, thus decreasing infection risk.    It also leads to a reduction in the number of venous access sites necessary.    These benefits allow savings in pharmacy and nursing time and additional advantages may also be seen with the reduced fluid and electrolyte load and the uninterrupted administration of drugs which are most effective when given continuously.    The practice can only be recommended for metabolically stable patients, which includes the large home PN patient population, who may experience the greatest benefits.

Before embarking upon the extensive stability testing of a drug-PN combination, the appropriateness of including the drug should be considered. Drugs which require frequent dose adjustment based on patient response or those which are better suited to intermittent administration should not generally be chosen for inclusion in PN.    Their use may result in suboptimal PN or drug dosing for the patient, or lead to compounded admixtures being wasted when they no longer meet the needs of the patient.    However, drugs with relatively stable doses and infusion rates can, if compatible and if clinical indications justify, be considered for inclusion.

In common with drug-free PN admixtures, robust admixture-specific stability

and compatibility data are required to form an opinion on the safety and appropriateness of adding a drug to an admixture. In addition to PN stability tests, it is necessary to assess the impact on the chemical stability of the drug by its inclusion in the admixture. Significant adverse effects on the stability of either the drug or the admixture are unacceptable.

There are currently no national guidelines relating to the addition of drugs to PN admixtures in the UK. Guidelines on administering drugs via enteral feeding tubes were launched by BAPEN in 2003, but as yet there are no corresponding guidelines for the parenteral route. The questionnaire detailed in Chapter 2 includes data on individual hospital practices and on the existence of some trust guidelines and highlights the lack of information and consensus in this area.

The necessary specificity of stability data has, in the past, diminished the potential advantages of adding drugs to admixtures. However, the advent of fixed PN environments such as standard regimens and MCBs may allow the advantages to be taken up. Driscoll et al. (1991) stated that:

> *"If standardised parenteral feedings were generally used clinically, such testing would have broader applicability."*

The aim of this thesis is to consider the pharmaceutical problems encountered when adding drugs to PN, and to describe and discuss the stability factors and parameters that must be investigated to enable the appropriate inclusion of drugs in PN admixtures.

# CHAPTER 2

# QUESTIONNAIRE

# CHAPTER 2 - QUESTIONNAIRE

## 2.1 INTRODUCTION

### 2.1.1 BACKGROUND

The advantages of administering drugs concurrently with PN admixtures have been detailed by numerous authors (Driscoll et al. 1991; Niemiec and Vanderveen 1984; Trissel et al. 1999). From a clinical practice viewpoint it can be beneficial because patients have finite venous access sites and these need to be preserved. The addition of drugs to PN can reduce the number of access sites and catheter manipulations, thus reducing infection risk and nursing time. Using PN as a drug vehicle also reduces the fluid and electrolyte load normally presented by the drug diluent.

The literature includes factors that must be considered before adding or Y-siting drugs and PN and parameters to monitor. All papers on this subject emphasise the need for complete stability assurance of both the drug and the PN before administration of the admixture to the patient, however the limited amount of stability information currently available means that the advantages of adding drugs to PN cannot yet be fully realised. Therefore before embarking upon an evaluation of the implications of drug additions to PN admixtures, it is necessary to determine whether the perceived advantages would be clinically relevant to UK hospitals.

The questionnaire set out to establish current practice in the area of drugs and PN mixing, and to find out whether the shortage of information and lack of national guidelines explains why the practice is not commonplace or conversely if the lack of information is due to lack of interest by hospital workers.

### 2.1.2 AIM

The aim of the study was to ascertain the extent to which hospitals added, or wanted to add, drugs to PN admixtures. If there was no demand for the

information to be generated then no patient would benefit from the proposed research work.

## 2.1.3 METHODOLOGY

### 2.1.3.1 Choice of Method

The method chosen to investigate current practice was a self-completion questionnaire. The recipients of the questionnaire were located throughout the UK so face-to-face interviews would have been impractical to carry out on a large scale. The use of a telephone interview would have required the interviewee to be available for up to half an hour and this is likely to be impractical in a hospital pharmacy work setting. Asking the respondent to be available for this extended period was likely to have had a detrimental effect on response rate.

The questionnaire was sent out as a Microsoft Word document as an e-mail attachment, reducing postage costs and administration time. It may have made completion of the questionnaire more convenient for some respondents and thus increased the likelihood that they would complete and return the questionnaire. Conversely, it limited the recipients to those who had an e-mail address. It is also impossible to send an e-mail addressed to "The Pharmacist" which can be done with a postal questionnaire using the main hospital address which is easily accessible.

### 2.1.3.2 Sampling

The group selected to receive the questionnaire consisted of all the pharmacists on the British Pharmaceutical Nutrition Group (BPNG) mailing list and all secondary care trust chief pharmacists in Wales and Scotland. The chief pharmacists were requested to forward the questionnaire to the pharmacist in charge of parenteral nutrition in their trust. Issuing questionnaires to the chief pharmacists with a request to forward them partially overcame the difficulty of not being able to e-mail a questionnaire addressed to "The Pharmacist".

## 2.1.3.3 Response Rates

Response rates are generally low (often around 50% in small surveys) in self-completion questionnaires, compared to alternatives such as interviews and telephone surveys (Cummings et al. 2001). Asch et al. (1997) reported a mean response rate of approximately 60% for mail surveys published in medical journals. The response rate achieved for this questionnaire was maximised by sending a follow-up copy of the questionnaire to non-responders four weeks after the first copy. Other techniques employed were the use of the covering letter, to explain the purpose of the questionnaire and to assure complete confidentiality, and the facility to access the questionnaire by various means, i.e. by filling in and returning the e-mail attachment, by printing and posting or faxing the attachment and by telephoning, faxing, e-mailing or writing to request a hard copy of the questionnaire and a prepaid, addressed envelope.

## 2.1.3.4 Practical Issues

The questionnaire was e-mailed to the selected recipients in blocks of ten e-mail addresses and each block was carbon-copied to the researcher to ensure there were no technological problems. Failed and invalid e-mail addresses were recorded and corrected where possible and the message resent on two separate days.

The covering letter stated the closing date for the questionnaire, which was six weeks after the questionnaire was sent. The second copy of the questionnaire was sent to those who had not responded two weeks before the closing date.

The e-mail addresses of the incoming replies were recorded and the respondent identified where possible to check if a reply had been received from a given hospital. The identification of the respondent was not used for any other purpose. As the questionnaire was confidential, it was not always possible to ascertain who had or had not replied, especially in the case of those questionnaires received by post. When the reminders were sent to all the repondents identified as possibly having not returned the questionnaire,

pharmacists who had submitted a completed questionnaire were requested not to fill out a further copy.

## 2.2 PILOT STUDY

### 2.2.1 METHODS

Initially, medical information executives from the pharmaceutical industry were asked about the queries they receive from healthcare professionals on the subject of using drugs with PN admixtures, and how often they receive this type of enquiry. This gave an overview of the current issues facing PN pharmacists. Following this, a number of aseptic pharmacists were engaged in informal, unstructured one-to-one discussions with the researcher about drug/parenteral nutrition practices in their hospitals, other practices they were aware of and their opinions on these practices.

Using issues brought up in the various interviews, a self-completion questionnaire was produced and piloted on practising hospital pharmacists to ensure all questions were relevant and appropriate and on pharmacists not practising in parenteral nutrition, to ensure clarity of questioning for a person without specialist knowledge (see Appendix 1).

### 2.2.2 RESULTS

Interviewing both medical information executives and pharmacists brought up a wide range of issues and questions to be addressed. It was apparent that some subjects introduced by medical information were hospital specific and of little global interest. These were identified during interviews with pharmacists and consequently disregarded for the final questionnaire.

The questionnaire was piloted and adjusted based on suggestions and comments made. It was noted by some that there were:

*"Too many questions that require detailed responses."*

In the pilot questionnaire there were a large number of open questions, to ensure that no response would be excluded. The most common responses were identified in the interviews and pilot questionnaire, and used to construct multiple choice questions wherever possible.

Other respondents felt that it was:

*"Not clear where to go after some questions"*

which meant reconstruction of the questionnaire layout was undertaken.

## 2.2.3 CONCLUSION

In response to the comments from the pilot questionnaire a number of questions were altered. Fewer open questions were included but all multiple choice questions had an "Other" option to allow respondents to give answers outside the available choices. The layout was changed to simplify completion and the questionnaire was broadly separated into four sections:

> Section A - Addition of drugs to parenteral nutrition admixtures
> Section B - Y-Siting drugs with parenteral nutrition admixtures
> Section C - Practical issues
> Section D - Guidelines

Sections A and B consisted of six and four questions respectively, both starting with the question:

*"Are drugs added/Y-sited with parenteral nutrition (PN) in your hospital?"*

A negative response directed the respondent to the next section.

Section C investigated the nature and frequency of any drug-PN related problems.

Section D explored the existence of any regional or local guidelines on the subject and asked respondents to enclose a copy if possible.

The final page invited further comments or questions.

## 2.3 MAIN STUDY

### 2.3.1 METHODS

A covering letter (see Appendix 2) explaining the reason for the questionnaire and instructions for its completion was e-mailed to the target group with the self-completion questionnaire attached as a Microsoft Word document (see Appendix 3).

### 2.3.2 RESULTS

#### 2.3.2.1 Response Rates

A response rate in excess of 60% was achieved at the final count: issuing a second copy of the questionnaire to non-responders at four weeks increased the response rate from 37.1 to 64.0%. Offering a number of alternatives for returning the questionnaire may have maximised returns. The use of the various methods is illustrated in Figure 2.1.

The questionnaire was issued to 47 aseptic pharmacists and 42 chief pharmacists. It was impossible to assess exactly how many of the questionnaires sent to chief pharmacists arrived with the pharmacist responsible for PN or aseptic compounding. With a sample size of 57 it cannot be concluded that the results are fully representative of practice throughout the UK but they give a useful insight into the practices of many trusts and of attitudes within the profession of pharmacy towards the inclusion of drugs in PN admixtures.
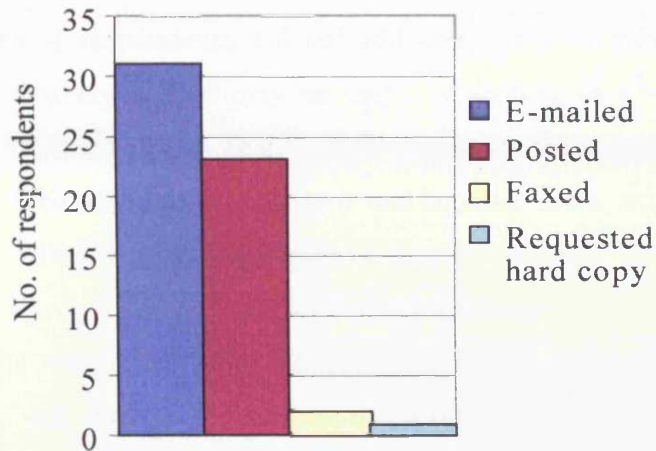
*Figure 2.1 Methods of Response*

2.3.2.2 Section A - Addition of Drugs to PN Admixtures

The majority of respondents (70.2%) did not add drugs to PN in their hospitals. Reasons cited are shown in Figure 2.2 and included:

> *"It's against hospital policy"*,
> *"Lack of aseptic facilities/trained personnel"*

and

> *"No need, we always use triple lumen [catheters]"*

but the most common response was lack of knowledge of compatibility and stability of drug and/or admixture, as shown in Figure 2.2.
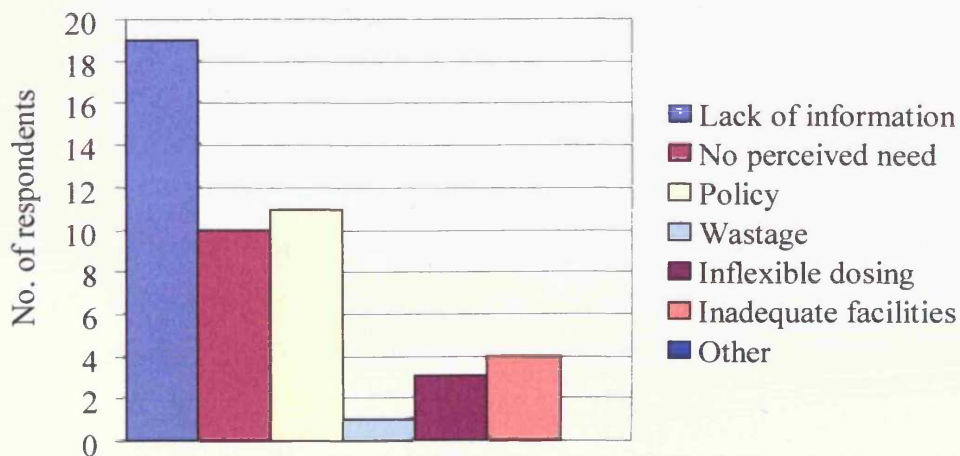


*Figure 2.2 Reasons for Not Adding Drugs to PN*

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Although these respondents did not add drugs to PN, they suggested some drugs that they considered may be useful to include in PN, such as insulin, histamine $H_2$ antagonists (e.g. ranitidine), analgesics (especially opioids), dopamine, midazolam, hydrocortisone and heparin. To a certain extent this list overlapped with the drugs used by those hospitals that did add drugs to PN.

Many of the respondents who did add drugs to PN indicated multiple reasons for doing so, hence the apparent inconsistency between numbers of respondents and responses in Figure 2.3. Few answers were given for "Other" namely:

> *"Less manipulations for home patients",*
> *"To decrease phlebitis risk to prolong peripheral access [using hydrocortisone]"*
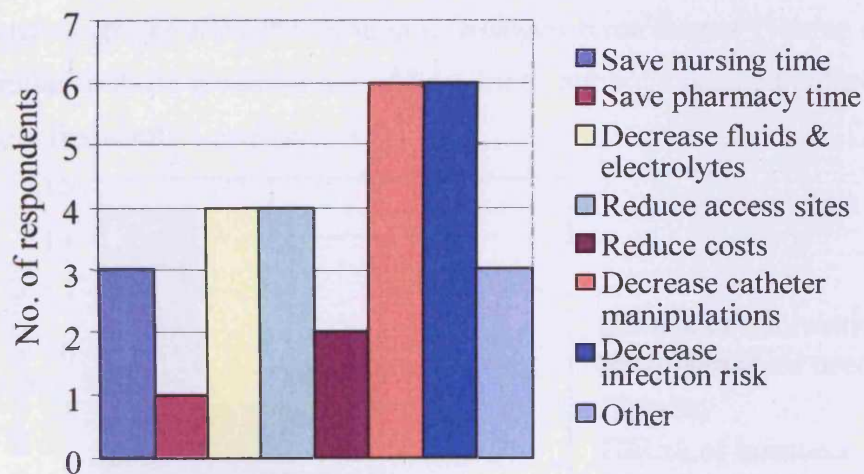


*Figure 2.3 Reasons for Adding Drugs to PN*

Reassuringly, all hospitals including drugs did so on the basis of stability information acquired most commonly from PN manufacturers (76.9%) and literature searches (53.8%) (see Figure 2.4).

Addition of drugs to PN was performed by pharmacists or pharmacy technicians in 92.3% of respondent's hospitals, with the remaining additions

being made by nurses.  Additions were made in an aseptic unit in every case.
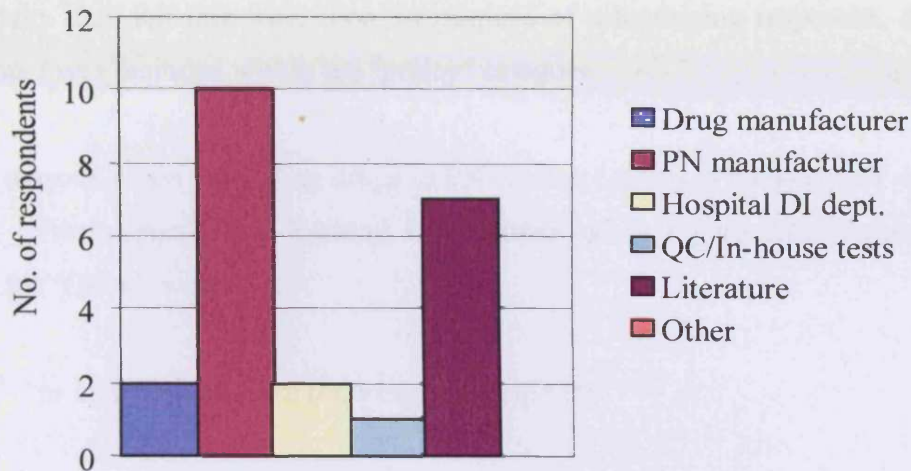


*Figure 2.4 Sources of Information (1)*

2.3.2.3 Section B - Y-Siting Drugs with PN Admixtures

Y-siting drugs was less common practice in the hospitals surveyed, with 75.4% of respondents never using the technique.  Reasons given for not Y-siting drugs were similar to those given for not adding drugs, although hospital policy was cited more frequently (see Figure 2.5).
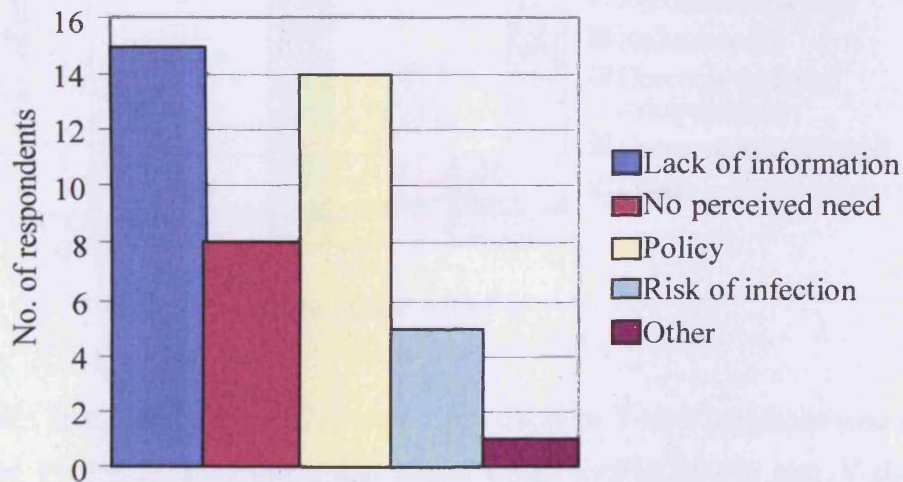


*Figure 2.5 Reasons for Not Y-Siting Drugs with PN*

Formal guidelines were not in place in most hospitals, but many trusts adhered to a general *"don't do it"* point of view.  Some respondents personally held this view and where no other arrangements were in place this became the default

policy for the respondent's hospital. Most of these respondents did not expand upon why they felt this way. For the purpose of categorising responses, this sentiment was included within the "policy" category.

Some reasons given for adding drugs to PN were not given as reasons to Y-site drugs. The responses for Y-siting are summarised in Figure 2.6. Reasons given for "Other" were:

*"to infuse a drug at a different rate to the PN"*

and

*"to administer glutamine if it hasn't been included in the PN"*



*Figure 2.6 Reasons for Y-Siting Drugs with PN*

Hospitals that added drugs to PN were more likely to Y-site than those who did not (see Figure 2.7); of those that added drugs to PN, 29.4% also Y-sited, whereas just 15.0% of those who did not add drugs Y-sited them. Again, all those engaged in the practice did so with stability information, although this time the drug manufacturer and literature sources were quoted as the most commonly used sources of information (each with 30.0%), followed by the PN manufacturer (20.0%), as shown in Figure 2.8.

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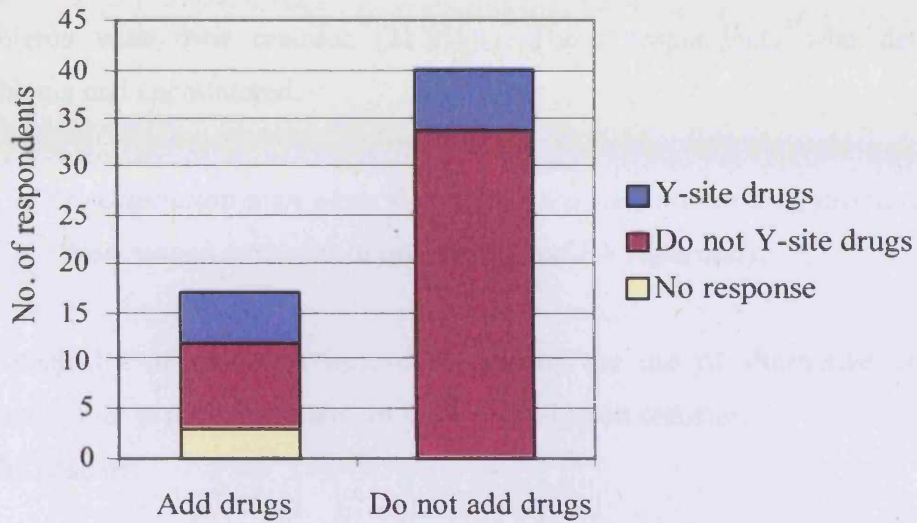
*Figure 2.7 Number of Respondents Adding and/or Y-Siting Drugs*
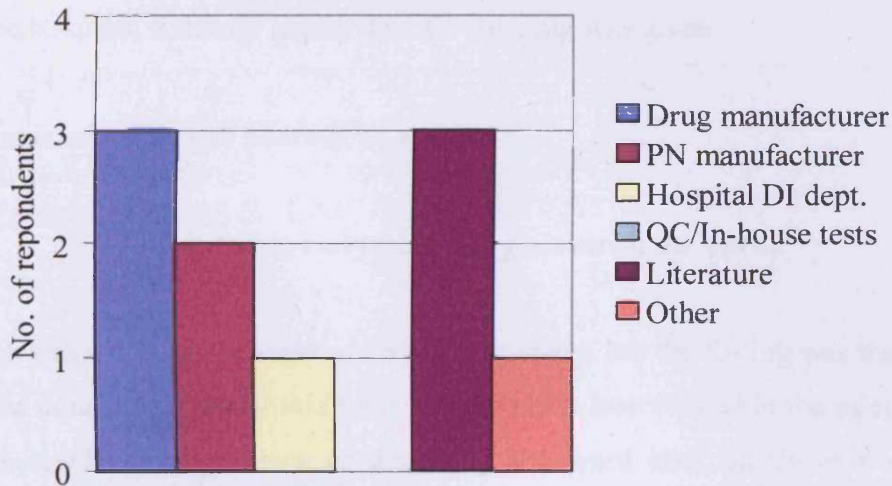


*Figure 2.8 Sources of Information (2)*

The drugs that were Y-sited included vancomycin, morphine, dopamine, dobutamine and insulin, overlapping significantly with those that most hospitals would like to add. Additionally drugs that were identified as desirable for Y-site administration were stated to be antibiotics, cyclosporin, inotropic drugs and *"Everything!"*.

2.3.2.4 Section C - Practical Issues

The majority of respondents who did add or Y-site drugs had experienced no

problems with their practice (71.9%). Those respondents who detailed problems had encountered:

*"Precipitation seen when Y-siting electrolytes, but no drug problems."*

*"Not enough iv access to give drugs and PN separately."*

The majority of problems were overcome by the use of alternative venous access, either at a different site, or via a multi-lumen catheter.

In the case of:

*"Drug degradation occurring in PN."*

at one hospital, a shorter expiry date for the drug was given.

An issue raised by one pharmacist was:

*"Not knowing everything that goes on on the wards."*

which was a concern brought up in the pilot study, but the feeling was that little can be done about this at this time. Pharmacists interviewed in the pilot study emphasised the importance of educating the ward staff on the role of the pharmacy and encouraging them to seek advice on matters of this type.

2.3.2.5 Section D - Guidelines

Guidelines were in existence in only 21 (36.8%) hospitals. Thirteen of these hospitals adopted a "blanket ban" approach on the addition of drugs to PN, and used this as their only guideline.

Of those with no guidelines, just 7 (12.2%) knew of plans to produce any. In all of these plans, the pharmacist was to be involved in writing the guidelines, clinicians were to be consulted in six cases, whilst five would involve nurses and dieticians and two would also confer with biochemists. Three respondents

mentioned that the nutrition support team would be likely to lead the production of the guidelines.

Five respondents included copies of their guidelines and three requested a summary of information gathered in the course of this research to compare with their practice.

## 2.3.3 DISCUSSION

### 2.3.3.1 Response Rates

It is quite possible that a number of questionnaires were not passed on by chief pharmacists to the appropriate person. It was not possible to obtain a list of the chief pharmacists in England so a large potential section of the appropriate working group were excluded from the study. The pharmacists on the BPNG list were by definition interested in clinical nutrition, which may have influenced the responses but these pharmacists are in a better position to comment upon current and potential future use of drugs in PN than pharmacists practising in other specialities. The sample size was not particularly large but, even though inferences should not be drawn about countrywide practice, useful data were gained and the comments and opinions elicited were very valuable.

### 2.3.3.2 Section A - Addition of Drugs to PN Admixtures

The number of respondents citing "lack of information" as a reason for not adding drugs to PN indicates that there is a need for such information. Many respondents who did not currently add drugs expressed an interest in doing so if the appropriate stability information became available to them. Those adding drugs did so with information from manufacturers in most cases which indicates that some stability information is available but is perhaps not easily accessible to all.

Trust policy was often cited as a reason for not adding drugs, but in many cases the policy was a non-specific ban on all additions.

Adding drugs to PN was used to improve clinical situations, for example to decrease catheter manipulations and therefore infection risk, or to bypass difficulties associated with the patient, for example to decrease the number of access sites or the fluid and electrolyte load. Cost and time savings were not as important to respondents as the aforementioned reasons.

Few different drugs were suggested for inclusion in PN, but those named were mentioned repeatedly.

Good practice dictates that any additions to PN should be made under aseptic conditions and all respondents adhered to this.

### 2.3.3.3 Section B - Y-Siting Drugs with PN Admixtures

Three quarters of the respondents did not Y-site drugs with PN and more hospitals had policies referring to this practice than for addition of drugs to PN, but these too were often just a "blanket ban" on the practice. Policy was cited nearly as frequently as lack of information as the reason for not Y-siting. Availability of information was as important a factor in decisions about Y-siting as it was for addition of drugs.

Patient-based reasons were more of a factor for Y-siting than for addition of drugs to PN but multiple reasons were usually given to justify the decision.

Patterns of availability of stability information were similar in Y-siting and drug addition.

### 2.3.3.4 Section C - Practical Issues

Few problems were reported by those pharmacists involved in the addition or Y-siting of drugs. Those encountered were dealt with in a pragmatic way and alternative approaches used to circumvent the problem. The responses suggest that pharmacists are inclined to look for practical ways to solve problems and

improve systems and this may explain some of the interest in adding drugs to PN.

Although it was not specifically asked about, concern about ward-based additions was raised by some respondents. All the guidelines submitted with the responses directed against this practice and it is not generally accepted as good practice but the pharmacists questioned in the pilot study felt that it was currently out of their hands. Investigation into this practice was not within the remit of this study but further work may ascertain its extent and implications.

### 2.3.3.5 Section D - Guidelines

That some hospitals have, and others are planning to write, guidelines implies that there is continuing interest in this subject. One respondent commented that:

> *"In my opinion the pharmacist is the only one qualified to write these guidelines."*

Most, however, would be relying on a multi-disciplinary team to write the guidelines and the involvement of all interested parties is more likely to result in the production of a document that is relevant and useful to all the healthcare professions involved in the care of patients needing parenteral nutrition.

## 2.3 CONCLUSION

The interest shown by the respondents to this questionnaire demonstrates that the research work to be undertaken would produce useful, relevant and much needed information on the use of drugs in conjunction with PN.

Attitudes within the profession of pharmacy are mixed towards the subject but pharmacists are not working in isolation in the care of the patient requiring PN, and the more drug administration options that are available the better the treatment of the patient may become.

# CHAPTER 3
# MATERIALS & METHODS

# CHAPTER 3 – MATERIALS & METHODS

## 3.1 PARENTERAL NUTRITION ADMIXTURE

### 3.1.1 CHOICE OF REGIMEN

The regimen used throughout the course of the study was Fresenius Kabi UK Ltd (FK) Regimen 5. This is a standard regimen suitable for patients with "moderately increased" nutritional requirements. It provides 13.5 g nitrogen and 2200 non-protein kilocalories in 2500 ml (Anon. 2002a). Standard doses of vitamins and trace elements were included in the regimen as were the maximum allowable additions of electrolytes (see Table 3.1). Regimen 5 was chosen after discussion with medical information staff at FK. It was proposed on the basis that it was a typical regimen suitable for use in a variety of clinical settings and was similar to regimens available from other manufacturers.

### 3.1.2 COMPOSITION OF REGIMEN

*Table 3.1 Composition of Regimen*

| Preparation | | Quantity / ml | Manufacturer |
|---|---|---|---|
| Vamin® 14 | Amino acid | 1000 | FK |
| Additrace® | Trace elements | 10 | FK |
| Glucose 10% | | 500 | FK |
| Glucose 50% | | 500 | FK |
| Addiphos® | Electrolyte | 15 | FK |
| Intralipid® 20% [A] | Lipid emulsion | 500 | FK |
| Vitlipid® N Adult [A] | Fat soluble vitamins | 10 | FK |
| Solivito® N [B] | Water soluble vitamins | 1 vial | FK |
| Sodium chloride 30% | | 49.2 | Martindale, Romford, UK |
| Potassium chloride 15% | | 150.3 | Antigen Pharmaceuticals Roscrea, Ireland |
| Calcium chloride 1 mmol/ml | | 7.5 | Martindale, Romford, UK |
| Magnesium sulphate 50% | | 2.2 | Torbay PMU, Torbay |
| Zinc sulphate 50 μmol/ml | | 3.0 | Sterile Products UHW, Cardiff |
| Water for Injections | | 10 [C] or 500 [D] | FK |

Notes:

[A] The lipid based preparations (Intralipid® and Vitlipid® N) were omitted from the test aqueous bags to allow stability testing in the aqueous phase.

[B] Reconstituted with Vitlipid® N for the lipid test admixtures and WFIs for the aqueous test admixtures.

[C] For reconstitution of Solivito® N.

[D] For volume replacement of lipid in some experiments.

See Appendix 4 for batch numbers and expiry dates.

## 3.1.3 PREPARATION OF REGIMEN

Regimens were prepared a maximum of three days before use and stored at 2 to 8°C until required. Previous stability testing ensured that the admixture would remain physically and chemically stable for the duration of each test. Bags were brought to room temperature before the start of each experiment and for the addition of drugs.

All admixtures were prepared under aseptic conditions in a Class II horizontal laminar flow cabinet (MDH, Andover, UK). Regimens were prepared in 3 litre ML infusion bags (FK) to minimise oxygen ingress during storage and experiment periods. Using an adaptation of the mixing protocol laid down in "Complete admixtures for parenteral nutrition" (Anon. 2002a) the admixtures were prepared in the following order:

1) The Vamin® solution was transferred into a sterile 3 litre ML bag.

2) Additrace® was transferred into the bag using a 10 ml syringe and 19 gauge needle.

3) Both glucose solutions were added to the bag.

4) Addiphos® was transferred into the bag using a 20 ml syringe and 19 gauge needle.

5) Sodium chloride 30% and potassium chloride 15% were added using 50 ml and 1 ml syringes and 19 and 21 gauge needles.

6)  Calcium chloride 1 mmol/ml was added using 10 ml and 1 ml syringes and 19 and 21 gauge needles.

7)  Magnesium sulphate 50% was added using a 2 ml syringe and 21 gauge needle.

8)  Zinc sulphate 50 $\mu$mol/ml was added using a 5 ml syringe and 21 gauge needle.

9)  Intralipid® 20% was transferred into the bag (lipid regimen only).

10) Solivito® N was reconstituted with 10 ml of either Vitlipid® N Adult or WFIs, and added to the bag using a 10 ml syringe and 19 gauge needle.

11) Air remaining in the bag was removed by compressing the bag, to minimise the potential for oxidation of components, and the bag was then sealed with the supplied locking device before storage.


Between each step the bag was gently agitated to ensure mixing of contents, and observed for signs of instability. The test drugs were the final addition to each bag, added aseptically at 0 hours for each experiment.


## 3.2 DRUGS

### 3.2.1 CHOICE OF DRUGS

The drugs in the study were chosen following analysis of the questionnaire replies. They are representative of the pharmacological classes of drugs that hospital pharmacists displayed an interest in adding to PN admixtures. Some, such as ranitidine, have already undergone some stability research (Baumgartner et al. 1997; Trissel 2001; Vehabovic et al. 2003; Williams et al. 1990) but others have very little data available.


As early as 1973, consideration was being given to including drugs in PN (Feigin et al.). There were many advantages to doing so including time savings, decreased infection risk and decreased fluid loads for patients. Driscoll et al. (1991) summarised the necessary characteristics of a drug in order for it to be considered for inclusion in PN. These were defined as:

- Stable dosage regimen over 24 hours;
- Appropriate pharmacokinetics for continuous infusion;
- Physical compatibility with PN components;
- Chemically stable over 24 hours;
- Stable infusion rate.

## 3.2.2 DOSES

The drugs were added to the test PN admixtures at an appropriate dose based on their clinical indication (Table 3.2) (BNF 2004).

*Table 3.2 Drug Doses and Manufacturers*

| Drug name | Presentation | Manufacturer | Study conc$^n$ mg drug/ml PN |
|---|---|---|---|
| Ranitidine (Zantac™) | 50 mg/2 ml | GlaxoSmithKline, Uxbridge, UK | 0.20 |
| Midazolam (Hypnovel®) | 10 mg/2 ml | Roche, Welwyn Garden City, UK | 0.15 |
| Tramadol (Zydol™) | 100 mg/2 ml | Searle, High Wycombe, UK | 0.08 |
| Hydrocortisone sodium phosphate (Efcortesol™) | 500 mg/5 ml | Sovereign, Basildon, UK | 0.02 |
| Hydrocortisone sodium succinate (Solu-Cortef®) | 100 mg/2 ml$^A$ | Pharmacia & Upjohn, Milton Keynes, UK | 0.02 |
| Heparin sodium (Monoparin®) | 1000 IU/ml | CP Pharmaceutical Wrexham, UK | 1 IU/ml PN |
| Dalteparin sodium (Fragmin®) | 2500 IU/ 0.2 ml | Pharmacia, Milton Keynes, UK | 1 IU/ml PN |

Note:

$^A$ when reconstituted with WFIs

See Appendix 4 for batch numbers and expiry dates, and Appendix 5 for drug degradation products.

## 3.3 REAGENTS

*Table 3.3 Reagents and Suppliers*

| Reagent | Supplier |
|---|---|
| Acetonitrile (HPLC Grade) | Fisher Scientific UK, Loughborough |
| Ammonium acetate (HPLC Grade) | Fisher Scientific UK, Loughborough |
| Hexane (Analytical Reagent Grade) | Fisher Scientific UK, Loughborough |
| Hydrogen Peroxide | Fisher Scientific UK, Loughborough |
| Methanol (HPLC Grade) | Fisher Scientific UK, Loughborough |
| Monobasic potassium dihydrogen orthophosphate (Analytical Reagent Grade) | Fisher Scientific UK, Loughborough |
| Monobasic sodium phosphate (Analytical Reagent Grade) | Fisher Scientific UK, Loughborough |
| Orthophosphoric acid (Analytical Reagent Grade) | Fisher Scientific UK, Loughborough |
| Sudan Red III | Sigma-Aldrich Ltd UK |
| Toluidine Blue O | Sigma-Aldrich Ltd UK |
| Triethylamine (HPLC Grade) | Fisher Scientific UK, Loughborough |

See Appendix 4 for batch numbers and expiry dates.

## 3.4 EXPERIMENTAL DESIGN

### 3.4.1 SAMPLING

Before sampling, the bag was turned end over end twenty times to ensure complete mixing. Prior to this, observations were made of the bulk admixture, to evaluate the admixture's colour and the extent of any of phase separation.

All samples were taken aseptically in the laminar flow cabinet to minimise the risk of microbial contamination, using a sterile needle and syringe and put into a clean container. The needle, syringe and sampling container varied with test and were of appropriate size and nature for the particular tests to be carried out. In each case, an excess amount of test admixture was removed from the bag via the injection port and the sampling container was rinsed three times with

sample before being filled.


## 3.4.2 EXPERIMENTAL SET-UP

Compounded drug-PN admixtures were hung at room temperature for 48 hours. In current practice a bag of PN is delivered over a maximum of 24 hours, but a longer test period was used to ensure admixture and drug stability for at least a 24 hour dosing period.

Physical tests were conducted hourly between 0 and 6 hours and then at 12, 24, 36 and 48 hours, as the samples were taken.

Samples for chemical testing were collected at 0, 3, 6, 12, 24, 36 and 48 hours, then frozen in microcentrifuge tubes (Fisher Scientific, Loughborough, UK) in a Nuaire -86°C Ultralow Freezer (GS Laboratory Equipment, Asheville, NC, US) and kept until all samples had been taken.

To ensure that freezing the samples would not damage the drug, frozen storage validation studies were undertaken. Three ampoules of each drug were selected from the same batch. One was stored intact in the original box and the remaining two were divided into two samples. One pair (one sample from each ampoule) was frozen, and the other pair stored at room temperature, protected from light. All samples were stored for one week, subjected to HPLC analysis and the results compared.

Control test admixtures had no drug added and were prepared and sampled at the same time as the test admixtures.

Light control samples were hung in a laboratory with fluorescent strip lighting and no direct sunlight. Admixtures that needed to be protected from light were overwrapped with aluminium foil and stored in a closed light protective container. Admixtures exposed to light were kept next to a south-facing window for the duration of the test.

In experiments carried out to ascertain the degree of light protection provided by the inclusion of lipid emulsion in a PN admixture, serial dilutions of Intralipid® 20% in glucose solution were scanned in a U-3000 Spectrophotometer (Hitachi, Tokyo, Japan) over the visible wavelengths (400 to 700 nm). The apparatus reports the amount of light transmitted through a sample as a percentage of the incident light. The results are presented in Table 3.4.

*Table 3.4 Percentage Light Transmission Through Lipid Emulsion*

| $\lambda$/nm | Concentration of Intralipid 20% in Glucose Solution |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.1% | 0.2% | 0.3% | 0.4% | 0.5% | 1% | 2% | 5% | 10% | 20% | 50% |
| 400 | 7.29 | 3.28 | 1.45 | 1.02 | 0.73 | 0.33 | 0.18 | 0.06 | 0.01 | -0.02 | -0.03 |
| 450 | 9.45 | 5.67 | 2.27 | 1.25 | 0.85 | 0.36 | 0.20 | 0.08 | 0.03 | -0.01 | -0.02 |
| 500 | 11.60 | 9.31 | 3.95 | 1.66 | 1.12 | 0.38 | 0.21 | 0.09 | 0.04 | 0.00 | -0.02 |
| 550 | 13.61 | 13.85 | 6.66 | 2.33 | 1.70 | 0.41 | 0.21 | 0.09 | 0.04 | 0.01 | -0.01 |
| 600 | 15.42 | 18.82 | 10.30 | 3.26 | 2.77 | 0.48 | 0.21 | 0.09 | 0.04 | 0.01 | 0.00 |
| 650 | 17.08 | 23.95 | 14.63 | 4.48 | 4.43 | 0.66 | 0.21 | 0.10 | 0.05 | 0.02 | 0.00 |
| 700 | 18.58 | 28.86 | 19.34 | 5.90 | 6.65 | 1.04 | 0.22 | 0.10 | 0.05 | 0.02 | 0.00 |

In the regimen used for these studies, Intralipid® 20% was present at approximately 20% by volume, so using the data from Table 3.4 light transmission would be minimal over the visible spectrum. However, the surface area of a three litre infusion bag is 1316 cm$^2$ and the apparatus makes its measurements in a cell with a 1 cm path length. Based on these approximations, half of the PN admixture will be exposed to UV light at any given time. Despite the low percentage of light transmission through most of the test mixtures, it is possible that a significant amount of light passes through some of the emulsion, but is not able to travel through the full 1 cm path.

The concentration of components in the aqueous bag was relatively higher than in the lipid test admixture due to the additional volume of the Intralipid®. Where this was thought likely to have an impact on stability, experiments were carried out with the Intralipid® replaced with an equal volume of WFIs. In some experiments, admixtures containing Intralipid® 10% in place of Intralipid® 20% were also prepared, as they have the same total volume but

decreased concentration of lipid emulsion, thus allowing evaluation of the impact of the concentration of lipid emulsion.

## 3.5 EXPERIMENTAL METHODS

### 3.5.1 INTRODUCTION

In order to ensure that a drug-PN admixture is safe to administer to a patient, it is necessary to carry out a full assessment of the stability and compatibility of the combination. There are many standard techniques used for stability testing each with its own attributes but none is suitable for use alone. By using a combination of techniques, it is likely that any incompatibilities will be detected.

There is little guidance from official sources regarding tests to be carried out and standards to be attained in those tests. The BP (2003) states that, in order to comply with their requirements:

> *"Emulsions for infusion do not show any evidence of phase separation."*

and

> *"Solutions for infusion, examined under suitable conditions of visibility, are clear and practically free from particles."*

There are two methods for the determination of particulate contamination detailed in the BP; light obscuration and microscopy. It states that light obscuration is to be preferred when examining injections and infusions for sub-visible particles, except in the case of preparations having reduced clarity, such as emulsions, in which case microscopy should be employed. The United States Pharmacopeia (USP) (2002) concurs, adding that:

> *"Any product that is not a pure solution having a clarity and viscosity approximately those of water may provide erroneous data when analysed by light obscuration counting methods."*

Beyond this, very little guidance is given, so definitions of instability used in this work have been taken from the literature.

## 3.5.2 VISUAL EXAMINATION

Whole bags of test admixtures were visually examined for signs of instability before sampling at each time point. For all types of test admixture, a record was kept of admixture colour and any changes in appearance noted.

The upper surface of each bag of test lipid admixture was closely examined for the presence of free oil, either as droplets or as a continuous layer, with depths of cream layers, where present, measured to the nearest millimeter. Any difficulty in redispersing cream layers was noted. It must be noted that the presence of a cream layer is not necessarily indicative of instability, but is the first, reversible, stage in the irreversible process of coalescence or "cracking" of the emulsion (Martin 1993). For both aqueous and lipid admixtures, 20 ml samples were syringed into clear glass sample tubes and examined for signs of instability against a black background, under standard fluorescent lighting conditions and using a cold fibre-optic light source (Schott KL1500 electronic, Schott, Wiesbaden, Germany), to aid visualisation of precipitation.

Examination for evidence of gross microbial contamination was carried out on all samples.

The major limitation of visual observation is the inability of the naked eye to detect the initial stages of emulsion instability. Indeed, enlarged lipid droplets are not seen unaided until they reach 40 to 50 $\mu$m in diameter in many cases (Driscoll 1990) but their presence is indicative of an unstable system (Driscoll et al. 1995) and they are potentially clinically harmful in that they can cause pulmonary embolism. However, even when considering this limitation, it is clear from the information that can be obtained that the technique is a useful one but is not to be used in isolation.

### 3.5.3 MICROSCOPY

Microscopy is the only particle sizing method in which individual particles can be observed. It requires little sample preparation, enabling examination of the particles as they are found in the test admixture. Dilution of samples, as is necessary in other particle sizing methods, can change the nature of the sample, for example by redispersing aggregates, but is not necessary when using this technique. Microscopy allows detailed inspection of a sample but should be used in conjunction with other particle sizing/characterisation techniques for full analysis, due to the small sample volume and subsequent statistical problems in finding isolated large droplets.

All samples (both aqueous and lipid) were examined using an Olympus BH-2 light microscope (Tokyo, Japan) with a phase contrast attachment to enhance the visibility of the particles in the sample. The microscope was calibrated using a stage micrometer. Signs of instability, characterised by precipitates or changes in lipid emulsion globule size and shape were noted, as were the maximum lipid globule size and the evidence of any other types of lipid emulsion instability, such as aggregation.

For the purpose of these studies, the term "aggregation" is used to describe the situation when a small number of particles (globules or crystals) attach to each other, but retain their individual boundaries (see Figure 3.1). "Flocculation" is used when particles are not directly connected to each other but more widespread areas of high particle concentration develop in the sample. The particles do not lose their boundaries but can lose globule shape in the case of lipid samples (see Figure 3.1).

Using a 1 ml syringe, one drop of sample was placed on a Thoma counting chamber welled slide (Weber, West Sussex, UK) and covered with a glass cover slip. Care was taken not to introduce any air bubbles into the samples when preparing the slides. Any excess sample was allowed to run into the well of the slide leaving a standard volume of admixture to be examined on the

calibrated grid, allowing accurate measurement of particles. Samples were examined at 100 times magnification initially, and at 200 or 400 times where necessary, to accurately record the size of particles or lipid globules greater than 5 µm in diameter.
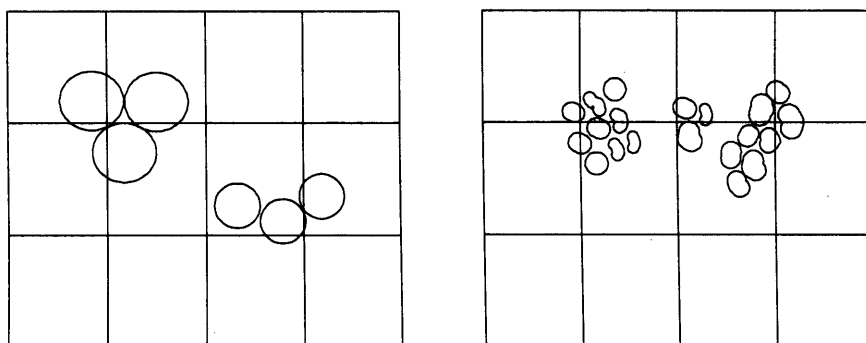


*Figure 3.1 a) Particle Aggregation   b) Particle Flocculation*

### 3.5.3.1 Neubauer Haemocytometer Slide Technique

Samples were also tested using the Neubauer slide technique (Minton et al. 1997), in which drug-free admixture was placed in one well of a Neubauer haemocytometer slide (Weber, West Sussex, UK) whilst the second well was filled with drug solution at stock strength. Cover slips were moved across the surface of the slide to allow the two phases to meet (see Figure 3.2). Interactions at the interface between the two liquids were then observed using the light microscope system described above and signs of lipid instability or precipitate formation noted. The tests were repeated with the drugs diluted to clinical doses.
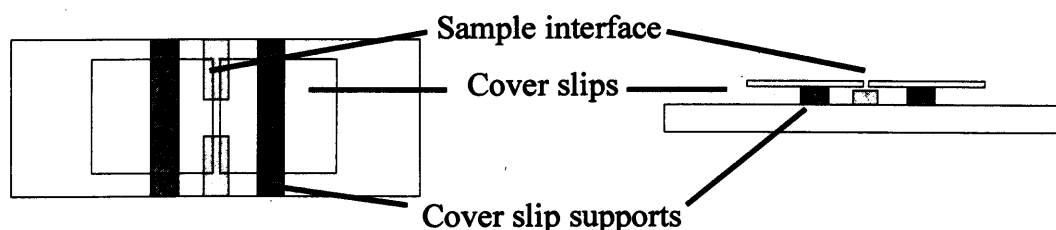


*Figure 3.2 Neubauer Haemocytometer Slide*

This technique allows rapid analysis of the interaction between drug solution

and PN admixtures at a 1:1 ratio, which simulates the interaction seen during Y-siting and tests the compatibility at the highest relative concentration. Repeating the experiments with solutions at the clinical concentration simulates the interactions occurring after the drug has been admixed with the PN. This has been shown to be a rapid and useful screening tool in drug-PN stability assessment (Price and Cosslett 2001) suitable for indicating whether a drug is likely to be compatible with an admixture, and thus saving time by quickly identifying unsuitable combinations.

### 3.5.4 LASER DIFFRACTION

Laser diffraction enables analysis of the particle size distribution of the lipid admixture-drug samples to be undertaken. The technique analyses the diffraction patterns from particles in a sample solution through which a laser beam is passed. From the patterns produced a determination of the size distribution of the particles can occur. It does not report oversized particles unless a significant volume of the sample is composed of them. This lack of sensitivity necessitates the use of other techniques such as microscopy for a full evaluation of particle size (Akasah 2002).

Laser diffraction was performed using a Malvern Mastersizer X (Malvern, UK) with a small volume sampler (SVS) attached. The calculations were performed using Version 2.19 of the Malvern software. The SVS was connected to the flow through measuring cell which has a low power helium-neon laser incident upon it. The SVS was filled with fresh particulate free water obtained from a Barnstead NANOpure Ultrapure water system (Dubuque, IA, USA) for use as the dispersant. The dispersant was then circulated at a constant flow rate through the measuring cell, ensuring air bubbles were not introduced. A measurement sequence involved a background reading being obtained, then the test samples gradually being introduced into the SVS until a light obscuration of approximately 20% was reached. When a particle passes through the analyser beam, light is scattered at an angle relative to the size of the particle as shown in Figure 3.3. All of the light reaches a receiver lens, where unscattered

light is monitored in order to calculate the sample volume concentration whilst the scattered light is gathered into groups of discrete angles of scatter by the detector, allowing size analysis of the particles present.

The instrument was set to perform 30000 "sweeps" per measurement and then to collate the data, before presenting a summary of the volume of sample of each particle size present. Three repeat measurements of each test sample at each time point were measured and the size distribution and maximum particle size assessed. The accuracy of the instrument was checked by measuring latex spheres of known diameter. For result comparison the D[4,3], the mean diameter of the volume distribution, was chosen as a suitable parameter for comparing particle size data for emulsions (Anon. 1993). Where relevant, the volume percentage greater than 5 μm and of any secondary peak was recorded.
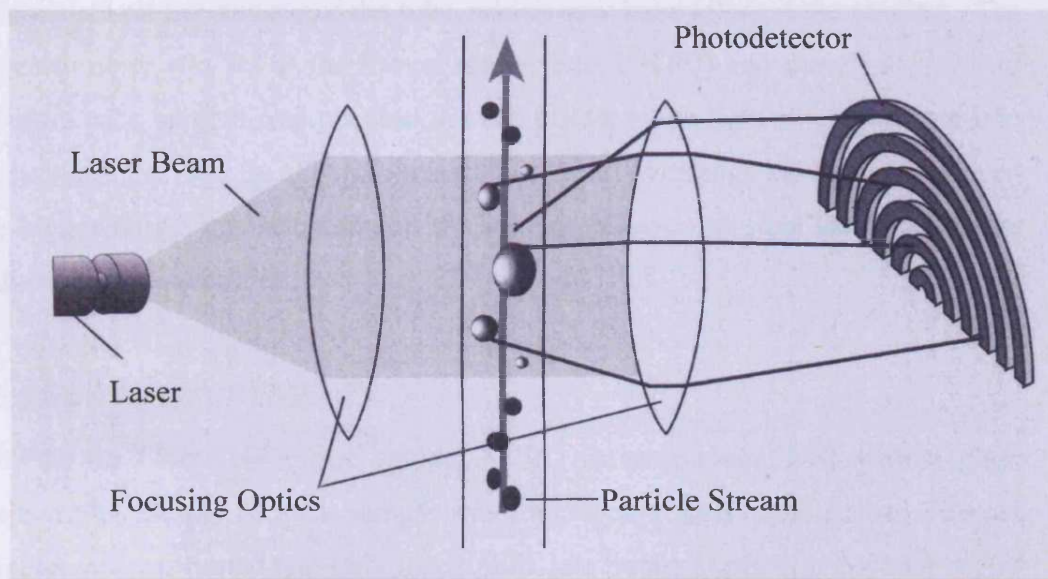


*Figure 3.3 Laser Diffraction*

Barber (1993)

### 3.5.5 NEPHELOMETRY

The Hach turbidity meter (Ratio XR, Loveland, Colorado, US) detects light obscuration caused by suspended particles in a non-opaque suspension, quantifying the degree of precipitation in a sample. A beam of light is shone through the sample, scattering as it encounters particles. The scattered light is

measured at right angles to the incident beam. As particle size and aggregation increases, light diffraction and scattering increases, causing a reduction in transmitted light and thus indicating an increase in turbidity (Hach et al. 1990). A change of greater than 0.5 NTU (nephelometric turbidity units) over time may indicate a change in the nature of the sample and was used as a definition of instability in this work. Nephelometry is sensitive to the presence of fine precipitates so it can detect those not seen during visual examination, however it is not suitable for lipid-containing admixtures as the lipid makes the sample opaque.

The undiluted aqueous sample was presented in a clean Hach sample cell. Samples were placed in an ultrasonic bath to remove any air bubbles and the sample cell was then coated with a thin film of silicone oil to fill any fine scratches on the surface of the tube, which may have affected the reading. The turbidimeter was set at the lowest range (0 to 2 NTU) and the display zeroed before each sample was put into the cell holder. The light shield was put into place and the reading allowed to stabilise. If an overrange value was produced a higher range was selected and the reading allowed to stabilise again before the value was recorded.

### 3.5.6 pH MEASUREMENT

Using an Orion pH meter (model 420A, Beverly, MA, US) with a glass electrode, the pH of each sample was measured at each time point. The pH meter was calibrated regularly using phthalate buffer at pH 4, phosphate buffer at pH 7 and borate buffer at pH 10 (Fisher Scientific, Loughborough, UK). A pH change of greater than 0.5 units over the test period was defined as indicating instability.

Large changes in admixture pH over time are uncommon but may cause or be a sign of instability (Stella 1986; Trissel 2001) and should be identified. The lipid emulsion used in Regimen 5 is formulated at a pH of 8 (Anon. 2002a) for maximum stability. The emulsion stability conferred by the adsorption of

phospholipids at the oil-water interface is mediated via electrostatic repulsion between lipid particles. The phospholipids are surface active agents having a finite pH range over which they function. In the case of the phospholipid systems used in parenteral lipid emulsions this is 5.5 to 9.0 (Driscoll et al. 1995). Inclusion of components such as glucose that decrease the pH of the admixture to below pH 5 will reduce the charge of the phospholipids, leading to destabilisation of the PN admixture (Driscoll et al. 1986). Conversely, allowing the pH to rise significantly will increase the likelihood of calcium phosphate precipitation. Knowledge and control of the pH of a PN admixture is therefore essential when considering its stability profile.

pH readings are less important in terms of identifying instabilities but a significant increase or decrease may indicate that physical or chemical changes are occurring within the PN admixture (Driscoll et al. 1995).

### 3.5.7 CENTRIFUGATION

Trissel et al. (1999) described a method for determining drug-PN compatibility in which the drug-PN admixture is centrifuged to accelerate precipitate formation or emulsion separation or cracking. In this adaptation of the technique, equal volumes of test admixture and drug (total volume approximately 30 ml) were placed in three centrifuge tubes and spun at 15000 rpm for 20 minutes at 20 to 24°C in a Sorvall Ultracentrifuge (Du Pont, Wilmington, Delaware, US). The rotation rate was necessarily increased from that of the original study, to ensure that the phases separated each time. A fourth tube containing PN alone was used to act as a control and to balance the rotor. Centrifuged samples were visually examined to determine the presence of free oil or precipitation. The experiments and observations were repeated using clinical doses of drugs shown to be unstable in the first experiments. This technique allows examination of precipitation in the aqueous phase of lipid-containing admixtures, which would otherwise not be possible due to the opacity of the samples.

## 3.5.8 OSMOLALITY

The Advanced Osmometer (Model 3D3, Advanced Instruments Inc., Norwood, Massachusetts, US) measures osmolality, a unit of concentration, using the principle of freezing point depression. This states that the freezing point of a pure solvent is lowered by the presence of a solute, by an amount directly proportional to the solute concentration. Osmolality values are clinically useful as they indicate whether a PN admixture may be suitable for peripheral administration. Hypertonic solutions i.e. those with an osmolality in excess of 1000 mOsm/kg (Madan et al. 1992) can cause thrombophlebitis when infused over an extended period, damaging the peripheral vasculature (Madan et al. 1992) so are not suitable for peripheral administration.

Samples (0.2 ml) were pipetted into sample tubes and placed into the osmometer. On starting the measurement sequence the sample tube entered a cooling chamber and a probe and stir/freeze wire were automatically lowered into the test sample. The sample was then supercooled i.e. cooled below its freezing point without solidifying, then induced to crystallise by the stir wire. The heat of fusion released as the water in the sample crystallises raises the temperature of the sample to its freezing point. The recorded freezing point was converted to a measurement in milliosmoles and presented.

Osmolality is concentration dependent and does not change over time so was measured only once for each experiment.

## 3.5.9 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

A ThermoFinnigan HPLC system with ChromQuest 3 Software and a Genesis C18 cartridge column (15 cm, 4 μm, 4.6 mm ID) with guard column (C18, 1 cm, 4 μm, 4.6 mm ID) from Jones Chromatography was used to perform HPLC analysis. Calibration curves were constructed (see Appendix 6) and degradation studies performed for all drugs analysed using this method to ensure that the assays were stability indicating and that degradation peaks did not overlap with peaks from undamaged drug.

All samples were suitably diluted before analysis in order to obtain an appropriate concentration of drug in the HPLC test sample. If insufficient drug is injected, it will be undetectable or the results unrepeatable, and if excessive amounts are used the samples can overload the column, giving erroneous results and possibly damaging the column. The dilution factor was often a compromise between injecting the highest possible amount of drug and not overloading the column with PN.

Frozen samples were defrosted as close to the time of analysis as possible, to minimise further drug degradation, and then diluted with mobile phase. Samples were measured using a pre-calibrated micropipette (Gilson, France), and made up to volume with mobile phase in a Class A, 10 ml volumetric flask (Fisher Scientific, Loughborough, UK). Diluted samples were placed in clear glass autosampler vials (Fisher Scientific, Loughborough, UK) and sealed. The amber autosampler lid minimised the amount of light entering the tray. The order of analysis was designed to minimise the wait time between HPLC runs and therefore reduce the possibility of further drug degradation (see Table 3.5).

*Table 3.5 Order of Analysis*

| Run Number | Sample time / hours |
|------------|---------------------|
| $1^{st}$ | $0^A$ |
| $2^{nd}$ | 3 |
| $3^{rd}$ | 6 |
| $4^{th}$ | 12 |
| $5^{th}$ | 24 |
| $6^{th}$ | 36 |
| $7^{th}$ | 48 |
| $8^{th}$ | $0^B$ |
| ... | ... |
| $35^{th}$ | 48 |

Note:

$0^A$ = 0 hour, $1^{st}$ repeat

$0^B$ = 0 hour, $2^{nd}$ repeat

Mean 0 hour peak areas were defined as being the 100% level (i.e. no drug degradation had occurred). This "external standard" method of calculating drug remaining was chosen over an "internal standard" method as the inclusion of another compound, when trialled, created further difficulties in assay development, such as overlapping peaks of the standard and the compound of interest, and may even have had an effect upon drug and/or PN stability. Solid phase extraction was attempted in order to remove the PN components from the admixture, but the degree of drug recovery was not reproducible so the technique was not utilised.

In practice, the shelf-life of a product is defined as the period during which the product remains stable and suitable for use. In quantitative terms, it is generally accepted as being the time from manufacture to when the drug reaches 90% of its original concentration ($t_{90}$), and this definition has been adopted for this study.

The $t_{90}$ given for a drug is an approximation based on the drug concentration recorded at the nearest time point available. Linear trendlines have been used for some assays to give an indication of the degradation rate but data cannot be extrapolated beyond the test period using the linear model, as drug degradation kinetics have not been studied in this work.

## 3.5.10 DATA ANALYSIS

For each drug, the mean of the repeat HPLC drug peak areas at 0 hours was calculated and set as 100%. Each peak area obtained was then compared to the mean 0 hour area to give the percentage drug remaining at that time point, the means of the repeats for each time point were then calculated. Relative standard deviation (RSD) at each time was calculated and used as error bars on the graphs of percentage drug remaining against time. A drug displaying degradation of 10% or less was defined as being stable over the time tested.

One-way analysis of variance (ANOVA), followed by Tukey's method, was performed using Minitab Statistical Software (Minitab Inc. Version 13.32),

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ANOVA being a statistical test used to determine if differences exist between groups, based on one variable in the case of one-way ANOVA. In each case, ANOVA determines whether a difference exists; Tukey's method was then used as a complementary test to ascertain where the differences lay. Statistical analysis was used to assess the significance of any change in drug concentration over time for each admixture and to assess differences between admixtures at any given time point. Where appropriate, p values have been cited; a p value $< 0.05$ was used as the level for significance.

## 3.6 SHELF-LIFE RECOMMENDATIONS

The shelf-life recommendations given for each drug are based on an assessment of all of the available evidence. It must be remembered that the shelf-life begins as soon as the drug is added to the admixture, and the time taken for the compounded admixture to reach the patient from the pharmacy must be taken into consideration, and this could result in an unacceptably short period over which to infuse the admixture.

# CHAPTER 4
# RANITIDINE

# CHAPTER 4 - RANITIDINE

## 4.1 INTRODUCTION

### 4.1.1 RANITIDINE IN PARENTERAL NUTRITION

Ranitidine is one of the histamine $H_2$ receptor antagonist group of drugs which reduce gastric acid output volume and acid and pepsin content via histamine $H_2$ blockade, thus relieving ulcerative conditions. Zantac™ (Ranitidine hydrochloride) is indicated for the treatment of ulcers, reflux oesophagitis and Zollinger-Ellison Syndrome and for prophylaxis of gastrointestinal haemorrhage from stress ulceration, acid aspiration (Mendelson's Syndrome) and recurrent haemorrhage in patients with bleeding peptic ulcers.

Patients receiving parenteral nutrition may develop gastric ulceration but this risk can be decreased by reducing gastric acid secretion and output (GlaxoSmithKline 2004) and by raising the pH of the stomach contents above pH 2.5 (Martindale 1999) by administering an $H_2$ antagonist (Grimble et al. 1991).

Numerous respondents to the questionnaire described in Chapter 2 noted either that their hospitals already included ranitidine in PN or that they would like to do so. It was the most commonly cited drug in the questionnaire and its physical compatibility with various clear fluid diluents (glucose solutions, normal saline and water) has been described in the literature (Trissel 2001). Some stability testing has also been performed in PN admixtures, but this testing, much of which was carried out in the US often using different solutions from those available in the UK, has not been comprehensive and conflicting results have been reported (Allwood and Martin 1995, Grimble et al. 1991, Trissel 2001).

Ranitidine is a specific, rapidly acting drug (GlaxoSmithKline 2004), given by a variety of administration methods, depending on patients' requirements. Intramuscular injection, slow intravenous injection, intermittent or continuous

intravenous infusion can be used. Continuous infusion is particularly advantageous in the treatment of PN patients because it maintains a stable blood level and therefore a stable gastric pH. This is not achieved by intermittent administration as $H_2$ antagonists cannot affect the pH of fluid already in the stomach. Doses vary with indication; for prophylaxis of stress ulceration in adult patients the manufacturer recommends a priming dose of 50 mg given over 2 minutes by slow intravenous injection, followed by a continuous intravenous infusion dose of 0.125 to 0.250 mg/kg/hour.

## 4.1.2 STABILITY ISSUES

### 4.1.2.1 Introduction

Zantac™ injection has been shown to be compatible with glucose, sodium chloride, sodium bicarbonate and Hartmann's solutions for up to 48 hours (Trissel 2001), although the manufacturer's recommendation limits this to 24 hours (GlaxoSmithKline 2004).

Many factors can influence the stability of ranitidine in a solution, some of which can be minimised or avoided, for example light and oxygen exposure, whereas others are fixed so can only be taken into consideration when admixing. This is especially the case in PN where admixture composition and therefore presence or absence of antioxidants, final admixture pH and buffering capacity, will be dictated by the patient's nutritional requirements and the hospital's formulary.
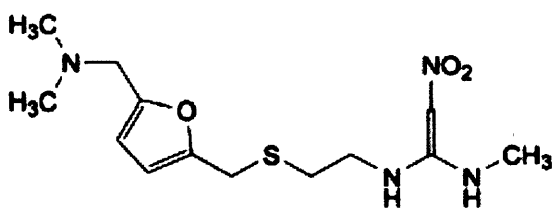


*Figure 4.1 Structure of Ranitidine*

(BP 2003)

## 4.1.2.2 pH

The pH of a solution containing ranitidine will exert an effect on the drug's stability. The drug is most stable at pH 7 and licensed injections are formulated between pH 6.7 and 7.3. pH mediated hydrolysis occurs via two mechanisms; one dominating at pH 2 to 4 and one at pHs greater than 9 (Inagaki et al. 1993). At intermediate pH both mechanisms operate (Haywood et al. 1987) but degradation is slow (Vehabovic et al. 2003). In PN admixtures, which are usually slightly acidic, some pH mediated degradation is likely but it is not expected to be the major factor influencing $t_{90}$.

## 4.1.2.3 Light

Ranitidine is known to be photolabile and as such both the drug and formulated medicines need to be protected from light (BP 2003; GlaxoSmithKline 2004; The Pharmaceutical Codex 1994; Trissel 2001). The published work on this subject has not investigated the impact of light on ranitidine in PN admixtures.

## 4.1.2.4 Air

Oxidation has been shown to be the main degradation mechanism of ranitidine (Allwood and Martin 1995; Trissel 2001) and the BP (2003) recommends storing the drug in an airtight container. When ranitidine is included in a PN admixture, a number of factors can influence the degree and rate of oxidation. The presence of trace elements, particularly copper, has been shown to have a detrimental effect on drug stability, by catalysing the oxidation reaction (Allwood and Martin 1995; Trissel 2001). The presence of antioxidants such as sodium metabisulphite from some amino acid formulations may have a protective effect on ranitidine by allowing preferential oxidation of the antioxidant instead of the drug, as is the case for antioxidant activity offered by vitamin preparations. Studies have also shown that ranitidine remained stable for longer periods when compounded in ML bags, compared to EVA bags which are semi-permeable to gases (Allwood et al. 1996).

### 4.1.2.5 Freezing

The Pharmaceutical Codex (1994) advises against freezing Ranitidine Injection USP. However, work carried out by several groups (Lampasona et al. 1986; Sarkar et al. 1991; Steward et al. 1990) showed no significant loss of drug when frozen for up to 100 days at -30°C. These studies did not investigate ranitidine in PN, necessitating the use of freezing studies as described in Chapter 3. The relative standard deviation between all results from the tests was less than 5% (RSD 3.34%), which was comparable to the deviation in the control of freshly opened ampoules (RSD 3.12%), so freezing the ranitidine in PN samples was deemed to be acceptable.

## 4.2 MATERIALS AND METHODS

### 4.2.1 MATERIALS

Zantac™ is an aqueous solution for injection, containing ranitidine hydrochloride HSE 56.0 mg/2 ml (equivalent to ranitidine 50.0 mg/2 ml ampoule), sodium chloride BP, potassium dihydrogen orthophosphate HSE, disodium hydrogen orthophosphate HSE and WFIs BP (see Table 3.2).

### 4.2.2 GENERAL METHODS

The methods described in Chapter 3 were used to assess the stability implications of adding ranitidine to parenteral nutrition.

### 4.2.3 METHOD SPECIFICS

#### 4.2.3.1 Experimental Specifics

The main mechanism of degradation of ranitidine has been shown to be oxidation (Allwood and Martin 1995; Trissel 2001), but since both light exposure and pH are also likely to have an influence on the stability of the drug in the PN admixture, experiments were designed to investigate all three influences.

In this study, two sets of admixture were prepared; one stored in ML infusion

bags and the other in EVA bags. All samples were protected from light and stored at room temperature.

Experiments were also set up to compare aqueous and lipid admixtures subjected to different lighting conditions. One of each type of admixture was protected from light, one was exposed to daylight and the control was exposed to fluorescent light.

The pH of the test admixture was fixed and dependent upon the pH of each constituent part and as such, it could not simply be adjusted to maximise the stability of a drug contained within it. Zantac™ injection is adjusted between pH 6.8 and 7.1 (GlaxoSmithKline 2004). The pH of the admixture tested was outside this range, and as such some pH mediated drug degradation may be expected. To examine this factor, ranitidine was added to two saline samples, one within the known stability range (pH = 6.9) and the other outside this range, and closer to the approximate pH of the PN admixtures tested (pH = 5.4). The samples were protected from light and stored at room temperature and HPLC analysis was carried out at 12 hour intervals.

## 4.2.3.2 HPLC Specifics

HPLC was performed using the system described in Chapter 3, using an adaptation of an assay described by Inagaki et al. (1993). The mobile phase consisted of 50% methanol and 50% 0.1 M ammonium acetate in water. This was used to dilute samples 1 in 10. A 20 μl injection loop was fitted to the injector. UV detection was set at 322 nm. The flow rate was set at 0.4 ml/min, leading to a retention time of the compound of interest of approximately 2.2 minutes. A peak attributable to PN was detected at approximately 1.7 minutes and this peak was clearly distinguishable from the ranitidine peak.

## 4.2.3.3 Other Methods

All physical tests were carried out as specified in Chapter 3 and compared to control admixtures containing no drug.

## 4.3 RESULTS

### 4.3.1 VISUAL EXAMINATION

Examination of the lipid based admixture containing ranitidine showed minimal creaming which never exceeded 3 mm in depth and was always easily redispersable. The admixture was off-white in colour and remained so throughout testing.

The test aqueous admixture was the same yellow colour as the control admixture throughout testing. Upon examination using a high intensity light source a fine green haze was seen. It did not visibly increase over time and was present to the same extent in both ranitidine-containing and control admixture.

### 4.3.2 MICROSCOPY

Very few enlarged lipid particles were seen in the ranitidine-containing admixture. One particle of 7.5 µm and one of 12.5 µm was observed at 24 hours. All other lipid globules were 5 µm or smaller at all time points. Control samples reached a maximum of 10 µm at 48 hours, with three particles having this diameter and one with a diameter of 7.5 µm. The only other time point with any particles greater than 5 µm was 0 hours when two particles of 7.5 µm were observed.

Microscopic examination of the aqueous admixture containing ranitidine showed a solution free from large particles.

#### 4.3.2.1 Neubauer Slide Technique

No evidence of lipid emulsion damage caused by ranitidine was seen, either at a 1:1 ratio or at the clinical dose.

One large crystal (25 µm) was observed when ranitidine was tested against the aqueous admixture at a 1:1 ratio. This was seen on only one occasion, and no

further evidence of incompatibility was seen using this technique.

### 4.3.3 LASER DIFFRACTION

No lipid droplets greater than 4.30 μm were detected in any ranitidine-containing samples, and many samples presented a maximum particle size of just 3.49 μm. The D[4,3] of samples was 0.37 μm or 0.38 μm in every case. There was no change in the profile of the particle size over time. The control samples shared the same profile as the drug-containing samples.

The values for the secondary peak, seen in all samples tested, consisted of the larger globules of the sample but these did not exceed 4.30 μm in diameter and the globules did not exceed 0.28% by volume for the ranitidine-containing samples and 0.21% by volume for the control samples. The peak constituted such a small percentage of the sample that it is not visible in Figure 4.2.
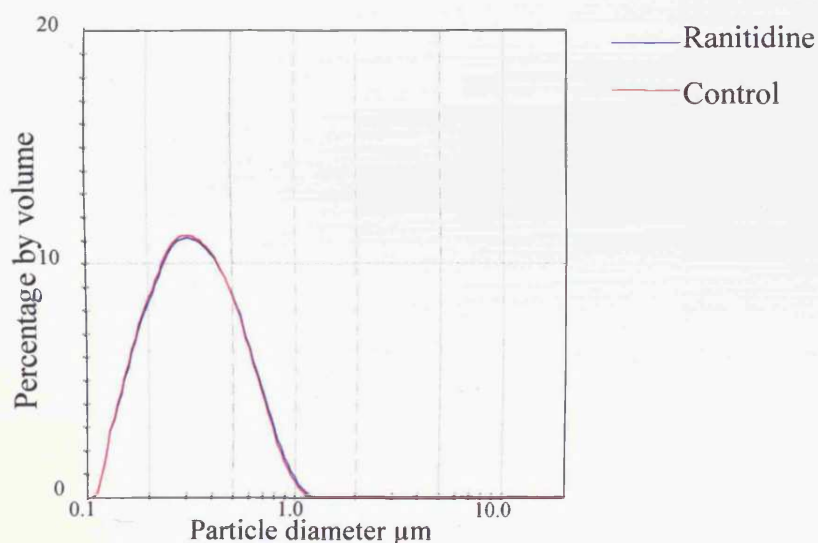


*Figure 4.2 Particle Size Distribution of Globules in Lipid Admixtures*

### 4.3.4 NEPHELOMETRY

Hach values fell steadily over the test period but the change over time was not greater than 0.5 NTU, the level used for indicating instability, as seen in Figure 4.3. Both ranitidine-containing admixture and control admixture decreased in

value over time, the control sample to a lesser extent.
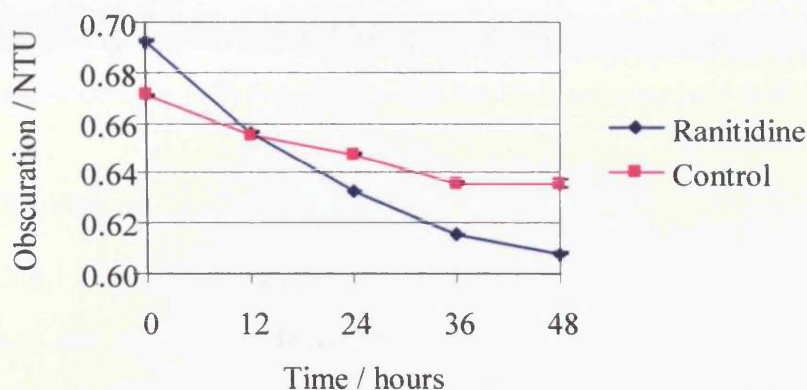


*Figure 4.3 Hach Values Over Time*

### 4.3.5 pH MEASUREMENT

Aqueous admixtures containing ranitidine had pH values ranging from 5.87 to 5.92, while the aqueous control ranged from 5.85 to 5.95.  Lipid admixtures containing drug varied between 5.93 and 5.99 and the comparable control values were between 5.90 and 5.97.

### 4.3.6 CENTRIFUGATION

In both the control and the ranitidine-containing lipid admixtures, centrifugation caused separation into one aqueous and one lipid layer.  A pellet was seen on the wall of the tube in both samples.  No free oil was liberated in any sample.  This was the case for both samples containing ranitidine at a 1:1 ratio and at a clinical dose.

Aqueous admixtures containing ranitidine, both at a 1:1 ratio and at a clinical dose, were visually identical to the control admixture.  The size of the pellet deposited on the wall of the tube was approximately the same in both samples.

### 4.3.7 OSMOLALITY

Osmolality values for aqueous samples containing ranitidine were slightly lower than values for aqueous control (ranitidine range 1605 to 1628

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mOsm/kg, control range 1634 to 1640 mOsm/kg) but the reverse was true for lipid-containing admixtures (ranitidine range 1411 to 1414 mOsm/kg, control range 1403 to 1406 mOsm/kg). The difference in the aqueous samples was not significant (p = 0.067) but it was for the lipid samples (p = 0.003).

## 4.3.8 HPLC

### 4.3.8.1 Degradation Studies

Acid and alkali degradation of ranitidine for 12 hours, resulted in split peaks and in a change in retention time, decreasing to approximately 1.65 and 1.79 minutes for the former and increasing to around 2.18 and 2.59 minutes for the latter, compared to a mean of 2.23 minutes for the control. Ranitidine was oxidised by treatment with hydrogen peroxide for 12 hours resulting in a chromatogram similar to that of the alkaline degraded sample, consisting of split peaks at approximately 2.20 and 2.57 minutes. The split peaks of the degraded samples were visibly distinguishable from those of the parent compound, as can be seen in Figure 4.4. Chemical structures of the known degradation products of ranitidine are shown in Appendix 5.
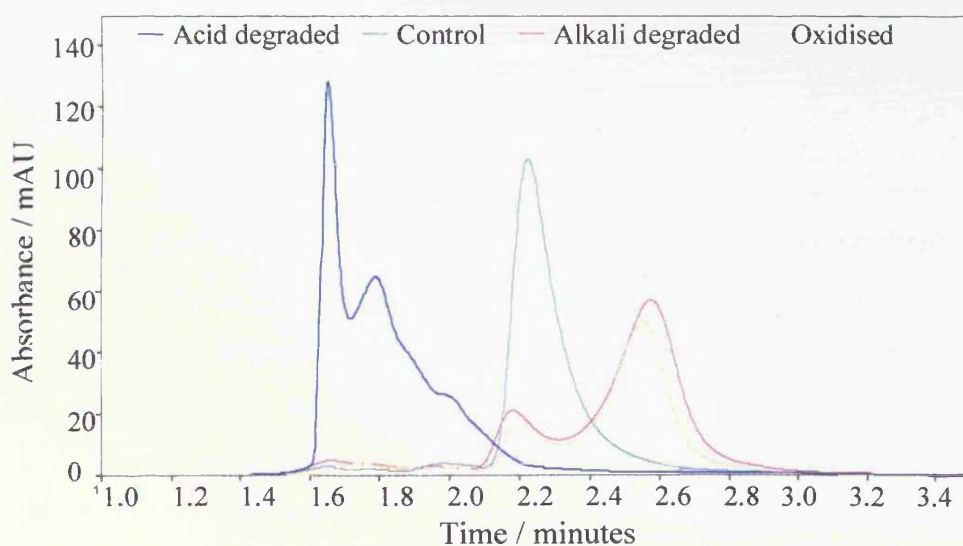


*Figure 4.4 Chromatogram of Degraded Drug Samples*

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4.3.8.2 Stability Studies

Lipid admixtures stored in both ML and EVA bags showed $t_{90}$s greater than 48 hours. The fall in drug concentration was initially more rapid in the ML (0 to 6 hours, rate = -1.15%/hour) than the EVA samples (-0.12%/hour) as seen in Figure 4.5. The difference in degradation rates caused a statistically significant difference between the two types of sample until 24 hours ($p \leq 0.012$), but when the degradation rate of the ranitidine in the ML samples slowed to -0.02%/hour there ceased to be a difference between ML and EVA bags ($p \geq 0.219$). Both storage conditions resulted in around 93% of drug remaining at 48 hours.
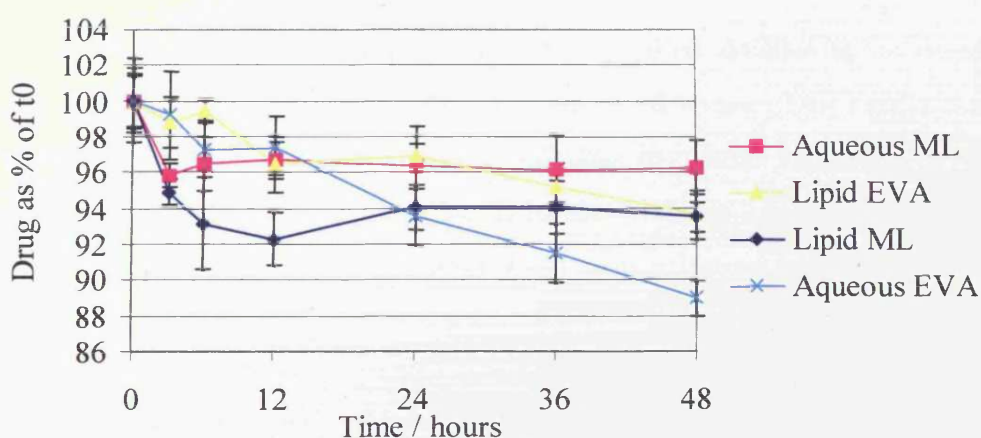


*Figure 4.5 Concentration of Ranitidine in Aqueous and Lipid Admixtures in Different Infusion Bags (± RSD)*

The degradation pattern seen in the lipid admixtures was repeated in the aqueous samples. A smoother line of degradation was seen in the EVA samples (-0.23%/hour) than the ML which again exhibited a large initial drop (-1.38%/hour) slowing to -0.01%/hour after 3 hours (see Figure 4.5). At 3 hours there was significantly more ranitidine remaining in the EVA bag than the ML ($p = 0.019$), but at the next two time points there was no difference ($p = 0.390$ and $0.518$). From 24 hours the ML bag contained significantly more ranitidine ($p \leq 0.009$). The ML samples had a $t_{90}$ in excess of 48 hours, by which time the level of ranitidine in EVA bags had dropped to 88.94% (RSD 0.99%).

Ranitidine concentrations in ML bags were consistently higher in the aqueous admixture than in the lipid admixture. However, this difference was not significant; it was within experimental error and did not lead to a different $t_{90}$.

Comparison of aqueous and lipid admixtures stored in EVA bags showed that ranitidine levels were consistently higher in the latter (see Figure 4.5). This difference was not significant until 24 hours, but it then remained so until the end of the test period ($p \leq 0.007$). The lipid admixture had a $t_{90}$ of 48 hours (93.60%, RSD 1.33%) compared to a $t_{90}$ of 24 hours (93.48%, RSD 1.54%) for the aqueous admixture.

Aqueous light controlled samples showed a gradual decline in ranitidine concentration, reaching 91.50% (RSD 1.27%) by 48 hours. This pattern was also followed by the light protected samples in which ranitidine levels degraded to 95.42% (RSD 1.52%) by 48 hours. Samples exposed to daylight showed very rapid degradation (-9.95%/hour) for the first six hours, slowing to -0.48%/hour from 6 to 48 hours. At no sampling point after 0 hours was the concentration of ranitidine greater than 90% in the samples exposed to daylight, as seen in Figure 4.6. These samples were significantly less stable than the other two admixtures at all times ($p = 0.000$). The light protected samples were significantly more stable than control at 36 and 48 hours ($p = 0.003$ and 0.002 respectively).
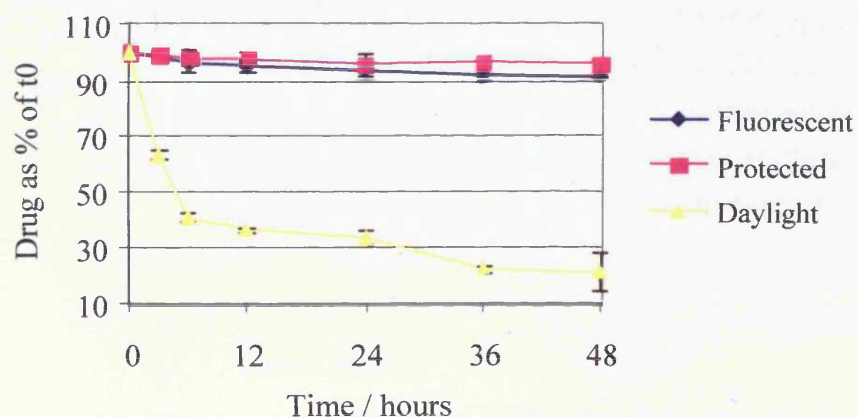


*Figure 4.6 Concentration of Ranitidine in Aqueous Admixtures Subjected to Different Lighting Conditions (± RSD)*

The concentration of ranitidine in lipid admixtures stored in the controlled light environment remained above 90% for the whole test period, reaching 96.04% (RSD 0.72%) at 48 hours. A similar degree of degradation was seen in the light protected samples, which reached 96.87% (RSD 0.69%) at 48 hours. There was no significant difference in ranitidine stability in these two storage conditions ($p \geq 0.090$). Levels of ranitidine in the admixture exposed to light dropped to below 90% by 3 hours (74.92%, RSD 1.52%). This was significantly lower than both of the previous samples ($p = 0.000$) (see Figure 4.7).
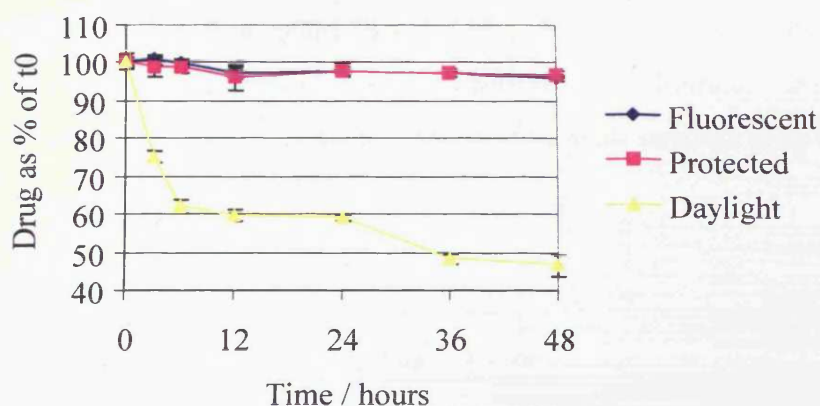


*Figure 4.7 Concentration of Ranitidine in Lipid Admixtures Subjected to Different Lighting Conditions (± RSD)*

Figure 4.8 shows the degradation of ranitidine exposed to the various lighting conditions in both aqueous and lipid samples.
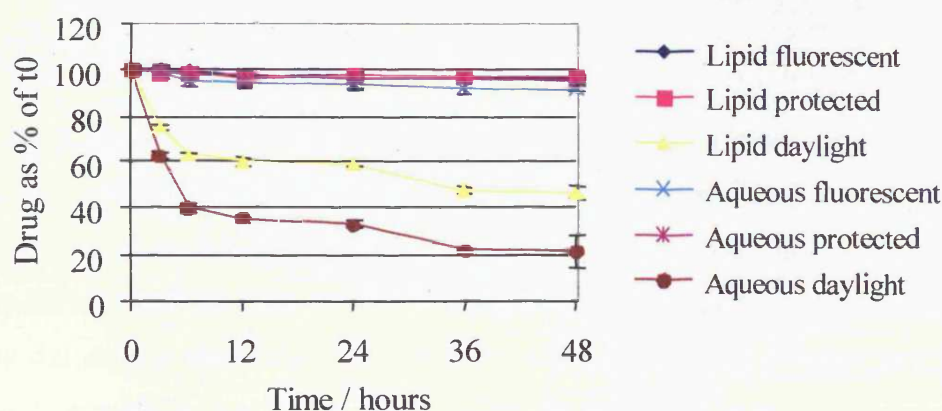


*Figure 4.8 Concentration of Ranitidine in Admixtures Subjected to Different Lighting Conditions (± RSD)*

Both control samples had $t_{90}$s in excess of 48 hours, but the aqueous had a steeper slope (-0.16%/hour) than the lipid sample (-0.08%/hour). The difference between the two samples became significant at 24 hours when $p = 0.003$. There was no significant difference between the two light protected samples ($p \geq 0.077$). In the samples exposed to daylight, levels of ranitidine were significantly higher in the lipid than the aqueous admixtures at all times ($p = 0.000$).

Analysis of the pH adjusted samples showed no significant difference between the samples at any time point ($p \geq 0.136$). Figure 4.9 shows that the drug degradation occurring over the test period was minimal and that the concentration of ranitidine remained above 96% in all samples at all times.
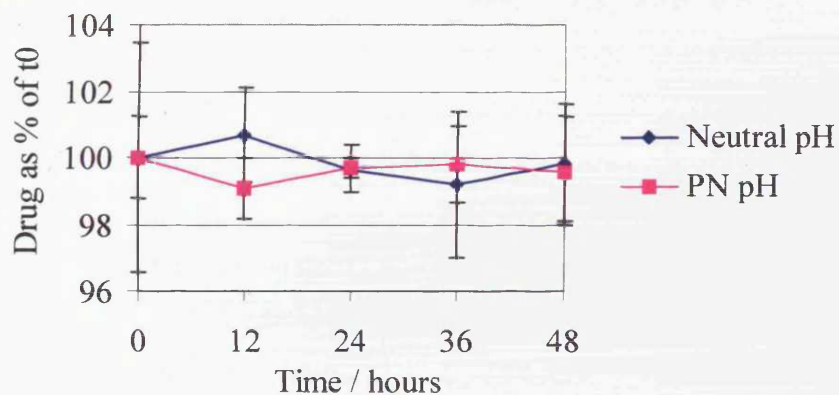


*Figure 4.9 Concentration of Ranitidine at Different pH Conditions (± RSD)*

## 4.4 DISCUSSION

The results of the physical assessments showed that the inclusion of ranitidine in the test admixtures had no detrimental effect on the physical stability of either the drug or the admixtures. In most tests, there was no significant difference between the control and the drug-containing admixture. Osmolality testing did show a slight difference, but this was not clinically relevant as the change in osmolality was insufficient to allow the admixture to be administered peripherally. Hach values for both the control and the ranitidine-containing admixture decreased over time, by less than the level indicative of instability, possibly due to the presence of undissolved solids from the reconstituted

vitamin preparation in the samples at the earlier time points.

The use of ML bags for storing lipid-containing admixtures did not seem to have an overall beneficial effect. The $t_{90}$ for both ML and EVA stored samples were in excess of 48 hours. The use of ML infusion bags has been shown to reduce oxidation of susceptible compounds by reducing air ingress (Allwood et al. 1996), but the rapid decrease in ranitidine concentration in the ML samples may be explained by the retention of more air in the ML bags than the EVA bags during compounding, this air then being available to interact with the drug. This did not occur to as great an extent in the EVA bags, either due to less air being introduced in the filling process or perhaps by the release of air into the atmosphere through the EVA bags. The exact amount of air introduced during compounding cannot be strictly regulated and may have caused the difference, although attempts were made to minimise this variation by following the mixing protocols detailed in Chapter 2. The slower rate exhibited in the second part of Figure 4.5 probably shows the stability profile after the excess air has been exhausted. The rate was then slower than the degradation rate of the EVA samples, demonstrating the reduction in air ingress by the ML bags. Future work should involve more detailed analyis of this, by measurement of the dissolved oxygen content, to confirm these results.

The shape of the graph seen in the aqueous samples stored in different infusion bags was the same as for the lipid samples, but unlike the lipid results the difference in degradation rate did result in different $t_{90}$s. The gradual decline in drug concentration in the EVA bag led to a $t_{90}$ of 24 hours, but the ML samples decreased at such a slow rate after the initial drop that a $t_{90}$ of 48 hours could be assigned.

Aqueous admixtures stored within EVA bags had the lowest concentration of ranitidine remaining at 48 hours and the shortest $t_{90}$. Ranitidine was significantly more stable in the lipid admixtures stored under the same conditions. If the presence of oxygen was likely to have a detrimental effect on

a drug in an admixture, preferential oxidation may occur with lipid emulsions, vitamin preparations or chemical antioxidants and may protect the drug, resulting in an increased $t_{90}$, at the expense of the stability of the oxidised component (see Section 1.2.3.2).

The omission of lipid emulsion from admixtures exposed to daylight caused a significant decrease in the amount of drug remaining when compared to the equivalent lipid-containing admixtures. The lipid offers some protection against UV-mediated degradation, but as can be seen from Figure 4.8, it is insufficient to be relied solely upon. The absence of light within the UV spectrum is clearly more important than the removal of all light, as can be seen by the difference between the control samples and daylight samples, compared to the difference between the control samples and light protected samples. However, the inclusion of lipid in the control samples (i.e. in the absence of UV light) becomes significant at 24 hours, suggesting improved drug stability with increased light protection. Although both the aqueous and lipid controls have a $t_{90}$ of 48 hours, it may be predicted by the slope of the graph that the aqueous admixture would not remain above 90% for as long as the lipid admixture. The presence of lipid appears to have had no other influence upon the stability of ranitidine in this admixture, as demonstrated by the lack of difference between the two types of light protected sample.

Although the drug is not within its optimum pH stability range HPLC analysis of the samples stored in different pH environments indicated that the increased acidity was not particularly harmful to the drug. The pH of the PN was therefore not thought to be the main factor in determining the $t_{90}$.

## 4.5 CONCLUSION

A number of conditions must be considered in assuring the safety of adding ranitidine to the test admixture and the relative importance of each of these has been evaluated in this study. Recommendations for shelf-lives of Regimen 5 admixture containing Zantac™ are given in Table 4.1.

Results from the physical tests show no detrimental interaction between ranitidine and the admixture at the clinical dose tested. Neubauer slide tests showed no incompatibility at a 1:1 ratio, indicating that the inclusion of higher doses than those tested in this study or the use of Y-siting may be possible, subject to results from all other physical stability tests at the increased dose.

According to the literature, oxidation is the main degradation mechanism of ranitidine and although this has not been supported by the results of this study, it is clear that this is one of several important factors in determining the chemical stability of the drug. Due to the potentially harmful degradation products of preferentially oxidised components, it may be preferable to use physical barriers, such as ML infusion bags, to protect drugs in admixtures, rather than relying on the protection offered by the admixture components.

Due to the impracticably short infusion period imposed by the $t_{90}$ of ranitidine in admixtures exposed to daylight, it is imperative that the drug is protected from the harmful effects of UV light. The inclusion of lipid emulsion does not adequately perform this function, it does however offer some protection to the drug in a UV light-free environment.

Although the pH of the admixture tested is outside the range at which Zantac™ injection is manufactured, decreasing the pH to the lowest value likely to be encountered in a PN admixture did not significantly decrease the stability of ranitidine. pH is not therefore likely to be one of the major influencing factors on the stability of ranitidine in standard PN admixtures.

*Table 4.1 Recommended Shelf-Life of Zantac™ in FK Regimen 5*

| Admixture type and storage condition | Recommended shelf-life / hours |
|---|---|
| Aqueous - Light protected, ML bags | 48 |
| Aqueous – Light protected, EVA bags | 24 |
| Aqueous – Unprotected | Not recommended |
| Lipid – Light protected, ML bags | 48 |
| Lipid - Light protected, EVA bags | 48 |
| Lipid - Unprotected | Not recommended |

# CHAPTER 5

# MIDAZOLAM

# CHAPTER 5 - MIDAZOLAM

## 5.1 INTRODUCTION

### 5.1.1 MIDAZOLAM IN PARENTERAL NUTRITION

Midazolam is a short-acting benzodiazepine central nervous system depressant. It is available as a solution for injection and is licensed for sedation, premedication and induction of anaesthesia. All benzodiazepines display sedative, amnesic, anxiolytic, muscle relaxant and anticonvulsant properties but have no inherent analgesic effect so are sometimes co-prescribed with an analgesic, commonly an opioid. The use of a drug combination can have a synergistic effect, enabling lower doses of each drug to be administered and therefore reducing patient tolerance, but this practice is not advocated in the UK because of the increased risk of cardiorespiratory effects (Martindale 1999).

Many patients, particularly those in intensive care, will need sedation at some point during their treatment. Sedatives will prevent the patient "fighting the ventilator" and make certain procedures, for example intubation, easier for the staff and more comfortable for the patient. The aim of sedation in the intensive care unit is to keep the patient "*asleep but easily rousable*" (Martindale 1999). A number of respondents to the questionnaire detailed in Chapter 2 mentioned that stability information on the inclusion of midazolam and other sedatives in PN would be useful for their practice.

Midazolam can be given by continuous infusion, which removes peaks and troughs in sedation and reduces the need for nursing interventions, or by intermittent bolus if necessary to decrease tolerance. It has a rapid onset and a short half-life of around two hours. Given peripherally, it will not cause thrombophlebitis or pain (Roche 1999). These characteristics make it a good choice for short-term sedation, as recommended by the Society of Critical Care Medicine (Anon. 2002b) and explains its replacement of diazepam for this indication. It is not an ideal drug as it can accumulate in peripheral tissues

when given for extended periods or to patients with liver failure, as its metabolism is primarily by the hepatic route. Accumulation results in increased elimination time but can be minimised by maintaining the lowest rate of infusion that achieves adequate sedation. Midazolam can also induce withdrawal symptoms if abruptly discontinued, but gradual dose reduction will obviate this problem. A loading dose may be necessary to rapidly initiate sedation but it is not necessary for previously sedated or anaesthetised patients. The manufacturer of Hypnovel® recommends a maintenance dose of 0.03 to 0.20 mg/kg/hour but notes that lower doses may be adequate in elderly patients and those receiving concurrent opioid analgesics. Doses should be titrated to achieve the desired level of sedation and assessed on a daily basis to ensure that the minimum effective dose is being used and therefore the risk of side effects reduced.

## 5.1.2 STABILITY ISSUES

### 5.1.2.1 Introduction

Midazolam is an imidazobenzodiazepine (see Figure 5.1). The presence of an ionisable nitrogen in the imidazole ring confers basicity, enabling the formation of salts (Andersin 1991). The free base has low water solubility, but the existence of water soluble hydrochloride and maleate salts, produced under acidic conditions, allows the production of stable, well tolerated solutions (Roche 2003).
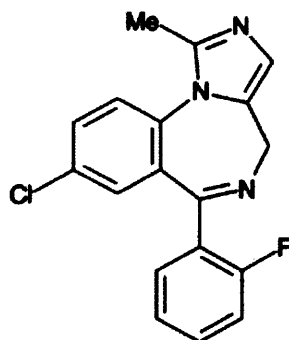


*Figure 5.1 Structure of Midazolam*
(BP 2003)

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Hypnovel® solution for injection (midazolam hydrochloride) is adjusted to pH 2.9 to 3.7 using hydrochloric acid and/or sodium hydroxide to maintain solubility and stability.

## 5.1.2.2 pH

Midazolam can exist in two forms: closed- and open-ring (see Figure 5.2). The closed-ring compound is predominant at higher pH, and above pH 5 over 99% of drug exists in this, the physiologically active, form. However, the closed-ring parent drug molecule is lipophilic so precipitates in aqueous solution, especially at near neutral pH. The open-ring compound is formed under acidic conditions, when the seven membered ring is hydrolysed and a water soluble salt formed. Hydrolysis of the ring is reversible between pH 1 to 4 and the equilibrium, and therefore solubility, is pH dependent.
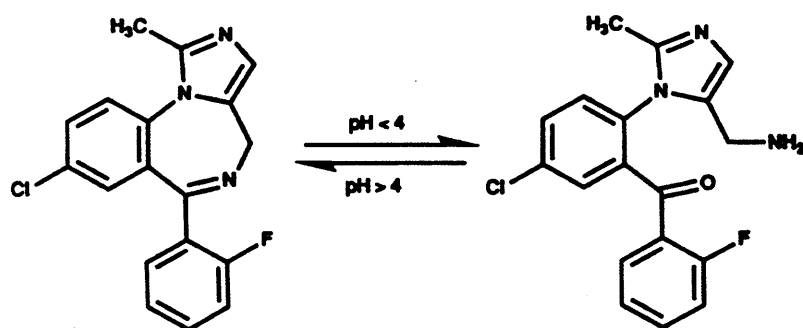


*Figure 5.2 Closed and Open Ring Forms of Midazolam*
(Andersin 1991)

## 5.1.2.3 Light

According to the BP, midazolam is light sensitive. Aqueous solutions have been shown to degrade under daylight and the degree of degradation was demonstrated to be pH dependent (Andersin and Tammilehto 1995). It is thought that increased photostability at lower pHs could be due to the formation of the open-ring compound, which may protect the molecule from degradation (Andersin and Tammilehto 1995). Conflicting information is given by the manufacturers of Hypnovel®, who state in the Summary of

Product Characteristics that no special storage precautions are required (Roche 1999) and supply the injection in clear glass ampoules. Also, work quoted in the Handbook on Injectable Drugs (Trissel 2001) indicates that:

> *"protection of midazolam solutions from light exposure is not necessary".*

## 5.1.2.4 Freezing

No special storage requirements are imposed by the manufacturer in the SPC, and although physical stability is assured for up to three days (Trissel 2001), no evidence of chemical stability on freezing is available in the literature.

To ensure freezing HPLC samples would not be detrimental to drug stability, freezing studies as described in Chapter 3 were carried out. The relative standard deviation between all results from the tests was less than 2% (RSD 1.95%), so freezing the samples was deemed to be acceptable.

## 5.2 MATERIALS AND METHODS

### 5.2.1 MATERIALS

Midazolam was obtained as Hypnovel® 10 mg/2 ml ampoules of sterile solution for injection (see Table 3.2). Each 2 ml of formulation contained 10 mg midazolam as the hydrochloride, plus sodium chloride, hydrochloric acid, sodium hydroxide and WFIs.

### 5.2.2 GENERAL METHODS

The methods described in Chapter 3 were employed to assess the stability implications of adding midazolam to parenteral nutrition.

## 5.2.3 METHOD SPECIFICS

### 5.2.3.1 Experimental Specifics

From the information available in the literature, it is clear that both pH and lighting conditions may influence the stability of midazolam in PN. Both factors were therefore investigated in the study.

Experiments were set up to compare aqueous and lipid admixtures subjected to different lighting conditions. One of each type of admixture was protected from light, one was exposed to daylight and the control was exposed to fluorescent light. Bags containing no drug were also prepared and stored under the same lighting conditions. The drug-free bags were used only for physical stability comparison.

PN admixtures usually have a pH above 5, due to the fact that the lowest pH of a commercially available amino acid solution is 5.2. This is outside the range for optimum midazolam stability. At this pH the drug exists as the lipophilic free base and may partition into the dispersed lipid phase of the emulsion. Alternatively, if it remains within the continuous aqueous phase, it may precipitate. Physical testing on aqueous admixtures assessed the likelihood of precipitation occurring at the clinical dose used.

To ensure that any stability changes caused by the difference in concentration due to the omission of the lipid emulsion volume from aqueous admixtures were accounted for, admixtures replacing Intralipid® 20% with WFIs and Intralipid® 10% were used, as described in Chapter 3.

### 5.2.3.2 HPLC Specifics

HPLC was performed using the system described in Chapter 3, using an adaptation of an assay described by Steedman et al. (1992). The mobile phase consisted of 60% acetonitrile and 40% 0.02 M monobasic sodium phosphate in water. This was used to dilute samples 1 in 10. A 20 µl injection loop was fitted to the injector. UV detection was set at 231 nm. The flow rate was set at

0.6 ml/min, leading to a retention time for the compound of interest of approximately 3.5 minutes. A peak attributable to PN was detected at approximately 1.5 minutes and this peak was clearly distinguishable from the midazolam peak.

### 5.2.3.3 Other Methods

All physical tests were carried out as specified in Chapter 3 and compared to control admixtures containing no drug.

## 5.3 RESULTS

### 5.3.1 VISUAL EXAMINATION

Lipid admixtures did not display signs of instability upon visual examination. Some creaming occurred but this was not excessive and was easily redispersed. Both control and drug-containing samples exposed to daylight became paler by 12 hours.

All aqueous admixtures were yellow at 0 hours, and no difference between control and drug-containing admixture was seen over time. The samples exposed to daylight became paler and darker yellow by 6 hours. This effect was also seen in samples not containing drug. No colour change over time was seen in the light protected or control samples.

### 5.3.2 MICROSCOPY

Few enlarged lipid particles were seen in midazolam-containing samples. The maximum droplet size observed was 15 μm, and one particle of this size was observed in two samples, at 0 and 12 hours. Numerous samples contained oversized particles, but always at a low frequency. The majority of lipid particles in samples were smaller than 5 μm (more than 99% by number). Control samples had comparable profiles. The maximum particle size seen was 15 μm, which occurred in one sample at one time point (24 hours).

No crystals larger than 5 μm were seen in either the control or the midazolam-containing aqueous admixtures.

### 5.3.2.1 Neubauer Slide Technique

Using the Neubauer slide technique, no evidence of lipid emulsion damage caused by midazolam, either at a 1:1 ratio or at the clinical dose, was seen. In the aqueous admixture, a white precipitate was formed immediately upon introduction of the drug at a 1:1 ratio. This could be seen without a microscope. Under the microscope, it was evident that the precipitate consisted of many large crystals, as seen in Figure 5.3, some up to 30 μm in diameter. When midazolam was introduced at the clinical dose, no evidence of precipitation was visible.



—10 μm
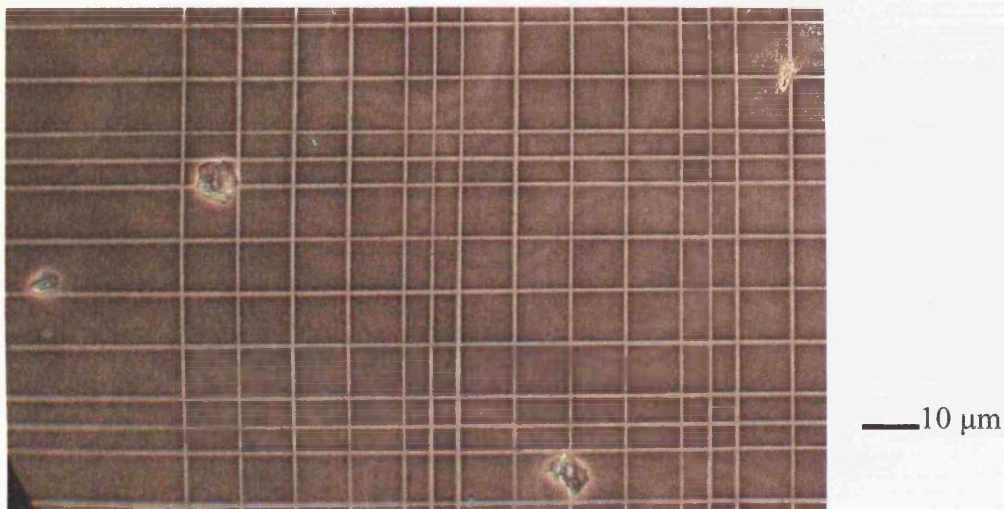
*Figure 5.3 Crystals in Midazolam-Containing Aqueous PN*

### 5.3.3 LASER DIFFRACTION

Control admixtures contained lipid droplets up to 4.30 μm. The D[4,3] of the samples were 0.37 μm. There was no change over time. Admixtures containing midazolam shared the same profile, also reaching a maximum diameter of 4.30 μm and having a D[4,3] of 0.37 μm.

A small secondary peak existed in both the control and the drug-containing sample, consisting of enlarged globules. It can be seen from Figures 5.4 and

5.5 that globules of this size made up a very small proportion of the sample (0.25% by volume for the midazolam-containing sample and 0.16% for the control in the examples shown).
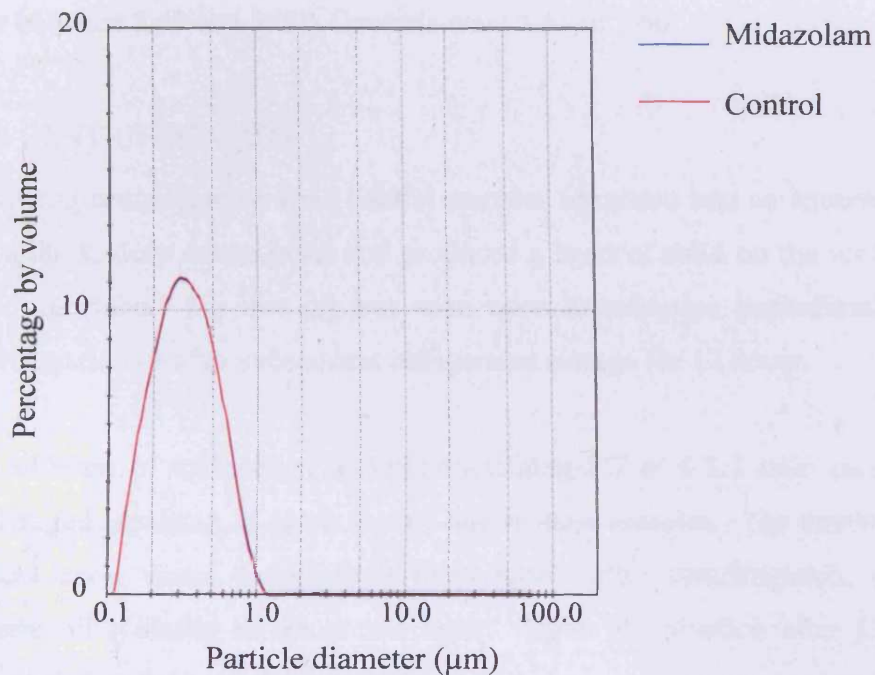


*Figure 5.4 Particle Size Distribution of Globules in Lipid Admixtures*
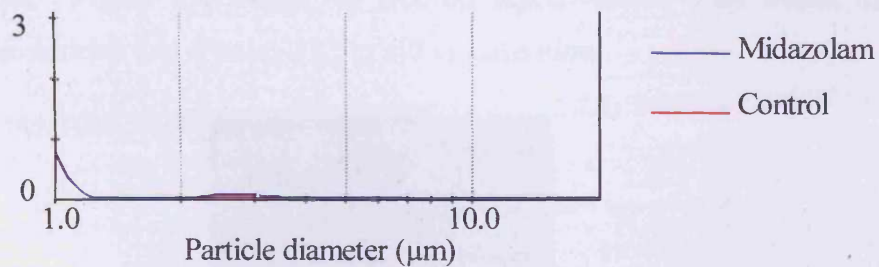


*Figure 5.5 Particle Size Distribution of Globules in Secondary Peak*

5.3.4 NEPHELOMETRY

Hach values for the midazolam-containing admixture ranged from 0.515 to 0.549 NTU over the period tested. Control values over the time period ranged from 0.505 to 0.578 NTU.

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## 5.3.5 pH MEASUREMENT

pH values for aqueous admixtures containing midazolam ranged from 5.68 to 5.75. Control values were 5.60 to 5.70. For drug-lipid admixtures, the values were between 5.63 and 5.70. Controls were 5.51 to 5.66.

## 5.3.6 CENTRIFUGATION

Following centrifugation lipid control samples separated into an aqueous layer and a thick, deep cream layer and produced a layer of solid on the wall of the centrifuge tube. No free oil was seen upon examination immediately after centrifugation or after subsequent refrigerated storage for 12 hours.

The addition of midazolam to lipid-containing PN at a 1:1 ratio caused the centrifuged emulsion to crack in two out of three samples. The cracking was evident upon visual examination immediately after centrifugation, seen as discrete oil globules in the cream layer. Upon examination after 12 hours refrigerated storage, all three samples had cracked; one into a complete layer of free oil, one as very large discrete globules and the final sample, which had apparently not cracked at the first examination, contained small discrete oil globules. Figure 5.6 shows the free oil layer, stained with Sudan Red III (Sigma-Aldrich Ltd, Poole, UK) to aid visualisation.



*Figure 5.6 Free Oil Layer in Midazolam-Containing Lipid Admixture after Centrifugation*

In common with the results from the Neubauer slide experiments, the inclusion of midazolam at a clinical dose did not cause cracking of the emulsion when centrifuged, either immediately or after storage.

Centrifugation of aqueous samples caused precipitate to be deposited on the wall of the tube in both the control and the drug-containing sample. There appeared to be more precipitate in the midazolam sample, but it was impossible to quantify accurately enough to draw a comparison. Aqueous samples with midazolam added at a 1:1 ratio caused immediate visible precipitation and, although a solid pellet was formed during centrifugation, the sample became turbid upon standing.

## 5.3.7 OSMOLALITY

Osmolality values between 1684 and 1693 mOsm/kg were obtained for aqueous admixtures and between 1922 and 1931 mOsm/kg for lipid admixtures. Control values were 1687 to 1708 mOsm/kg, and 1925 to 1953 mOsm/kg, respectively.

## 5.3.8 HPLC

### 5.3.8.1 Degradation Studies

Acid degradation of midazolam for 12 hours resulted in no peak being seen at or around the expected retention time. There was a peak seen in the samples subjected to basic conditions for the same period, but it was very much smaller than that seen in the control sample. Chemical structures of the known degradation products of midazolam are shown in Appendix 5.

The degraded samples were introduced into the HPLC system undiluted to maximise the possibility of the drug peak being detected, but the control sample was diluted 1 in 10 with mobile phase. In Figure 5.7 the peak area of midazolam in the control sample was 37.87% of the total and in the alkali-degraded sample, which contained ten times as much drug at the beginning of

the experiment, the percentage of the chromatograph occupied by the peak was 11.75%.
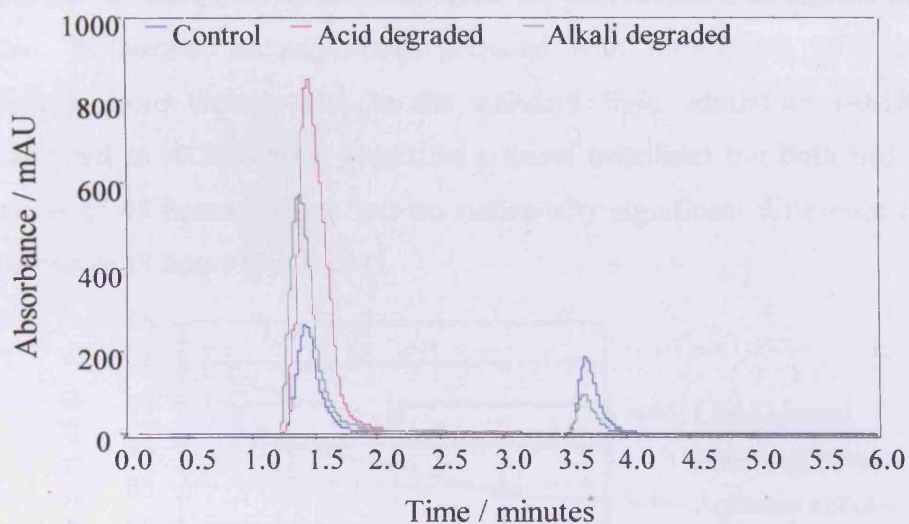


*Figure 5.7 Chromatogram of Degraded Drug Samples*

5.3.8.2 Stability Studies

Analysis of aqueous PN containing midazolam stored under standard lighting conditions showed that midazolam had degraded to approximately 90% (90.22%, RSD 0.23%) of the original concentration by 12 hours. Under the same storage conditions, midazolam in the lipid admixture stayed above 90% for the full test period (93.35% at 48 hours, RSD 0.36%). The results are shown in Figure 5.8.
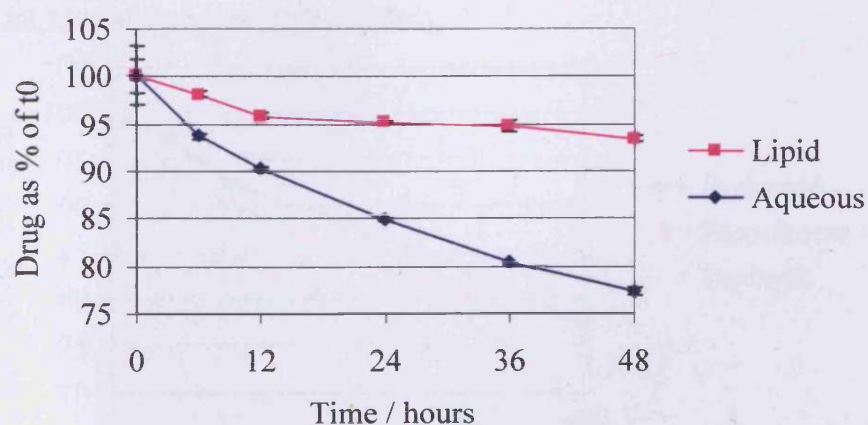


*Figure 5.8 Concentration of Midazolam in Aqueous and Lipid Admixtures Over Time (± RSD)*

Midazolam in aqueous admixtures containing extra WFIs was stable for the same period as the standard aqueous admixture, lasting 6 hours, but degraded at a faster rate (-0.59%/hour compared to -0.33%/hour), as shown in Figure 5.9. Midazolam in admixtures prepared with Intralipid® 10% degraded slightly more slowly than in the standard lipid admixture (-0.18%/hour compared to -0.20%/hour, based on a linear trendline) but both had a $t_{90}$ in excess of 48 hours. There was no statistically significant difference between the two at 48 hours ($p = 0.134$).
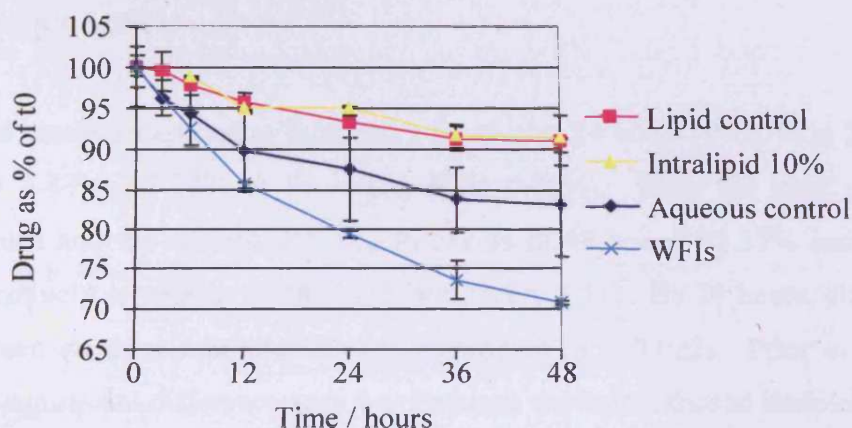


*Figure 5.9 Concentration of Midazolam in Various Admixtures Over Time (±
RSD)*

Comparisons of data for aqueous admixtures are shown in Figure 5.10, demonstrating that the longest $t_{90}$ was seen in the light protected samples, which remained above 90% for over 12 hours (92.36% at 12 hours, RSD 0.11%; 88.33% at 24 hours, RSD 0.14%).
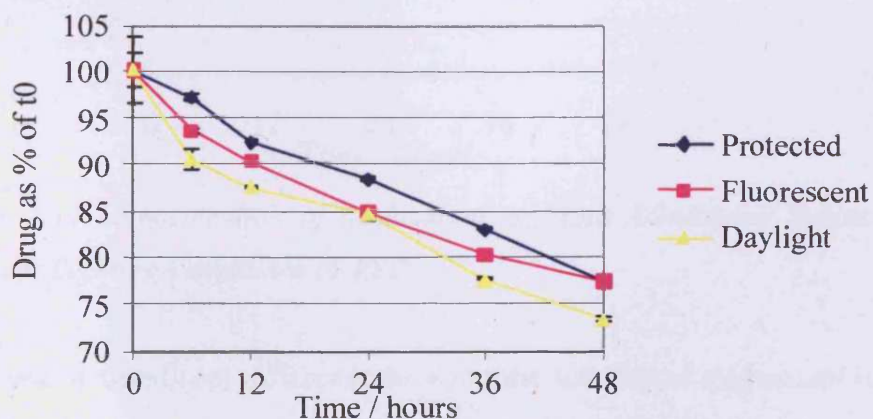


*Figure 5.10 Concentration of Midazolam in Aqueous Admixtures Subjected to
Different Lighting Conditions (± RSD)*

The samples exposed to fluorescent light in the laboratory (control) also remained above 90% until 12 hours (90.22% at 12 hours, RSD 0.23%), but degraded slightly faster. The gradients of the slopes, calculated using a linear trendline, were -0.46%/hour for the light protected sample and -0.45%/hour for the control sample. The samples exposed to daylight had fallen to 90.46% by 6 hours (RSD 1.16%). Statistical analysis showed that the differences between all three samples were significant at all time points, except between control and light protected at 48 hours (p = 0.561) and between control and daylight at 24 hours (p = 0.591).

Lipid samples exposed to light had a $t_{90}$ of over 24 hours (91.87% at 24 hours, RSD 0.46%; 81.78% at 36 hours, RSD 0.06%). Both the light protected samples and the control had a $t_{90}$ in excess of 48 hours; 91.37% and 93.35% respectively remaining at 48 hours (see Figure 5.11). By 24 hours, differences between all three admixtures were significant (p ≤ 0.022). Prior to this, the only significant difference seen was between daylight-exposed samples and the other two samples at 6 hours (p ≤ 0.001).
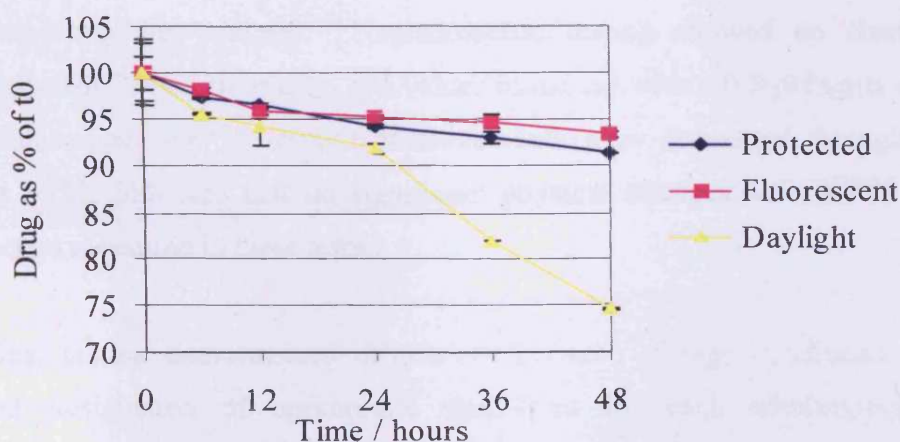


*Figure 5.11 Concentration of Midazolam in Lipid Admixtures Subjected to Different Lighting Conditions (± RSD)*

There was a significant difference between the stability of midazolam in lipid control and aqueous control admixtures from 6 hours (the lipid having a longer $t_{90}$). This was also the case for lipid protected and aqueous protected samples

from 12 hours, and for lipid daylight and aqueous daylight samples, for all time points.

## 5.4 DISCUSSION

Physical stability analysis of admixtures containing midazolam has shown concentration dependent instability, seen when the drug was included at a 1:1 ratio, as would be the case when Y-siting the drug (Minton 1998). Formation of crystals and damage to the lipid emulsion may have clinical significance due to the size of the particles produced. Incompatibilities were not evident at the lower doses, i.e. those clinically appropriate for inclusion in an admixture. The physical analytical methods employed were not capable of demonstrating differences between the stability of the aqueous and lipid admixtures, but it was possible to draw conclusions about the impact of the inclusion of midazolam in admixtures.

Quantifiable physical changes were minimal and usually less than inter-sample variation and variation in control samples over time was often greater than in the drug-containing samples. Nephelometric testing showed no changes greater than 0.5 NTU over time, pH values remained within 0.5 pH units of 0 hour values and laser diffraction measurements were consistent throughout testing. This indicates that no significant physical changes occurred in the parameters measured in these tests.

Chemical testing demonstrated differences between storage conditions and allowed designation of appropriate shelf-lives for each admixture-drug combination (see Table 5.1).

Midazolam concentration remained above 90% for a longer period in the lipid admixture than in the aqueous. The physicochemical characteristics of the two admixtures were similar and none of the parameters tested was an obvious cause for the difference in $t_{90}$. The tests using aqueous admixture containing extra WFIs were designed to control for the concentration factor but as the

drug degraded at a faster rate in the admixture containing extra WFIs this factor was eliminated. Changing the 20% lipid emulsion for a 10% formulation did not cause any significant difference in drug stability.

The light protection studies showed that the $t_{90}$ of midazolam in both aqueous and lipid PN can be extended by excluding light, despite there being no recommendation to do so in the Hypnovel® Summary of Product Characteristics (Roche 1999).

## 5.5 CONCLUSION

When considering whether midazolam was a suitable additive to be included in the test admixture a number of factors have been investigated. Examination of the literature suggested that both light exposure and the pH of the admixture would play an important role in determining the stability period of the drug. The decrease in $t_{90}$ from 12 to 6 hours for aqueous admixtures exposed to light and from 48 to 24 hours for lipid admixtures leads to the conclusion that light protection should be provided in order to maximise the shelf-life of the compounded admixture. The light protection conferred by the inclusion of lipid emulsion in the admixture is inadequate to obtain the full benefits. In the complete absence of light protection the stability of midazolam cannot be assured beyond 6 hours, which is not a sufficient period of time over which to infuse the PN.

The pH of the admixture is fixed and outside of the optimum range for midazolam stability but despite this, it is possible to assure drug stability for 48 hours in the lipid admixture when light protection is provided. As the equilibrium between the two forms of midazolam is reversible, inclusion of the drug in this relatively high pH environment is acceptable; the drug is present as the lipophilic form which, although less stable than the open-ring form, has proved to be adequately stable for the infusion period and is physiologically active.

The difference between the two forms of midazolam is likely to result in the drug partitioning between the two phases of the PN admixture. The shelf-life of the drug in each type of admixture was quite different (see Table 5.1) and with further work it may be possible to elucidate the reasons and perhaps link them to the partitioning behaviour.

Examination of the physical results shows that concentration dependent incompatibilities exist but the drug does not damage the admixture at the dose tested. The dose should not be increased without further physical testing. The formation of crystals and free oil droplets when midazolam is included at a 1:1 ratio makes this drug unsuitable for Y-siting with this regimen.

*Table 5.1 Recommended Shelf-Life of Hypnovel® in FK Regimen 5*

| Admixture type and storage condition | Recommended shelf-life / hours |
|---|---|
| Aqueous - Light protected | 12 |
| Aqueous - Unprotected | 6 |
| Lipid - Light protected | 48 |
| Lipid - Unprotected | 24 |

# CHAPTER 6
# TRAMADOL

# CHAPTER 6 - TRAMADOL

## 6.1 INTRODUCTION

### 6.1.1 TRAMADOL IN PARENTERAL NUTRITION

Tramadol is a centrally acting analgesic which operates via two synergistic mechanisms; an opioid effect and a noradrenaline and serotonin reuptake inhibition mechanism (Dayer et al. 1997). It is an effective and well-tolerated analgesic (Kmetec and Roskar 2003) licensed for the treatment and prevention of moderate to severe pain. It has less addiction potential than other opioid analgesics, but is less effective in treating severe pain (BNF 2004).

Tramadol has been used for many clinical applications including post-operative pain, patient-controlled analgesia, gynaecology and obstetrics, chronic pain, neuropathic pain and pain associated with cancer. Compared to other opioid analgesics, tramadol displays fewer side-effects, meaning that it is particularly suitable for use in patients in whom the cardiac and respiratory depressant effects generally associated with opioids would be hazardous.

Zydol™ injection (tramadol hydrochloride) is suitable for administration by intramuscular or slow intravenous injection, or by continuous or intermittent infusion after dilution with a suitable infusion fluid. Post-operative doses allow up to 250 mg in the first hour, followed by 50 or 100 mg four to six hourly, up to a total daily dose of 600 mg (Searle 1997). The dose is adjusted according to the severity of the pain and patient response.

### 6.1.2 STABILITY ISSUES

#### 6.1.2.1 Introduction

Tramadol is a synthetic analogue of codeine existing as a racemic mixture of two enantiomers (see Figure 6.1); one acts as an opioid receptor agonist and is a preferential inhibitor of serotonin reuptake and the other tends to inhibit noradrenaline reuptake (Dayer et al. 1997).

H3C CH3
N H

and enantiomer . HCl

OH

OCH3
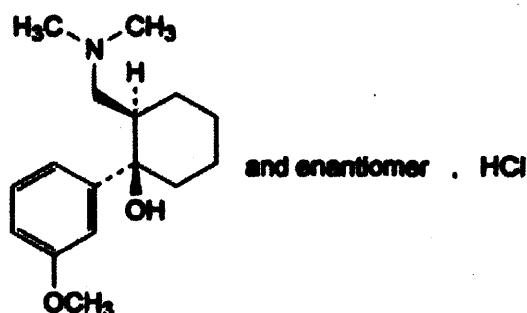
*Figure 6.1 Structure of Tramadol*

(BP 2003)

Little stability data for this drug are reported in the literature. The Summary of Product Characteristics for Zydol™ injection reports that the drug is physically and chemically compatible with sodium bicarbonate 4.2% and Ringer's solution for up to 24 hours and for up to five days with sodium chloride 0.9%, sodium lactate compound and glucose 5% solutions, plus several other infusion fluids. Physical incompatibilities with some injectable drugs are also mentioned. Stability data in the Handbook on Injectable Drugs (Trissel 2001) rely on information from just two sources; the data sheet and one paper (Muller and Berg 1997) which investigated tramadol stability in glucose 5% and sodium chloride 0.9%, under different storage conditions. It showed that light exposure was not detrimental to the stability of tramadol and that minimal drug loss or visual changes were experienced upon storage of up to 14 days, or exposure to 40°C temperatures. Due to the paucity of literature, it cannot be assumed that because an incompatibility has not been reported, that it does not exist.

6.1.2.2 pH

Zydol™ injection is adjusted to pH 6.0 to 6.8 during manufacture (Pfizer 2004). No further information relating to the influence of pH on the stability of tramadol was found in the literature.

### 6.1.2.3 Light

The BP (2003), in its monograph on the parent drug, recommends that tramadol be stored protected from light. The Summary of Product Characteristics for Zydol™ injection does not mention this requirement. Tramadol infusion solutions were tested for light stability by Muller and Berg (1997) and were found to be stable.

### 6.1.2.4 Freezing

Freezing studies as described in Chapter 3 were carried out. The relative standard deviation between all results from the tests was less than 2% (RSD 1.64%), which was less than the deviation in the control of freshly opened ampoules (RSD 2.07%), so freezing the samples was deemed to be acceptable.

## 6.2 MATERIALS AND METHODS

### 6.2.1 MATERIALS

Zydol™ injection is a solution containing tramadol hydrochloride 100 mg/2 ml and sodium acetate, dissolved in WFIs (see Table 3.2).

### 6.2.2 GENERAL METHODS

The methods described in Chapter 3 were employed to assess the stability implications of including tramadol in a parenteral nutrition admixture.

### 6.2.3 METHOD SPECIFICS

#### 6.2.3.1 Experimental Specifics

There has been little investigation of the factors influencing stability of tramadol in solution. Physical incompatibility between tramadol and other injectable solutions has been reported; when incompatible injections are mixed in a syringe, precipitation occurs (Searle 1997). Physical stability testing will reveal whether the incompatibility noted in the Summary of Product Characteristics (Searle 1997) occurs with the test PN admixture.

The pH of the admixture tested was below the range at which the injection is formulated and may therefore be detrimental to drug stability. However, the admixture was less than 0.5 pH units outside this range so additional testing was not planned, although an unacceptably short shelf-life for tramadol in the test admixture in initial studies would necessitate pH effect testing.

Light stability was a potential problem according to the BP (2003), however this view was not upheld in the study by Muller and Berg (1997). Experiments were therefore designed to compare the stability of tramadol in aqueous and lipid admixtures under different lighting conditions. One of each type of admixture was protected from light, whilst one was exposed to daylight and the control exposed to fluorescent light only.

### 6.2.3.2 HPLC Specifics

HPLC was performed using the system described in Chapter 3, using an adaptation of an assay described by Gan and Ismail (2001). The mobile phase consisted of 35% acetonitrile containing 0.1% triethylamine and 65% 0.01 M phosphate buffer. The mobile phase was adjusted to pH 5.9. This was used to dilute samples 1 in 10. A 20 µl injection loop was fitted to the injector. UV detection was set at 218 nm. The flow rate was set at 0.5 ml/min, leading to a retention time of the compound of interest of approximately 2.3 minutes. A peak attributable to PN was detected at approximately 1.7 minutes and this peak was clearly distinguishable from the tramadol peak.

### 6.2.3.3 Other Methods

All physical tests were carried out as specified in Chapter 3 and compared to control admixtures containing no drug.

## 6.3 RESULTS

### 6.3.1 VISUAL EXAMINATION

No gross visible difference between both lipid and aqueous control and the

drug-containing admixtures was observed.

Creaming of up to 5 mm in depth was seen in lipid admixtures containing tramadol, and up to 4 mm in the control admixtures. All creaming was redispersable with little effort. No free oil was seen in any admixture. The same degree of haziness was seen in both the control and the tramadol-containing aqueous admixtures.

## 6.3.2 MICROSCOPY

Isolated lipid globules of up to 15 μm in diameter were observed in some of the lipid samples that contained tramadol. These oversize globules were in the minority, with over 99% of globules seen in each sample smaller than 5 μm, and most of the remaining globules between 5 and 7.5 μm in diameter. In approximately half of the samples no globules or particles larger than 5 μm were seen, whilst no globules larger than 7.5 μm were observed in over three-quarters of the samples.

Microscopic examination of the aqueous admixture containing tramadol showed a solution free from particles greater than 5 μm.

### 6.3.2.1 Neubauer Slide Technique

Results from Neubauer tests for tramadol with lipid admixture in a 1:1 ratio were inconclusive. Some tests showed flocculation and aggregation of the lipid emulsion, with individual globules of up to 30 μm in diameter observed, whereas other samples showed no adverse effects from the inclusion of high doses of tramadol. In the tests at the clinical dose of tramadol no samples displayed flocculation or oversized globules.

Aqueous admixtures tested with tramadol in a 1:1 ratio contained many small crystalline particles, which reached a maximum of 10 μm in diameter in one sample but were smaller than 5 μm in all other samples. These particles were not seen in the aqueous admixture mixed with tramadol at its clinical dose.

## 6.3.3 LASER DIFFRACTION

Laser diffraction results were very consistent, both over time and between samples. The maximum particle size reached by the samples containing tramadol was 4.30 $\mu$m and the D[4,3] was 0.37 $\mu$m at all times. A small secondary peak, which never exceeded 0.20% by volume, was present in all samples; its size was not influenced by time. This was also true for the control samples, in which the maximum volume of the secondary peak was 0.21%.

## 6.3.4 NEPHELOMETRY

The turbidity of both control and drug-containing admixtures changed by less than 0.5 NTU over the test period, the former falling from 0.569 NTU (SD 0.006) to 0.519 NTU (SD 0.011) and the latter from 0.554 NTU (SD 0.002) to 0.515 NTU (SD 0.005).

## 6.3.5 pH MEASUREMENT

The pH range within all sample types varied by less than 0.5 pH units over time. Readings for the aqueous control samples ranged from 5.51 to 5.66, whilst for tramadol-containing aqueous samples the range was from 5.61 to 5.68. Lipid control samples had the widest pH range, from 5.50 to 5.70; the equivalent drug-containing admixture ranging from 5.68 to 5.76.

## 6.3.6 CENTRIFUGATION

Subjecting lipid samples containing tramadol at a 1:1 ratio to the centrifugation conditions described in Chapter 3 was insufficient to cause complete destruction of the lipid emulsion, but a small amount of free oil was liberated in all of the samples. This was seen as several discrete globules floating on the top of the admixture. No free oil was seen either in the samples containing the clinical dose of the drug nor in the control samples.

Centrifuged aqueous samples containing tramadol were indistinguishable from the centrifuged controls at both the 1:1 ratio and the clinical dose, with no signs

of instability noted.

## 6.3.7 OSMOLALITY

Osmolality values for samples containing tramadol were approximately the same as those for the control samples. The aqueous samples ranged from 1631 to 1634 mOsm/kg for the tramadol-containing samples and 1634 to 1640 mOsm/kg for the control. The inclusion of lipid emulsion lowered the osmolality of the lipid admixtures to between 1404 and 1406 mOsm/kg for the tramadol-containing samples and between 1403 and 1406 mOsm/kg for the control.

## 6.3.8 HPLC

### 6.3.8.1 Degradation Studies

Overnight (12 hour) degradation of tramadol using acid and alkali conditions did not result in a change in the shape of the drug peak. It did, however, cause a shift in retention time (see Figure 6.2) of 9.70% for the acid samples and 2.32% for the alkali treated samples. These shifts were adequate to ensure that the undegraded drug was being detected, as the inter-sample variation of retention time for undegraded samples was 0.20%. Chemical structures of the known degradation products of tramadol are shown in Appendix 5.
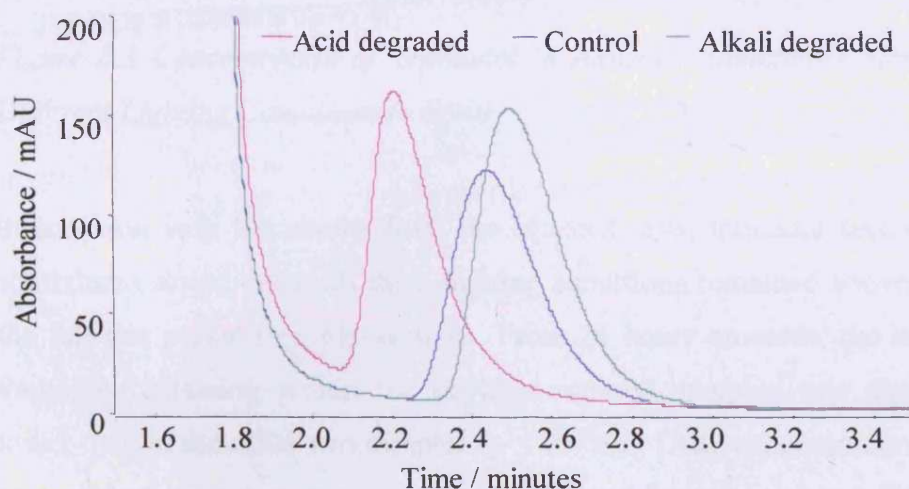


*Figure 6.2 Chromatogram of Degraded Drug Samples*

## 6.3.8.2 Stability Studies

The percentage of tramadol in aqueous admixtures stored under control light conditions remained above 90% for the whole test period, and as such a $t_{90}$ of 48 hours was assigned. This was also the case for both the light protected and the daylight-exposed samples, however greater degradation was seen in the daylight-exposed samples than with the other two types of test light condition (see Figure 6.3). After 24 hours the difference between the light protected samples and those exposed to daylight became significant ($p \leq 0.006$), whilst a significant difference between control and daylight-exposed samples was detected from 36 hours ($p = 0.029$). There was no significant difference between control and light protected samples at any time ($p \geq 0.074$).
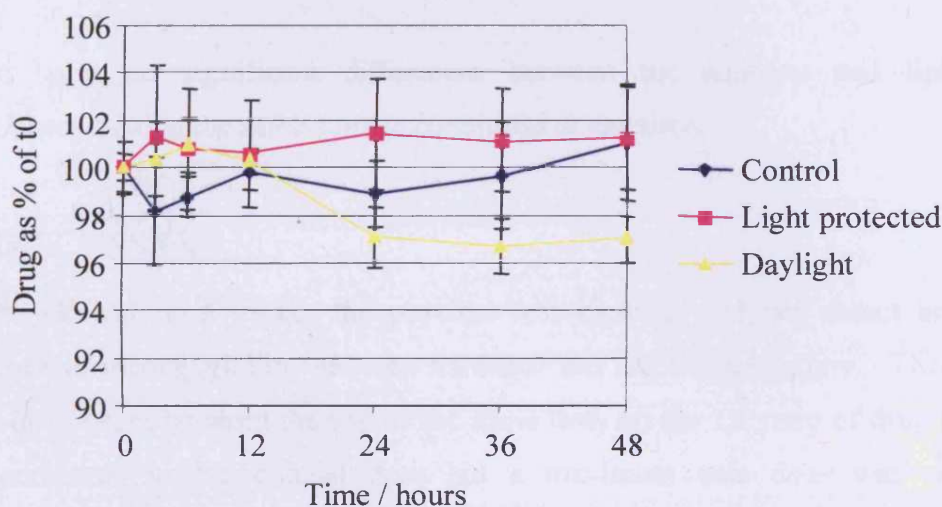


*Figure 6.3 Concentration of Tramadol in Aqueous Admixtures Subjected to Different Lighting Conditions ($\pm$ RSD)*

In common with the results from the aqueous tests, tramadol levels in lipid admixtures stored under all three lighting conditions remained above 90% for the full test period (see Figure 6.4). From 24 hours onwards, the amount of tramadol remaining within the daylight-exposed samples was significantly lower than in the other two samples ($p \leq 0.030$). Drug concentrations in light protected samples were not significantly different from control ($p \geq 0.183$).
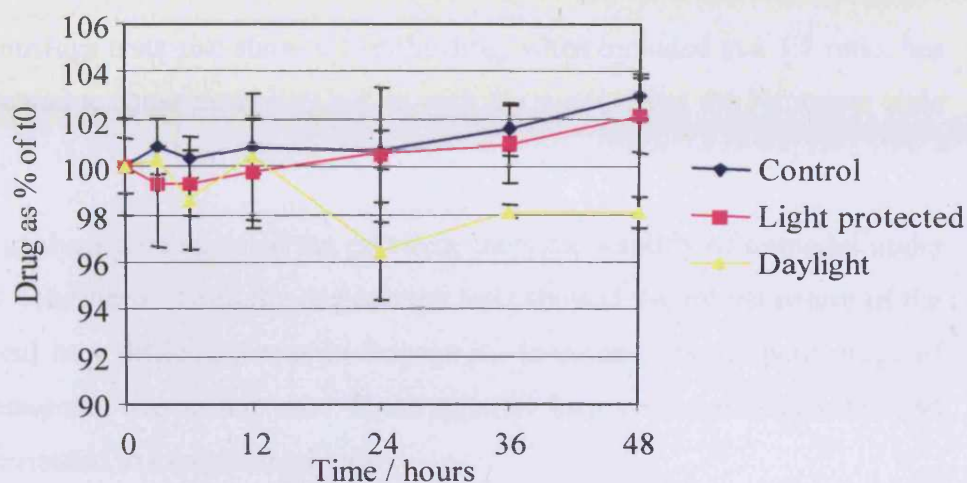
*Figure 6.4 Concentration of Tramadol in Lipid Admixtures Subjected to Different Lighting Conditions (± RSD)*

There were no significant differences between the aqueous and lipid admixtures sharing the same storage conditions at any time.

## 6.4 DISCUSSION

At the clinical dose tested, the physical test methods did not detect any evidence of incompatibility between tramadol and the test admixture. There were differences between the results for some tests on the 1:1 ratio of drug to PN compared to the clinical dose but a maximum safe dose was not established.

Microscopy was one of the tests that detected potential problems, however the presence of a small number of oversized particles is common in PN (Bethune et al. 2001), even when no drug additions are made. The use of filters, as recommended by the BPNG (Bethune et al. 2001), would protect patients from the potential dangers presented by this contamination.

Results from the Neubauer slide technique showed dose-related incompatibility. Although not all 1:1 samples were unstable, flocculation and the presence of oversized lipid globules in some requires that this combination be deemed incompatible. This was not the case for the clinical dose tests.

The centrifuge tests also showed that the drug, when included at a 1:1 ratio, has the potential to cause instability but, as with the results from the Neubauer slide technique, this was dose related.

HPLC analysis demonstrated the excellent chemical stability of tramadol under the test conditions. Even the degradation tests showed the robust nature of the drug, and how difficult it was to damage it. In some tests the percentage of drug remaining was seen to rise. These apparent increases were very small and were attributed to experimental error.

Tramadol, when included in the admixtures tested, was subject to significant but slow light-mediated degradation. The inclusion of lipid emulsion in the admixture did not significantly improve the stability, either by offering light protection or by any other mechanism.

## 6.5 CONCLUSION

Tramadol has been demonstrated to cause dose dependent instability in the admixture tested and as such should not be included in this regimen at doses above those tested. It is likely that increased doses may be suitable for inclusion but these must be thoroughly analysed before use.

HPLC analysis showed tramadol to be very stable in the test regimen, sustaining less than 10% degradation over the test period under all test conditions. Due to the slow rate of degradation it is not necessary to protect the admixture from light to keep the concentration of tramadol above 90% for 48 hours. However, as there is a significant increase in the amount of drug remaining in the samples protected from UV damage, it is preferable to protect the admixture.

*Table 6.1 Recommended Shelf-Life of Zydol™ in FK Regimen 5*

| Admixture type and storage condition | Recommended shelf-life in hours |
|---|---|
| Aqueous – Light protected | 48 |
| Aqueous – Unprotected | 48 |
| Lipid – Light protected | 48 |
| Lipid – Unprotected | 48 |

# CHAPTER 7

# HYDROCORTISONE

# CHAPTER 7 - HYDROCORTISONE

## 7.1 INTRODUCTION

### 7.1.1 HYDROCORTISONE IN PARENTERAL NUTRITION

Hydrocortisone is one of the body's naturally occurring corticosteroids. As its main glucocorticoid it acts on carbohydrate, protein and fat metabolism, suppresses tissue inflammation and immune response, and increases the body's ability to cope with stress (Constanti et al. 1998). Due to hydrocortisone's wide-ranging actions in the body, it can be used clinically for replacement therapy in adrenal insufficiency and for the treatment of shock, hypersensitivity reactions (such as anaphylactic shock), rheumatic diseases, inflammatory bowel disease, inflammatory skin disorders (especially eczema), inflammatory conditions such as tennis elbow and tendinitis, and conditions affecting the eye, outer ear and mouth (BNF 2004). Most relevant to this work is the inclusion of hydrocortisone in PN to reduce phlebitis. Polak (1956) investigated hydrocortisone as a means of preventing peripheral vein thrombophlebitis with favourable results. This practice has since been used and investigated and was mentioned by respondents to the questionnaire detailed in Chapter 2.

To optimise treatment for the various indications hydrocortisone is available in a number of formulations, allowing administration via several routes. The chemical form of hydrocortisone used in each is dependent upon the characteristics necessary for the formulation. Hydrocortisone for intravenous injection can be formulated as one of two esters, and both hydrocortisone sodium succinate (HSS) (Figure 7.1) and hydrocortisone sodium phosphate (HSP) (Figure 7.2) were analysed in this work.

Doses of hydrocortisone vary with indication and range from 20 mg daily by mouth for replacement therapy to 500 mg injected or infused up to four times daily in severe conditions and emergencies (Martindale 1999). Doses used for the reduction of phlebitis are subphysiological and vary between practitioners but are commonly around 10 mg/litre in clear infusion fluids (Payne-James and

Khawaja 1993). The dose used in this study was equivalent to 20 mg/litre, as some questionnaire respondents indicated the use of this higher dose in their PN patients.
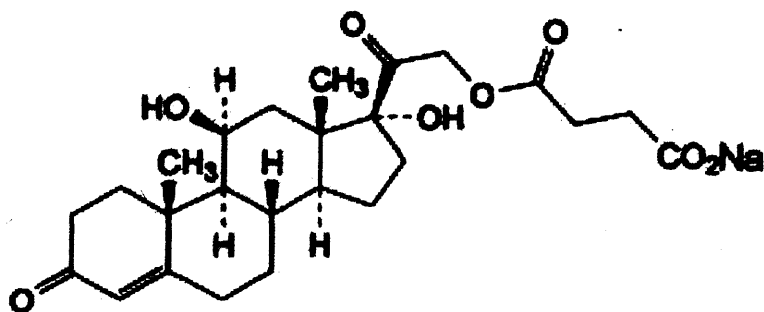


*Figure 7.1 Structure of Hydrocortisone Sodium Succinate (HSS)*
(BP 2003)
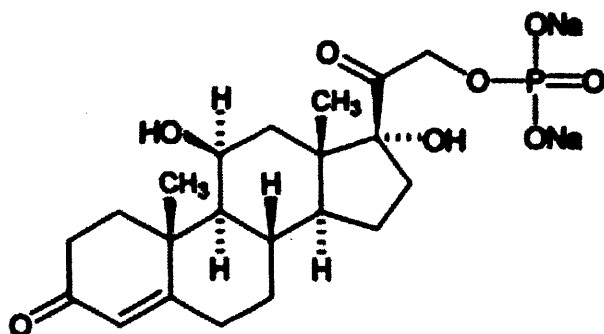


*Figure 7.2 Structure of Hydrocortisone Sodium Phosphate (HSP)*
(BP 2003)

## 7.1.2 STABILITY ISSUES

### 7.1.2.1 Introduction

Hydrocortisone is practically insoluble in water, and soluble only 1 in 40 in ethanol. These properties necessitate the use of more soluble esters to produce solutions for injection. HSS is soluble 1 in 3 and HSP 1 in 4 of water (Moffat 1986).

After parenteral administration of HSS it is hydrolysed to the

pharmacologically active parent drug, a change which also occurs upon storage (Dix Smith and Hoffman 1979). This instability means HSS must be manufactured as a lyophilised powder, and used immediately after reconstitution.

HSP is sufficiently stable to be presented as a solution for injection, but is less clinically useful than HSS due to comparatively lower tissue permeability (Garrett 1962) and increased irritation on injection (BNF 2004).

### 7.1.2.2 pH

The optimum pH range for HSS stability is 7 to 8 (Trissel 2001). Studies have shown that the solution remains stable for 72 hours at pH 6 and 12 hours at pH 5. Below this, precipitation occurred (Edward 1967). HSP injections are manufactured to pH 7.5 to 8.5 (Trissel 2001) and may be expected to decrease in stability below this, in a similar way to HSS.

At different pHs, each ester may be expected to display different characteristics, explaining the difference in tissue permeability. At physiological pH, HSP is in equilibrium between two forms, one with a double charge and the other with a single charge. HSS is in equilibrium between the sodium salt (bearing one charge) and the uncharged hemiester. Uncharged molecules are more able to enter lipid membranes and therefore have clinical activity (Garrett 1962).

### 7.1.2.3 Light

Reconstituted solutions of HSS should be protected from light (Trissel 2001). Monographs in the BP (2003) and Martindale (1999) recommend light protection for both drugs.

### 7.1.2.4 Air

The BP (2003) and Martindale (1999) recommend that both HSS and HSP are

stored in airtight containers. Monder (1968) also showed that oxidation rates of hydrocortisone are greatly increased at pH 9.

### 7.1.2.5 Freezing

The Handbook on Injectable Drugs (Trissel 2001) advises that HSP should be protected from freezing, but this comment is not made in the Summary of Product Characteristics (Sovereign 2000). No further information on the stability of frozen HSP was found in the literature, however, there is evidence of stability for HSS when frozen for up to four weeks in glucose 5% (Trissel 2001).

To ensure freezing HPLC samples would not be detrimental to drug stability, freezing studies as described in Chapter 3 were carried out. The relative standard deviation between all results from the tests was less than 2% (RSD 1.09% for HSS and 1.17% for HSP), so freezing the samples was deemed to be acceptable.

## 7.2 MATERIALS AND METHODS

### 7.2.1 MATERIALS

HSS was obtained as Solu-Cortef® 100 mg ampoules containing powder for reconstitution with 2 ml WFIs (see Table 3.2). The formulation also contained sodium biphosphate and sodium phosphate as excipients.

HSP was obtained as Efcortesol™ 500 mg/5 ml aqueous solution (see Table 3.2). Excipients in the formulation were disodium edetate, disodium hydrogen phosphate (anhydrous), sodium acid phosphate, sodium formaldehyde bisulphite monohydrate, phosphoric acid and WFIs.

### 7.2.2 GENERAL METHODS

The methods described in Chapter 3 were used to assess the stability implications of adding hydrocortisone to PN. The hydrocortisone preparations

were added to separate PN admixtures, to allow comparison between the stability and PN compatibility of the two drug formulations.

## 7.2.3 METHOD SPECIFICS

### 7.2.3.1 Experimental Specifics

The mechanism of degradation responsible for the short shelf-life of reconstituted HSS is thought to be hydrolysis (Ho and Goeman 1970), but this cannot be avoided once the solution has been reconstituted, so was not investigated. According to the literature low pH, light and oxygen may all degrade hydrocortisone to some extent. As these variables may be controlled, studies were performed to investigate their influence on hydrocortisone and PN compatibility.

The hydrocortisone preparations tested were maximally stable between pH 7 and 8.5 (Trissel 2001), which is higher than the pH of the test admixture. Evidence in the literature (Edward 1967) indicates a decrease in hydrocortisone solubility with decreased pH. Physical testing on aqueous admixtures assessed the likelihood of precipitation occurring at the drug concentration and admixture pH used.

Experiments were also set up to compare aqueous and lipid admixtures subjected to different lighting conditions. One of each type of admixture was protected from light, one was exposed to daylight and the control was exposed to fluorescent light.

To assess the impact of oxygen on hydrocortisone stability, two sets of admixture were prepared for each drug formulation; one stored in ML infusion bags and the other in EVA bags. All samples were protected from light and stored at room temperature.

To ensure that any stability changes caused by the difference in concentration, due to the omission of the lipid emulsion volume from aqueous admixtures,

were accounted for admixtures replacing Intralipid® 20% with WFIs and Intralipid® 10% were used, as described in Chapter 3.

### 7.2.3.2 HPLC Specifics

HPLC was performed using the system described in Chapter 3, using an adaptation of an assay in the BP (2003). The mobile phase consisted of 330 ml acetonitrile, 600 ml water, 1 ml orthophosphoric acid, made up to 1000 ml with water. Samples containing HSS were diluted 4 in 10 with mobile phase and those containing HSP were diluted 2 in 10. A 20 µl injection loop was fitted to the injector. UV detection was set at 254 nm. The flow rate was set at 1 ml/min for HSS and 0.5 ml/min for HSP, leading to retention times of approximately 4.5 and 2.4 minutes respectively. A peak attributable to PN was detected at approximately 0.8 minutes for HSS and 1.5 minutes for HSP and was clearly distinguishable from the drug peak in both cases.

### 7.2.3.3 Other Methods

All physical tests were carried out as specified in Chapter 3 and compared to each other and to control admixtures containing no drug.

## 7.3 RESULTS

### 7.3.1 VISUAL EXAMINATION

Visual examination of the bulk admixtures containing hydrocortisone showed no difference from control samples. Cream layers were present at all times except 0 hours in all lipid bags and reached the maximum depth of 6 mm, in the HSS-containing sample, at 24 hours. All cream layers were easily redispersable and no free oil was seen in any sample. Aqueous bulk samples containing hydrocortisone were visually indistinguishable from the control test admixtures. Small volume samples examined under high intensity light showed a slight haze in control and HSP samples, but a more dense cloudiness in the HSS samples (see Figure 7.3).

*Control*  *HSP*  *HSS*

*Figure 7.3 High Intensity Light Examination of Control, HSP and HSS Samples*

## 7.3.2 MICROSCOPY

Lipid admixture samples containing HSS contained few enlarged lipid droplets, and those which were present did not exceed 15 μm at any time. The majority of globules (more than 99% by number) were smaller than 5 μm. Typically, up to four enlarged lipid droplets per sample were seen under microscopic examination, which was comparable to control samples. Lipid globules in samples containing HSP reached a maximum diameter of 25 μm at 48 hours, but only one globule of this size was seen. In total, there were five globules with diameters greater than 10 μm observed at this time. The number of globules greater than 5 μm at all other time points were lower than in the 48 hour test period. From 24 hours, a small number of amorphous globules were seen.

Very few crystals were seen in the aqueous admixture containing HSS. At three time points (0, 6 and 24 hours) a small number of crystals (four or fewer)

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up to 5 μm in diameter were seen. No crystals were seen in the control or HSP samples.

### 7.3.2.1 Neubauer Slide Technique

The interaction between both HSS and HSP and lipid-containing admixture at a 1:1 ratio caused flocculation of the lipid and production of many globules greater than 10 μm in diameter (see Figure 7.4). When the drugs were tested against the aqueous admixture, particles up to 10 μm in diameter were evident.

Testing at the clinical dose did not show any of the incompatibilities seen with the 1:1 testing.
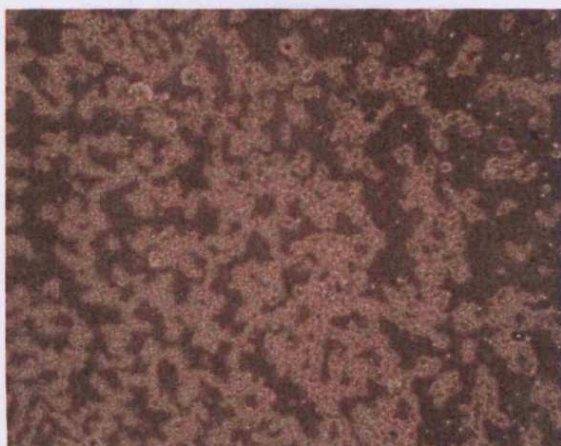


*Figure 7.4 Flocculation of Lipid Admixture by HSS at a 1:1 Ratio*

### 7.3.3 LASER DIFFRACTION

Control samples contained lipid droplets up to 4.30 μm and had a D[4,3] of 0.37 μm. No changes over time were seen. The same values were obtained for samples containing HSS. Samples containing HSP reached a maximum particle diameter of 5.29 μm and a maximum D[4,3] of 0.36 μm. These results are shown in Figure 7.5.

Secondary peaks reached a maximum of 0.30% by volume in the HSS-containing samples and up to 0.92% by volume in HSP-containing samples (as seen in Figure 7.6).
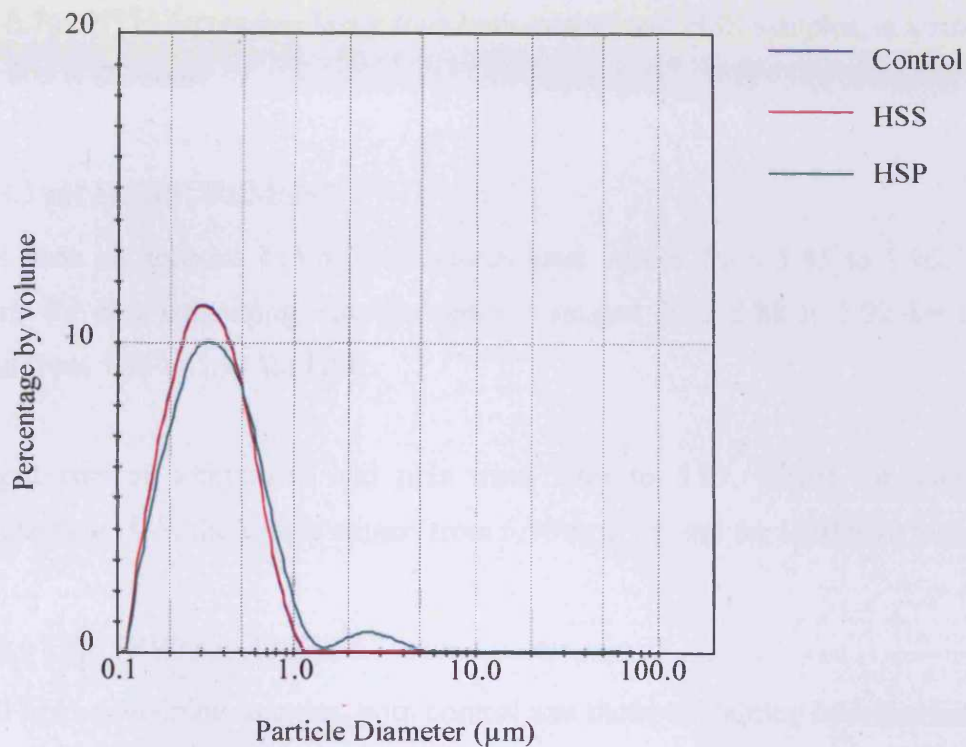
*Figure 7.5 Particle Size Distribution of Globules in Control Lipid Admixtures and those containing HSS or HSP*
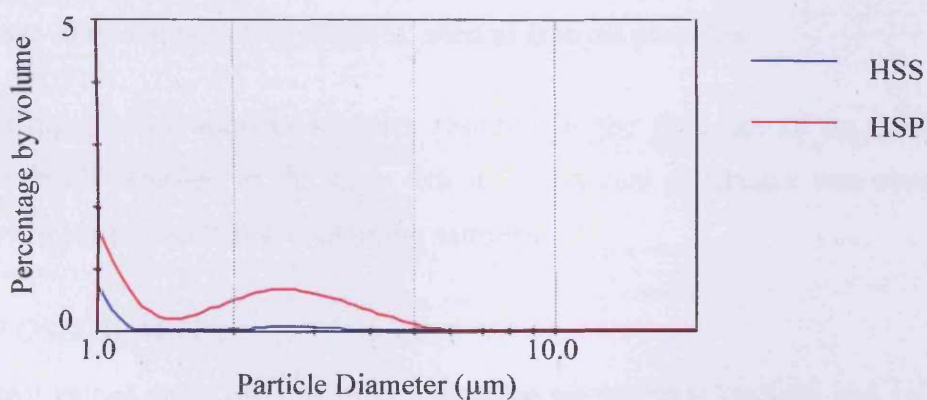


*Figure 7.6 Secondary Peaks in Admixtures containing HSS or HSP*

## 7.3.4 NEPHELOMETRY

Turbidity measurements for control samples ranged from 0.578 to 0.505 NTU, gradually decreasing over time at a rate of -0.001 NTU/hour. For the HSS samples turbidity again decreased over time, from 0.571 NTU to 0.511 NTU, at the same rate as the control samples. HSP turbidity values ranged from 0.895

to 0.749 NTU decreasing faster than both control and HSS samples, at a rate of -0.003 NTU/hour.

## 7.3.5 pH MEASUREMENT

pH tests on aqueous control admixtures gave values from 5.85 to 5.95. pH tests for drug-containing aqueous samples ranged from 5.88 to 5.92 for HSS and from 5.84 to 5.91 for HSP.

Lipid control admixtures had pHs from 5.90 to 5.97, whilst for samples containing HSS, the values ranged from 5.93 to 5.98, and for HSP 5.90 to 5.95.

## 7.3.6 CENTRIFUGATION

All lipid-containing samples, both control and those containing hydrocortisone, separated into aqueous and lipid phases when centrifuged. An insoluble pellet, thought to consist of the salts in the admixture, was seen on the wall of the tube in all samples. Control sample lipid phases consisted of a thick layer of intact lipid emulsion, whilst HSS and HSP samples showed evidence of emulsion damage in two out of three samples, seen as free oil globules.

Centrifugation of aqueous samples resulted in the presence of an insoluble pellet in all samples, to the same extent. No visual difference was observed between control and drug-containing samples.

## 7.3.7 OSMOLALITY

Control values were 1403 to 1406 mOsm/kg for aqueous samples and 1636 to 1640 mOsm/kg for lipid samples. For HSS, aqueous values were 1409 to 1411 mOsm/kg and for lipid 1627 to 1631 mOsm/kg. For HSP, aqueous values were 1785 to 1788 mOsm/kg and for lipid 1579 mOsm/kg consistently.

## 7.3.8 HPLC

### 7.3.8.1 Degradation Studies

Exposure of all samples to the degradation conditions was for 12 hours. Alkali

degradation of HSS samples resulted in a change in retention time to around 2 minutes. The peak height was approximately the same as that of the undegraded drug. Acidification and heating of HSS caused a decrease in retention time of approximately 30 seconds and a decrease in peak height to around half the height of the undegraded compound, as seen in Figure 7.7.
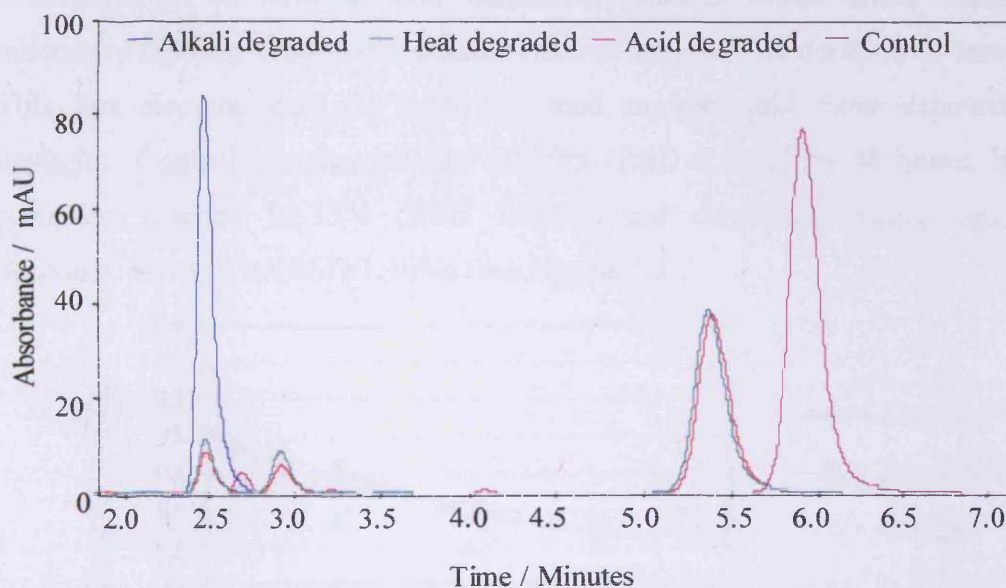


*Figure 7.7 Chromatogram of Degraded HSS Samples*

Acid-, alkali- and heat-degradation of HSP samples caused a shift in retention time of approximately 15 seconds and, more notably, a reduction in peak height from over 230 mAU to approximately 60 mAU (see Figure 7.8).
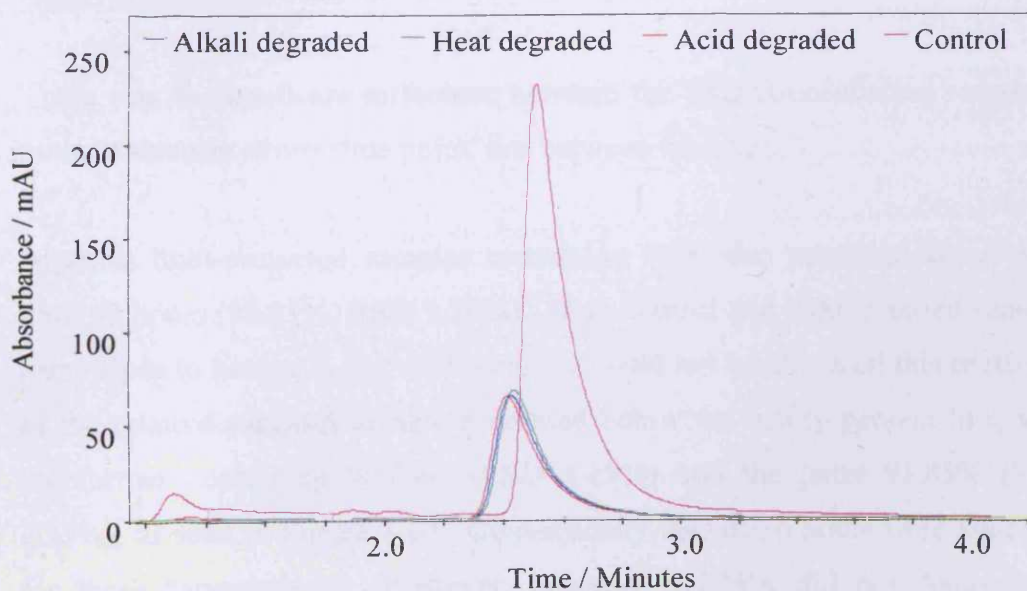


*Figure 7.8 Chromatogram of Degraded HSP Samples*

Chemical structures of the known degradation products of HSS and HSP are shown in Appendix 5.

### 7.3.8.2 Stability Studies

Concentrations of HSS in lipid admixture samples stored under standard laboratory lighting conditions remained above 90% for the duration of testing. This was also the case for light protected samples and those exposed to daylight. Control samples reached 90.79% (RSD 0.53%) by 48 hours, light protected reached 92.33% (RSD 0.60%) and daylight-exposed samples decreased to 93.12% (RSD 1.40%) (see Figure 7.9).
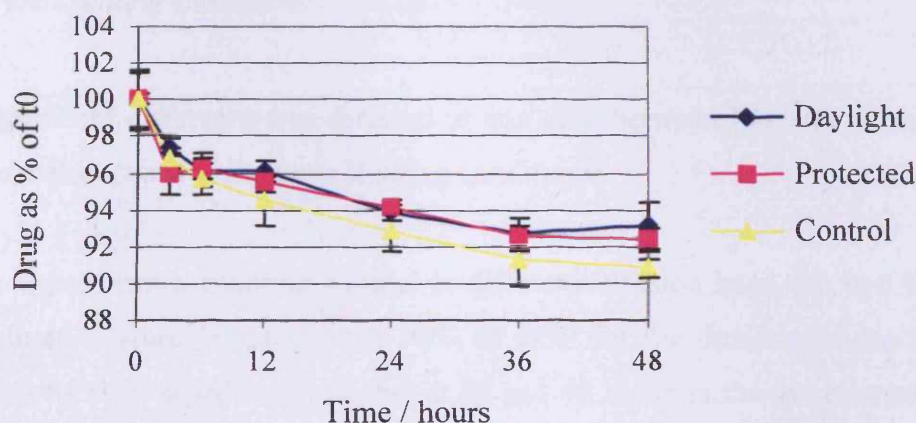


*Figure 7.9 Concentration of HSS in Lipid Admixtures Subjected to Different Lighting Conditions (± RSD)*

There was no significant difference between the drug concentration remaining in these samples at any time point, nor between their $t_{90}$s.

Aqueous light-protected samples containing HSS also remained above 90% until 48 hours (92.81%, RSD 1.26%). Both control and light-exposed samples came close to having $t_{90}$s of 48 hours, but could not be allocated this shelf-life, as the relative standard deviation crossed below the ninety percent line, with the former containing 90.80% (RSD 1.15%) and the latter 91.85% (RSD 2.15%), as seen in Figure 7.10. Consequently, $t_{90}$s of 36 hours were allocated for these formulations. However, one-way ANOVA did not demonstrate

significant differences between the three admixtures.
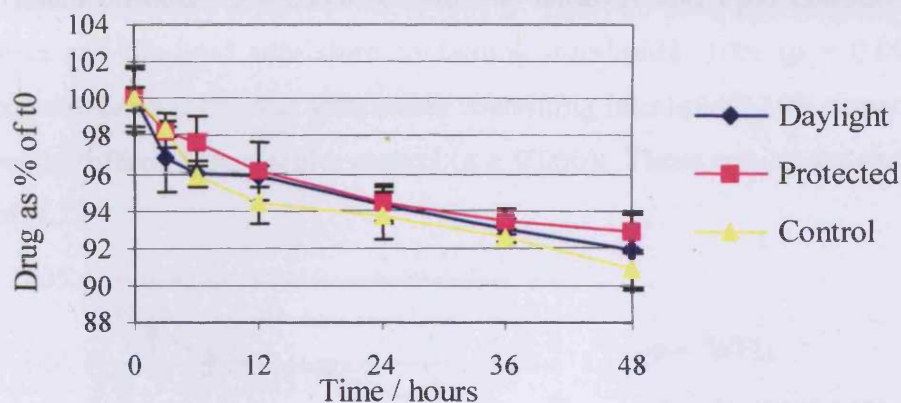


*Figure 7.10 Concentration of HSS in Aqueous Admixtures Subjected to Different Lighting Conditions (± RSD)*

No significant difference was detected at any time between lipid and aqueous admixtures exposed to the same lighting conditions.

In the experiment comparing storage in different infusion bags, the two lipid admixtures retained levels of over 90% of HSS for the duration of the test. Drug levels were significantly higher at 36 and 48 hours in the samples stored in ML bags ($p \leq 0.001$). HSS levels in aqueous samples remained above 90% for 36 hours in the ML bags, but only 24 hours in the EVA bags.
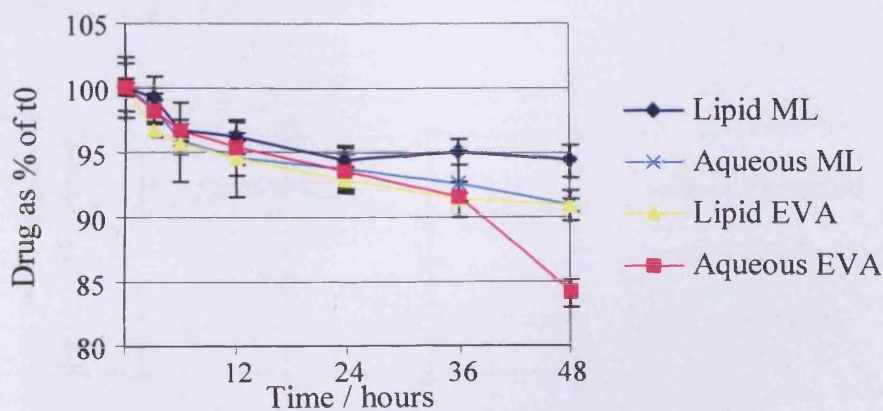


*Figure 7.11 Concentration of HSS in Aqueous and Lipid Admixtures in Different Infusion Bags (± RSD)*

The inclusion of extra WFIs, to assess the concentration effects detailed in

Section 3.4.2, resulted in a significantly higher level of HSS being detected from 3 hours onwards, compared to both the aqueous and lipid control test admixtures and the lipid admixture containing Intralipid® 10% (p = 0.000). HSS concentrations in the test admixtures containing Intralipid® 10% were not significantly different from either control (p ≥ 0.050). These results are shown in Figure 7.12.
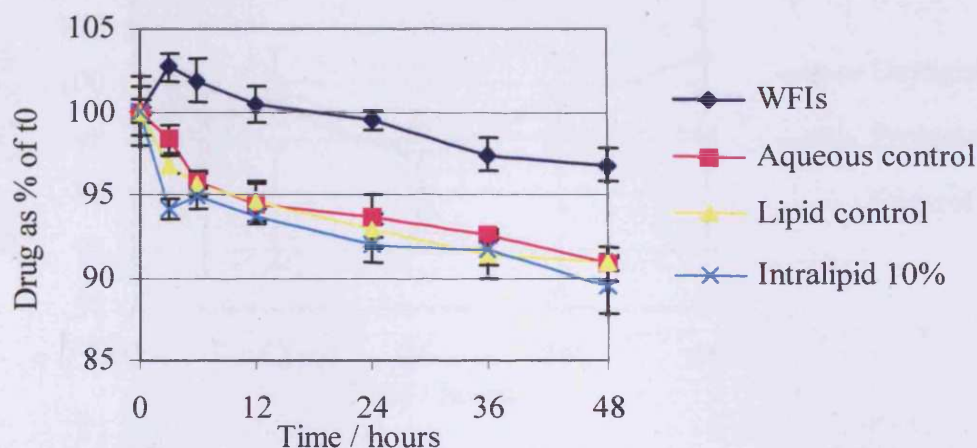


*Figure 7.12 Concentration of HSS in Various Admixtures Over Time (± RSD)*

All lipid samples in the light-exposure experiments contained levels of HSP in excess of 90% throughout the test period and accordingly were assigned $t_{90}$s of 48 hours. It can be seen from Figure 7.13 that drug levels were very stable and no significant difference between any of the lighting conditions tested could be identified.
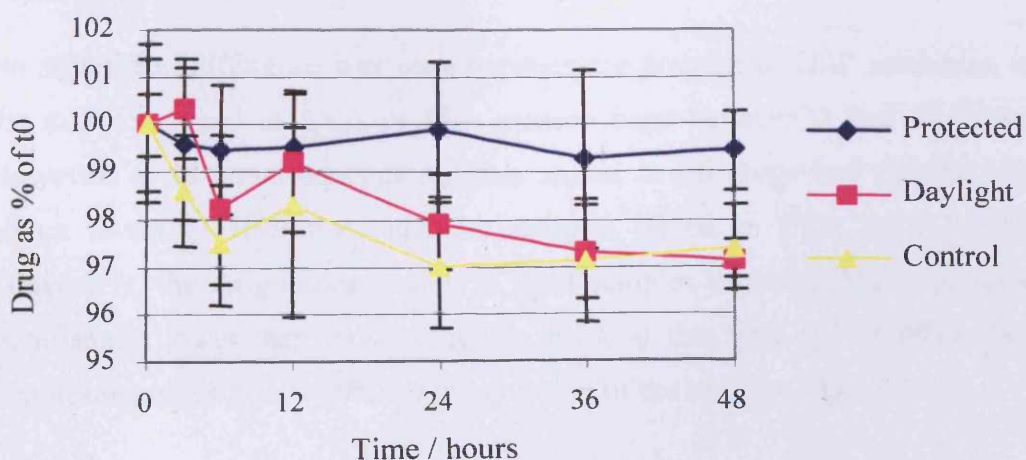


*Figure 7.13 Concentration of HSP in Lipid Admixtures Subjected to Different Lighting Conditions (± RSD)*

In the aqueous test admixtures, there were significantly higher levels of HSP in both the daylight-exposed and light-protected samples, compared to the control samples ($p \leq 0.042$), except at 24 hours, when $p \geq 0.094$ (see Figure 7.14). No significant difference between daylight-exposed and light-protected samples was detected ($p \geq 0.059$).
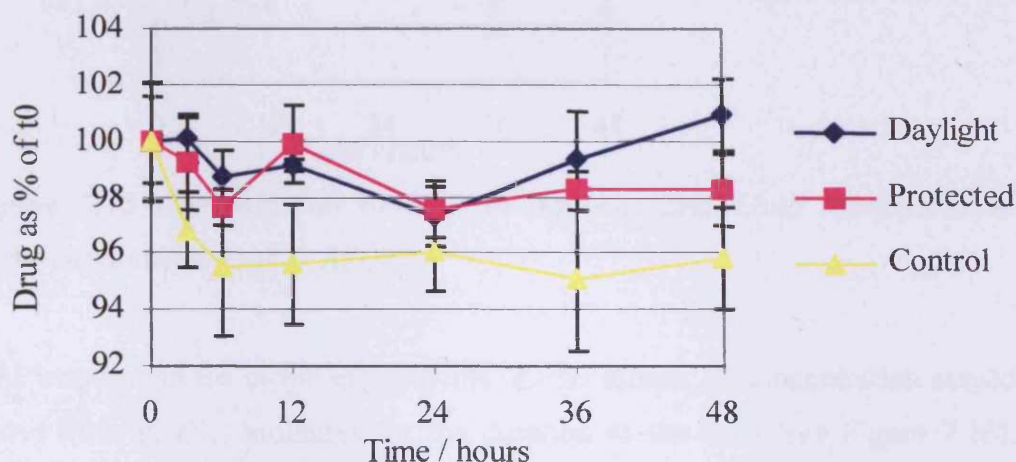


*Figure 7.14 Concentration of HSP in Aqueous Admixtures Subjected to Different Lighting Conditions (± RSD)*

Both lipid and aqueous control admixtures were statistically equivalent at all times, as were the light-protected samples. The concentration of HSP in aqueous samples exposed to light was significantly higher than in the equivalent lipid samples, but only at the 48 hour test period, when $p = 0.000$.

No significant difference was seen between the amount of HSP remaining in the samples stored in EVA or ML infusion bags between 0 and 36 hours. However, at 48 hours aqueous samples stored in ML bags had significantly higher levels of HSP than aqueous samples stored in EVA ($p = 0.043$). Conversely, the drug concentration in lipid samples stored in ML bags were significantly lower than those stored in EVA at this time ($p = 0.003$). All samples remained above 90% for the duration of the test (see Figure 7.15).
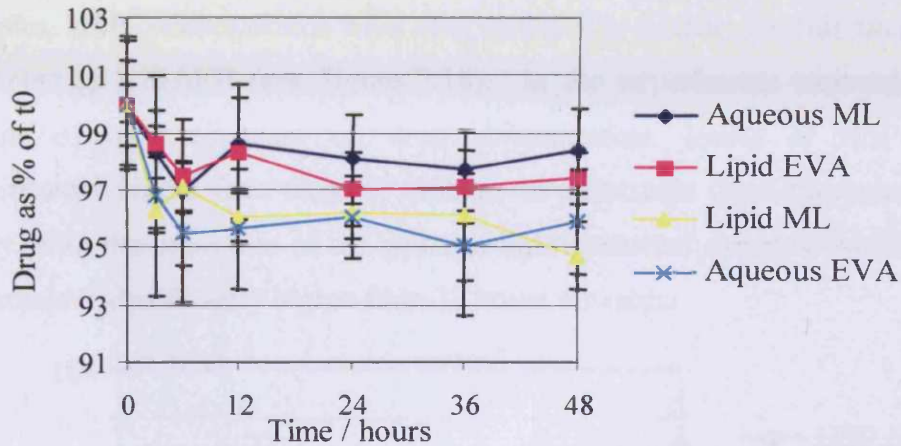
*Figure 7.15 Concentration of HSP in Aqueous and Lipid Admixtures in Different Infusion Bags (± RSD)*

HSP concentrations in the experiments on the effects of concentration stayed above 90% in all admixtures for the duration of the tests (see Figure 7.16). HSP levels in samples containing extra WFIs were significantly higher than aqueous control from 24 hours ($p \leq 0.038$), but not than lipid control or the admixture containing Intralipid® 10% ($p \geq 0.078$). There was no statistically significant difference between HSP concentrations in the admixture containing Intralipid® 10% and the two controls ($p \geq 0.090$).
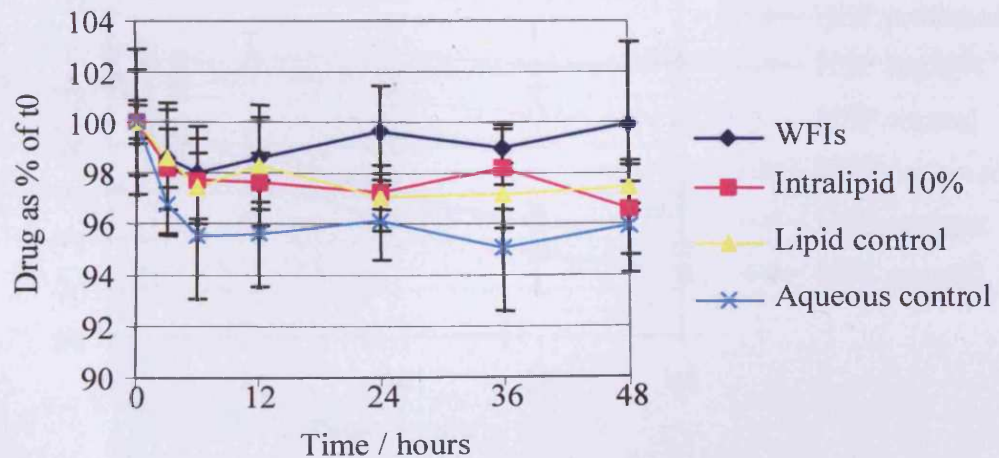


*Figure 7.16 Concentration of HSP in Various Admixtures Over Time (± RSD)*

HSP levels in aqueous control admixtures were significantly higher than HSS levels from 24 hours ($p \leq 0.027$) (see Figure 7.17). For the lipid control

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samples, HSP concentrations were also statistically higher, but this time from 12 hours ($p \leq 0.013$) (see Figure 7.18). In the experiments examining the effects of light exposure on drug concentration, levels of HSP were significantly higher than those of HSS in all admixture types throughout the study, with the exception of the aqueous light-protected samples, which were nonetheless significantly higher from 12 hours onwards.
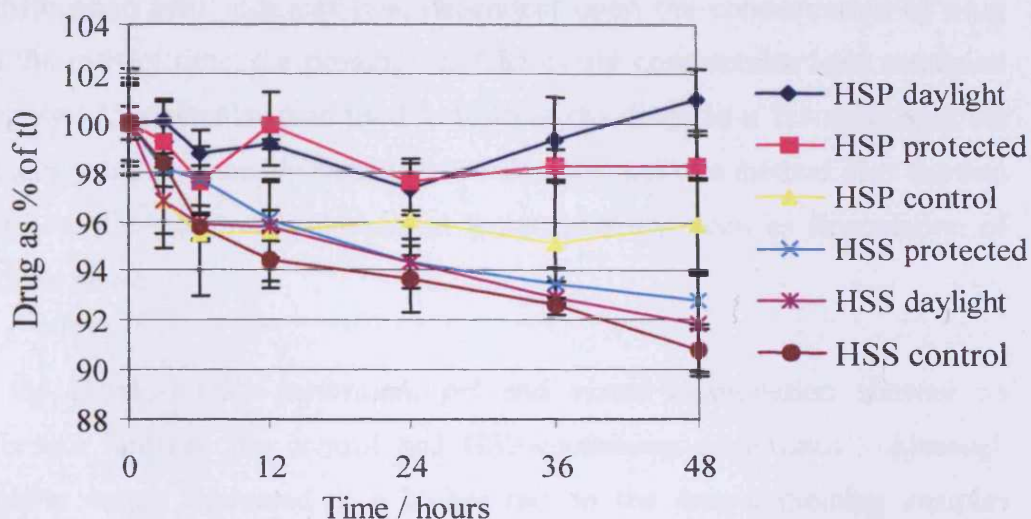


*Figure 7.17 Concentrations of HSS and HSP in Aqueous Admixtures (± RSD)*
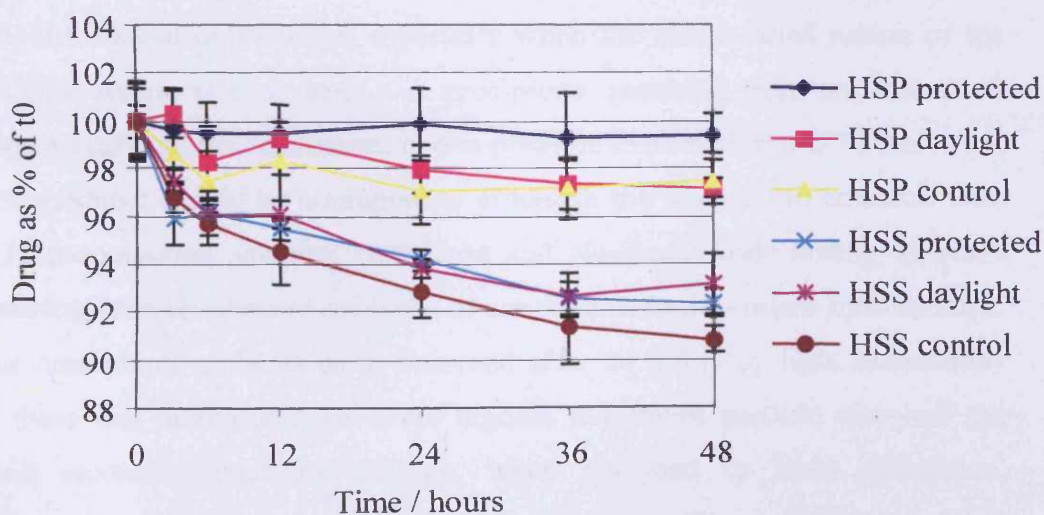


*Figure 7.18 Concentrations of HSS and HSP in Lipid Admixtures (± RSD)*

## 7.4 DISCUSSION

Analysis of the results from the physical testing of HSS-containing samples

showed no difference from control samples in the laser diffraction, nephelometry and pH tests. Visual examination of the aqueous test admixture showed the presence of a more dense haze in the HSS-containing sample than the control or HSP sample. This may be because the HSS formulation used is a powder for reconstitution. However, this apparent increase in haze intensity was not reflected in the nephelometry readings. The liberation of free oil in the centrifugation tests indicates that, dependent upon the concentration of drug and the contact time, the presence of HSS could compromise lipid emulsion integrity. The other method used to analyse the drugs in a 1:1 ratio with the test admixture was the Neubauer slide technique and this method also showed evidence of concentration-dependent incompatibility, seen as flocculation of the lipid phase.

Of the physical tests performed, pH and visual examination showed no difference between the control and HSP-containing admixtures. Although turbidity values decreased at a higher rate in the drug-containing samples compared to the control, the change over time was within the acceptable range. It is unclear why the HSP-containing admixture showed a higher turbidity than either the control or the HSS, especially when the freeze-dried nature of the HSS formulation is considered. A precipitate, resulting from an interaction between HSP and the admixture, is one possible explanation and further work on this subject should be attempted to elucidate the reason. In common with the HSS-containing samples, centrifuge and Neubauer slide testing of HSP-containing samples showed evidence of concentration-dependent lipid damage. Some amorphous globules were observed after 24 hours by light microscopy and these test admixtures gave the highest maximum particle size and the largest secondary peak by volume, when analysed by laser diffraction. However, the D[4,3] was not significantly different to those of HSS or control samples.

The pH of the admixtures tested were found to be between 5.80 and 6.00, lower than the optimum range for hydrocortisone stability and tissue

permeability, but stability periods have been ascertained in these experiments and the structure of the hydrocortisone salt will change and tissue permeability improve when exposed to the higher pH of the physiological environment (Garrett 1962).

It should be noted that both the HSS and HSP formulations contain inorganic phosphate salts, which could potentially interact with calcium ions in the same way as phosphate preparations added to the PN admixture. Although the quantities are very small (4.35 mg, or $4.58 \times 10^{-5}$ moles, phosphate per ml HSP; data unavailable for HSS), these excipients must be considered when significant amounts of the hydrocortisone preparations are to be added to the admixture, which already contained the maximum phosphate concentration allowed by the manufacturers (37.5 mmol) (Anon. 2002a). The formulations also contain sodium (1.83 mg, or $7.96 \times 10^{-5}$ moles, per ml in HSP; data unavailable for HSS) which could potentially interact, although this is extremely unlikely at this dose.

There was no significant difference in the HSS concentration levels of the two control admixtures at any time, showing that the inclusion or omission of lipid emulsion in this admixture is not an important factor in determining HSS stability. Light exposure of admixtures containing HSS did not increase drug degradation and the inclusion of lipid emulsion in the test admixtures did not significantly change the amount of drug remaining at 48 hours. However, control and daylight-exposed aqueous samples remained above 90% for only 36 hours compared to 48 hours for all other samples in this test but this difference in $t_{90}$ was not statistically significant, and it can be seen from Figure 7.10 that the difference is likely to be due to experimental variation.

When comparing light-protected samples with the controls, levels of HSP in light-protected lipid samples were consistently higher than all others, followed by lipid control, aqueous light-protected and aqueous control respectively, but the only significant difference lay between the two extremes, i.e. lipid light-

protected and aqueous control. This may suggest that light could potentially damage HSP, but the high levels of drug retained in all admixtures and the lack of difference between most samples may indicate that light is not a major cause of HSP degradation in this test admixture. This is supported by the fact that the manufacturer of Efcortesol™ does not mention the use of light protection in its Summary of Product Characteristics (Sovereign 2000).

The inclusion of extra WFIs to decrease the relative concentration of the aqueous test admixture to mimic that of the lipid-containing test admixture resulted in significantly higher levels of HSS being detected throughout testing compared to all other admixtures in the experiment (see Figure 7.12). This pattern was also seen in the HSP tests (Figure 7.15), although the difference was only significant between admixtures containing extra WFIs and aqueous control samples. The difference between hydrocortisone levels in the controls and the admixtures containing extra WFIs may be explained by the more dilute environment of the latter. However, that the levels of hydrocortisone in the admixture containing extra WFIs were higher than those in the lipid-containing admixtures implies that concentration effects are not the only reason for the difference and shows that the admixture containing extra WFIs was not an ideal model to simulate the lipid admixtures.

Results comparing infusion bags showed that higher levels of hydrocortisone were retained in the ML than the EVA bags, demonstrating that oxygen is a factor in hydrocortisone stability (see Figures 7.11 and 7.15). For the HSS samples, significant differences were seen between the bags at 48 hours. No significant difference was seen between the HSP-containing admixtures, implying that oxygen protection is not as important for HSP stability as it is for HSS. Overall, it appears that oxygen exposure is not the major factor controlling hydrocortisone stability in this test admixture.

Results comparing HSS and HSP under various test conditions have shown that levels of HSP are consistently higher as time increases. This is to be expected,

as HSP is available as a solution for injection, whereas stability problems with HSS have led to its formulation as a powder for reconstitution, therefore it may be expected to display a shorter stability period.

## 7.5 CONCLUSION

There appear to be many interrelated and complicated factors dictating the stability of hydrocortisone in PN. However, HSP levels remained stable and above 90% for 48 hours in all variations tested; the shallow degradation curves suggest that the assurance of longer stability periods may be possible with further experimentation. This may also allow the differences between storage conditions to be elucidated, which was not possible with the current data set.

Lipid admixtures retained levels of HSS greater than 90% for the test duration in all light and air permutations tested. Levels in aqueous admixture varied with light and air conditions and because these factors may be interrelated, the recommended shelf-lives have been reduced to take account of the $t_{90}$ under the least stable storage conditions (see Table 7.1). Although not all admixtures retained levels of HSS above 90% for the duration of testing, the expected infusion period of the PN admixture allows the use of this hydrocortisone preparation.

Neither formulation was precluded from addition to the test admixture by the results of the physical tests. Fewer differences in the physical stability data were seen between HSS and control than between HSP and control, which suggests that HSS should be the formulation of choice for inclusion in this PN admixture, as does the observation in the BNF (2004) that the administration of HSP injection is more irritant than HSS injection. However, the shorter $t_{90}$ of many of the HSS-containing admixtures tested and interaction of several stability factors lead to the conclusion that HSP may be the preferred hydrocortisone preparation for inclusion in the test PN admixture.

*Table 7.1 Recommended Shelf-Life of Solu-Cortef® (HSS) in FK Regimen 5*

| Admixture type and storage condition | Recommended shelf-life / hours |
|---|---|
| Aqueous – Light protected, ML bags | 48 |
| Aqueous – Light protected, EVA bags | 36 |
| Aqueous - Unprotected (ML bags) | 36 |
| Lipid - Light protected | 48 |
| Lipid - Unprotected | 48 |

Note: for lipid samples, storage in different infusion bags did not alter shelf-life.

*Table 7.2 Recommended Shelf-Life of Efcortesol™ (HSP) in FK Regimen 5*

| Admixture type and storage condition | Recommended shelf-life / hours |
|---|---|
| Aqueous - Light protected | 48 |
| Aqueous - Unprotected | 48 |
| Lipid - Light protected | 48 |
| Lipid - Unprotected | 48 |

Note: storage in different infusion bags did not alter shelf-life.

# CHAPTER 8
# HEPARIN

# CHAPTER 8 – HEPARIN

## 8.1 INTRODUCTION

### 8.1.1 HEPARIN IN PARENTERAL NUTRITION

Heparin is a naturally occurring anticoagulant, produced by mammals, primarily in the mast cells (Quader et al. 1998). It is an anionic polysaccharide composed of a repeating disaccharide unit of alternating sulphated d-glucosamine and one of two uronic acid residues (either d-glucuronic acid or l-iduronic acid) (Desai 2000; Martindale 1999) (see Figure 8.1). The strong negative charges on the molecule are important in heparin's mechanism of clotting factor inactivation.
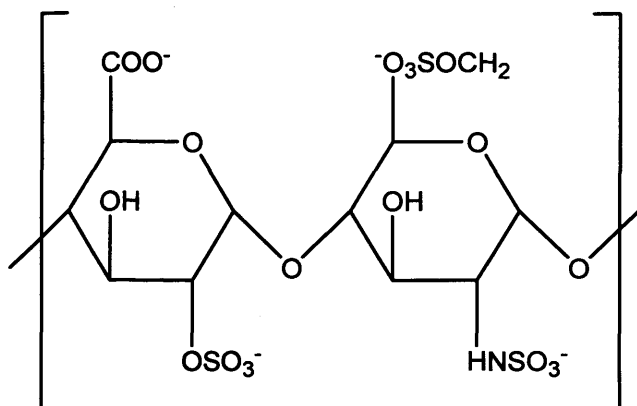


*Figure 8.1 Disaccharide Heparin Unit*

There are two classes of heparin available for clinical use, distinguished by their size; standard or unfractionated heparin, which ranges from 3000 to 30000 daltons, with most commercially available preparations between 12000 and 15000 daltons (Desai 2000), and the low molecular weight (LMW) heparins, which have an average molecular mass of 3000 to 7000 daltons (Quader et al. 1998). Standard heparin is prepared from the intestinal mucosa of oxen, pigs or sheep, or from the lungs of oxen and is commercially available as the sodium or calcium salt, which display little difference in their clinical effects (Hirsh 1991). LMW heparins are salts of standard heparin fragments, produced by enzymatic or chemical fractionation or depolymerisation

(Verstraete 1990). Due to the different methods of preparation and consequent heterogeneity of the structure of the LMW heparins, differences are seen in their molecular masses and clinical activity (Verstraete 1990).

Heparin's anticoagulant properties are employed principally for the treatment and prophylaxis of thrombo-embolic disorders. These uses include the treatment of deep vein thrombosis, pulmonary embolism, myocardial infarction, unstable angina and acute peripheral arterial occlusion, and surgical prophylaxis especially in orthopaedic surgery and in patients at high risk of developing thrombosis, for example obese patients and those with a history of deep vein thrombosis or pulmonary embolism (BNF 2004). Heparin is also used in extracorporeal circuits during haemodialysis and cardiopulmonary bypass and to maintain the patency of intravenous catheters and cannulae. Maintaining the patency of indwelling infusion devices using heparin may be attempted in one of two ways; firstly, by using the heparin solution as an intermittent lock or flush, or secondly, by including the heparin solution in the intravenous fluid to be administered. The evidence for the use of heparin as a lock or flush is inconclusive and several studies have been unable to demonstrate a benefit compared to sodium chloride 0.9% (Randolph et al. 1998). However, it has been shown that the inclusion of a low dose of heparin (1 IU/ml) in an infusion to be administered continuously may prolong the lifespan of the catheter, decrease the risk of thrombus formation (Randolph et al. 1998) and reduce phlebitis (Payne-James and Khawaja 1993), hence the appeal to hospitals of adding heparin to PN admixtures.

Standard heparin offers rapid anticoagulation, but is short acting and known to cause a number of adverse effects. LMW heparins generally have improved bioavailability and predictability (therefore requiring less monitoring) (Quader et al. 1998) and a longer duration of action, allowing doses to be administered once a day (BNF 2004), although variations in both these properties are seen between products. The risk of adverse effects seen upon administration of standard heparin, namely haemorrhage, thrombocytopenia (reduced platelet

count) and osteoporosis (with long-term therapy), although still present with LMW heparins, may be reduced (Quader et al. 1998). The main disadvantage associated with the use of LMW heparins is the increased initial financial outlay, but some studies have shown that this may be balanced by increased cost-effectiveness in certain clinical situations (O'Brien 2000; Segal et al. 2003).

For the purpose of this investigation, one standard heparin preparation (heparin sodium, as Monoparin®) and one LMW heparin (dalteparin sodium, as Fragmin®) was chosen. Dalteparin was selected as the LMW heparin as it is widely used in practice and is licensed for intravenous use, as well as the more usual subcutaneous route. It must be realised that data should not be extrapolated between heparin brands because differences in formulations exist even though heparins are manufactured to comply with pharmacopoeial standards, for example those of the British Pharmacopoeia (2003) which states that:

*"The potency of heparin sodium intended for parenteral administration is not less than 150 IU/mg, calculated with reference to the dried substance."*

In the case of LMW heparins this may be even more important due to differences in each product's anticoagulant activity, resulting from the different methods of manufacture.

## 8.1.2 STABILITY ISSUES

### 8.1.2.1 Introduction

Heparin has been the subject of many studies, although most have only examined visual compatibility (Trissel 2001). These studies have shown incompatibilities between heparin and a great many drugs, however, physical compatibility has been demonstrated between heparin and many of the commonly used infusion fluids, and the BNF recommends administration of

heparin by continuous intravenous infusion in sodium chloride 0.9% or glucose 5% solution (2004).

It is stated in the Handbook on Injectable Drugs (Trissel 2001) that heparin is compatible with FreAmine®, an amino acid solution, and that the activity of heparin in an amino acid and glucose solution was retained for 24 hours. No evidence suggesting that heparin is incompatible with other amino acid solutions is presented. Other components of PN admixtures, however, have elicited reports of incompatibility with heparin.

The activity of heparin has been seen to change when added to glucose solutions, but this has not been the case in every report. Apparent losses of up to 60% in six hours have been reported (Okuno and Nelson 1975), whereas some authors noted no loss of activity for up to 60 hours (Hodby et al. 1972), or even an apparent loss then total recovery at a later time point (Anderson et al. 1977). A review of the literature and further studies by Anderson and Harthill (1982) showed a "dextrose effect" which explained the apparent loss of activity and later rebound as a consequence of fluctuating conditions in the solution. Factors thought to be important included pH, heparin concentration, differences in the glucose product used and the container. It was thought that heparin may, under the influence of glucose, undergo reversible conformational changes leading to loss of activity. Despite the different findings, there appears to be a general consensus that glucose solutions are suitable diluents for heparin (Martindale 1999).

Potentially detrimental interactions between lipids, calcium and heparin have been documented; gross flocculation of lipid emulsion was seen in a paediatric admixture (Raupp et al. 1988) and white deposits were seen in catheter lines (Rattenbury et al. 1988). It has been suggested that the positively charged calcium ions form a bridge between the negatively charged groups on the heparin molecule and the phospholipids (Raupp et al. 1988), thus reducing the surface charge on the lipid globules and increasing the possibility of

aggregation occurring (Minton 1998).

## 8.1.2.2 pH

pH ranges at which heparin is reported to be stable vary with drug concentration and diluent (Trissel 2001). The most limited range cited within the Handbook of Injectable Drugs is 5 to 8.5, and, although heparin was also autoclaved in the reported study, the values given are close to those of the European Pharmacopoeia (2001) and USP (2002) which state that a 1% solution of heparin should have a pH of between 5.5 and 8.0, and 5.0 and 7.5 respectively, with stability not assured outside of these ranges. The test PN admixture for this study falls within this range and should not, therefore, be detrimental to the stability of heparin. However, heparin has been seen to raise the pH of some diluents in a concentration-dependent manner (Trissel 2001). This is unlikely in the case of a PN admixture due to the buffering capacity of the amino acids but if, upon pH testing of the admixture, it is apparent that the pH of the admixture has been significantly changed and is no longer within the stability range of heparin, further investigations will be performed.

## 8.1.2.3 Air

Both the BP (2003) and Martindale (1999) state that both standard and LMW heparins should be stored in airtight containers. There is no further information given in the SPCs, but both preparations are presented in such a way as to minimise contact with the air.

## 8.1.2.4 Freezing

Neither heparins' SPC mentions the need to protect the drug from freezing, however the Handbook on Injectable Drugs states that heparin should not be frozen (Trissel 2001). Freezing studies are therefore necessary to ensure that storage under these conditions did not affect chemical test results.

### 8.1.2.5 Calcium Concentration

The interaction between calcium, lipid and heparin is well documented (see Section 8.1.2.1) and it has been suggested that incompatibilities between these PN components are likely to occur when the levels of heparin and calcium exceed 1 IU/ml and 1 $\mu$mol/ml respectively (Rattenbury et al. 1989).

### 8.1.2.6 Heparin Type

A comparison of the destabilisation of an AIO admixture by Monoparin® and Fragmin® showed that the LMW heparin caused less damage to the lipid emulsion component (Durand and Barnett 1992), and this will be investigated in these studies.

### 8.1.2.7 Container Type

A report by Tunbridge et al. (1981) suggested that loss of heparin activity may occur upon contact with glass. However, this has not been proven and heparin is presented in glass ampoules by manufacturers. Loss of heparin due to sorption to PVC containers has been shown (Sautou et al. 1994), but good compatibility with polyethylene and polypropylene syringes has been demonstrated (Simmons and Allwood 1981; Sautou et al. 1994). No information on the compatibility of heparin with EVA was reported.

## 8.2 MATERIALS AND METHODS

### 8.2.1 MATERIALS

Monoparin® is a solution for injection containing heparin sodium (mucous) BP, 1000 IU/ml ampoule (see Table 3.2). The solution also contains WFIs, hydrochloric acid and sodium hydroxide as excipients.

Fragmin® is a solution for injection containing dalteparin sodium 2500 IU/0.2 ml, sodium chloride Ph. Eur. and WFIs Ph. Eur. (see Table 3.2).

## 8.2.2 GENERAL METHODS

The physical methods described in Chapter 3 were used to assess the stability implications of adding heparin to parenteral nutrition.

Due to the nature of heparin's structure, chemical analysis is not straightforward. A literature search for heparin assays produced the following suggestions:

### 8.2.2.1 HPLC

The HPLC assay method described by Sautou et al. (1994) used a C18 column, a mobile phase consisting of 65% phosphate buffer (0.01M) and 35% acetonitrile, a flow rate of 1.3 ml/min and a detection wavelength of 220 nm. The concentration of heparin assayed was between 125 and 500 IU/ml.

### 8.2.2.2 ELISA

ELISA (Enzyme-Linked Immunosorbent Assay) is a rapid test used to measure very small quantities of a substance. An antibody linked to an enzyme is used to detect the substance of interest. The enzyme catalyses a reaction between the antibody and the antigen (the analyte) which produces a coloured product, the amount of which is proportional to the quantity of analyte present. The ELISA test is very specific and sensitive.

### 8.2.2.3 Chromogenic Assay

Heparins increase the activity of antithrombin which inhibits factors IIa and Xa (pro-clotting factors). When both factor Xa and antithrombin are present in excess in a sample, the rate of factor Xa inhibition is directly proportional to the heparin concentration. Residual Xa activity, which is inversely proportional to the heparin concentration, is quantified using a specific Xa substrate, detected by the use of a spectrophotometer. Detection limits for heparin using this test are between 0.1 and 0.8 IU/ml and outside this range, linearity may be lost.

### 8.2.2.4 Toluidine Blue Dye

The addition of toluidine blue dye to a solution containing heparin results in the formation of a complex, which can be partitioned into hexane. The concentration of the residual dye is quantified using a spectrophotometer and the quantity of heparin present deduced using a calibration curve (Smith et al. 1980).

### 8.2.2.5 Summary of Chemical Assays

The HPLC assay tested was insensitive to the low levels of heparin present in the test admixtures. Other HPLC assays were considered but these too were inappropriate due either to their limits of detection (De Vries 1989; Thanawiroon and Linhardt 2003) or lack of equipment (Barrowcliffe et al. 1989; Hennink et al. 1987; Toida et al. 1997), the latter difficulty also precluded the use of the ELISA technique.

As stated in Section 8.2.2.3, linearity of response for the chromogenic assay is lost when the heparin concentration tested is outside the range 0.1 to 0.8 IU/ml. In addition to this, the presence of the lipid emulsion in the test samples rendered them opaque, thus precluding the use of a spectrophotometer.

Trials of the toluidine blue assay indicated that the concentration of heparin present was too low to complex with a significant amount of dye and consequently the decrease in the amount of dye was immeasurable.

Due to time and cost constraints, no other suitable assay for the chemical stability of heparin at the required test concentration could be found.

### 8.2.3 METHOD SPECIFICS

### 8.2.3.1 Experimental Specifics

Good practice dictates that a PN admixture is thoroughly mixed between additions and this is particularly important after the addition of heparin, as it is

known to pool unless the container is inverted a number of times (Trissel 2001). This pooling can result in a high concentration of the drug being delivered to the patient in what is effectively a bolus dose, even possibly an overdose, and therefore the advantages of continuous administration are lost.

The known interaction between calcium, lipid emulsion and heparin (Section 8.1.2.6) led to the construction of a stability matrix to test the physical compatibility of three doses of heparin against three concentrations of calcium in the test PN admixture. Concentrations of lipid emulsion were kept constant throughout testing, because to change this would change the regimen. High level test concentrations of heparin and calcium were the usual continuous infusion dose for heparin and the maximum allowable in the admixture for calcium, low levels were set at zero for both to act as a control (this necessitated the use of an electrolyte-free amino acid solution in the case of calcium) and medium levels were half the standard dose for heparin, and the calcium concentration contained in the standard admixture before the addition of electrolytes (see Table 8.1 for concentrations and Table 8.2 for combinations).

*Table 8.1 Concentrations of Calcium and Heparin in Compatibility Matrix Tests*

| Concentration Level | Calcium Concentration / μmol/ml PN | Heparin Concentration / IU/ml PN |
|---|---|---|
| Low | 0 | 0.0 |
| Medium | 2 | 0.5 |
| High | 5 | 1.0 |

*Table 8.2 Key to Calcium/Heparin Matrix Combinations*

| Abbreviation | Calcium Concentration Level | Heparin Concentration Level |
|---|---|---|
| L/L | Low | Low |
| L/M | Low | Medium |
| L/H | Low | High |
| M/L | Medium | Low |
| M/M | Medium | Medium |
| M/H | Medium | High |

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| H/L | High | Low |
|-----|------|-----|
| H/M | High | Medium |
| H/H | High | High |

## 8.3 RESULTS

Note: Results from the compatibility matrix tests are given after those for the standard heparin dose (1 IU/ml) in the standard test admixture results, and are identified as such.

### 8.3.1 VISUAL EXAMINATION

Visual examination of lipid admixtures showed a small amount of creaming in all samples, from 12 hours onwards. The depth of the cream layer did not exceed 4 mm at any time, in any sample, and was always easily redispersable.

Aqueous admixtures containing heparin were indistinguishable from the control. All samples had become a duller yellow colour by 12 hours. Under examination with a cold light source, each sample showed a haze at all times.

*Matrix Tests*

Visual examination did not reveal any differences between the samples in either the aqueous or lipid matrix tests.

### 8.3.2 MICROSCOPY

The maximum globule diameters observed for control lipid samples were 5 $\mu$m at all time points except 24 hours when three enlarged globules of up to 15 $\mu$m were observed. In the samples containing Monoparin®, for times of up to 12 hours, all observed globules were 5 $\mu$m or smaller and there was no evidence of flocculation. At the later time points, some amorphous globules were seen and some areas of the sample appeared to have begun to flocculate. The maximum globule sizes seen at these times were 20 $\mu$m at 24 and 36 hours (which included ten and seven globules greater than 10 $\mu$m respectively) and 7.5 $\mu$m at 48 hours. Globules in Fragmin®-containing samples reached a maximum of

5 μm at all times, but some samples included small areas of flocculation from 12 hours.

Examination of aqueous admixtures showed the presence of a small number of crystals in both the control and heparin-containing samples, however, the control samples contained just two particles of up to 10 μm whereas the Monoparin®-containing samples contained six aggregates of up to 25 μm in diameter and the Fragmin®-containing samples contained three aggregates of 20 to 30 μm in diameter.

*Matrix Tests*

A degree of flocculation was evident by 6 hours in all lipid-containing samples with medium or high levels of Monoparin®. In some samples (H/M and H/H), flocculation was seen at 0 hours but not at 3 hours, when it was seen in the L/M and L/H samples. From 12 hours onwards, flocculation was seen in all samples with medium or high levels of Monoparin®. Aggregates were seen in M/M, M/H, H/M and H/H samples from 6 hours, however these aggregates did not exceed 7.5 μm until 24 hours, when they reached 20 μm in the M/M and H/H samples. Samples containing low levels of Monoparin® reached a maximum globule diameter of 7.5 μm, except in the case of the M/L sample in which one 25 μm globule was observed at 24 hours, but at no other time point.

Aqueous test admixtures containing Monoparin® with low levels of calcium reached a maximum particle diameter of 10 μm. No more than two crystals were seen in any of these samples. The particles observed in the samples containing medium levels of calcium were all smaller than 5 μm, and reached a maximum count of six. The H/L sample was similar in appearance to the samples with low calcium concentration, containing two crystals, one 7.5 μm and one 10 μm in diameter. Aggregation of crystals was evident in both the H/M and the H/H samples, the former containing five aggregates of 20 to 30 μm, and the latter six aggregates with a maximum diameter of 25 μm.

Microscopic examination of all Fragmin®-containing lipid admixtures with low concentrations of calcium consistently showed low numbers of enlarged globules. The maximum size observed was 15 μm (seen in the L/L sample at 0 hours), and this was the only globule larger than 10 μm observed. In samples containing medium concentrations of calcium the largest globule observed was 20 μm, one of five globules greater than 10 μm in diameter seen in the M/L samples. Globules greater than 10 μm were seen in M/L samples at 24 hours only, and not at all in M/M samples. At 24 hours, some evidence of flocculation was seen in M/H samples; some globules had changed shape, but no aggregation had occurred. The maximum globule size seen in this sample was 10 μm, observed at 0 hours. Samples containing high levels of calcium and low levels of Fragmin® reached a maximum globule size of 15 μm, at 24 hours, with three globules larger than 10 μm observed. At all other time points, the maximum globule size in this combination was 5 μm. Two 15 μm globules were observed in the H/M sample at 0 hours, but the maximum size reduced to 5 μm in the 3 hour test. However, flocculation appears to have begun by 6 hours, and was more pronounced by 24 hours. Aggregates of up to 7.5 μm, constructed of multiple globules smaller than 5 μm, were present from 12 hours. Flocculation was evident in the H/H samples from 3 hours, but aggregates did not exceed 10 μm in diameter.

Crystals were observed in all Fragmin®-containing aqueous samples, except the M/H sample. For the low and medium calcium concentration samples, crystals reached a maximum diameter of 10 μm, and maximum count of two per sample. This was also the case for the H/L sample. The H/M sample contained approximately twenty crystals with a maximum diameter of 5 μm, and the H/H contained three crystalline aggregates of between 20 and 30 μm.

## 8.3.2.1 Neubauer Slide Technique

Large crystals and crystalline aggregates of up to 50 μm were seen in both the Monoparin®- and Fragmin®-containing lipid admixture. Extensive flocculation was also observed (see Figure 8.2), with many of the globules

losing their characteristic round shape. Little aggregation was evident. Crystals of up to 30 μm were also seen in the aqueous admixture when combined in a 1:1 ratio with heparin. However, aggregates were not observed.

When heparin was included in lipid admixtures at a 1 IU/ml dose, localised flocculation was evident, with associated change in globule shape. A small number of crystals of up to 10 μm were seen in the aqueous admixture tested against 1 IU/ml heparin.



*Figure 8.2 Flocculation Caused by Monoparin® in Lipid Admixture at 1:1 Ratio*

*Matrix Tests*

Due to the need for a 1:1 interaction between drug and test admixture in the Neubauer slide technique, it was not possible to perform the full matrix test. A comparison between low and high dose heparin was carried out, using WFIs to represent 0 IU/ml heparin, and the standard solutions for the high dose, 1:1 examination.

No aggregation or flocculation of the lipid emulsion was evident at any calcium concentration with the low heparin concentration samples. Isolated globules of up to 15 μm and 25 μm in the Fragmin®- and Monoparin®-containing samples respectively were seen, but over 99% of globules were 5 μm or smaller. Crystal formation and flocculation was seen with all three calcium concentrations tested against both heparin formulations at a 1:1 ratio. Flocculation was more extensive in the medium and high calcium

concentration test admixtures, but the number and size of crystals formed did not appear to vary with calcium concentration. Crystals seen in the aqueous admixture combined with heparin in a 1:1 ratio were of the same order of magnitude as those seen in the lipid admixture.

A small amount of flocculation was seen in the Monoparin® H/M and H/H samples at the clinical dose. This was not seen with the Fragmin®-containing samples. Combination of each heparin with the aqueous admixtures produced a small number of crystals up to 10 μm in diameter.

8.3.3 LASER DIFFRACTION

The maximum recorded diameter in the control samples was 4.30 μm at all times. In both of the heparin-containing admixtures, the maximum diameter was 4.30 μm from 0 to 36 hours, but increased to 5.29 μm at 48 hours. The D[4,3] of all three types of admixture varied between 0.36 and 0.37 μm. Secondary peaks of between 0.96 and 1.26% volume of the sample were recorded in the control samples. Monoparin®- and Fragmin®-containing samples had secondary peaks of between 0.88 and 1.41% and 0.84 and 1.34% respectively. No obvious trend between the volume of the secondary peak and time was detected.

*Matrix Tests*

Except for the H/H samples of both brands of heparin, all samples measured gave a maximum diameter of 4.30 μm. The H/H samples measured 5.29 μm, but this was only at 48 hours.

For the Fragmin® experiments, the minimum D[4,3] recorded was 0.35 μm, which was recorded with each of the low calcium concentration samples at various time points, while the maximum D[4,3] achieved by these samples was 0.36 μm. The maximum D[4,3] for any of the Fragmin®-containing samples was 0.37 μm which was recorded for at least one of the high calcium concentration samples at all time points and one medium calcium concentration

sample at one time point. All other medium and high calcium concentration samples measured 0.36 μm.

No clear correlation between either heparin or calcium concentration was observed for the volume of the secondary peak, although there appeared to be a slight increase with increasing additions in the Monoparin®-containing samples (see Figure 8.3) and with increasing calcium in the Fragmin®-containing samples (see Figure 8.4).
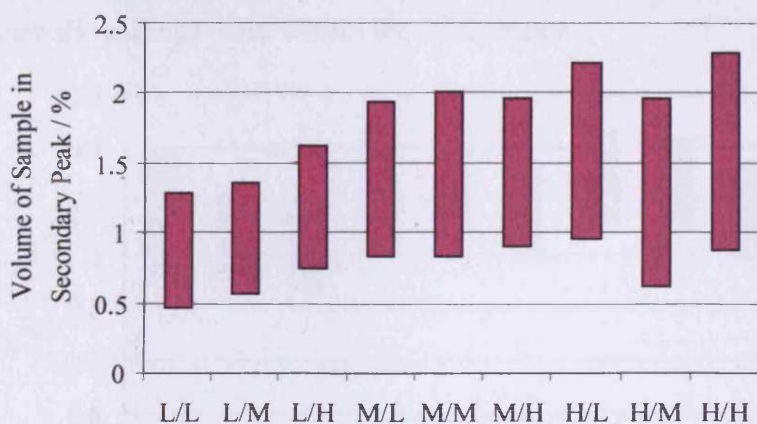


Figure 8.3 Secondary Peak Volume Ranges for Monoparin®-Containing Admixtures
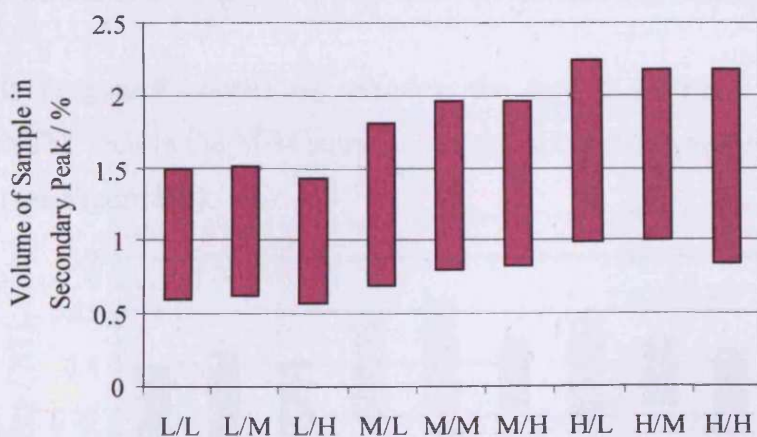


Figure 8.4 Secondary Peak Volume Ranges for Fragmin®-Containing Admixtures

8.3.4 NEPHELOMETRY

Turbidity values for the control samples ranged from 0.744 to 0.866 NTU,

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decreasing steadily at a rate of -0.0019 NTU/hour. Samples containing Monoparin® ranged from 0.745 to 0.837 NTU, and those containing Fragmin®, from 0.732 to 0.811 NTU, decreasing at -0.0016 NTU/hour and -0.0014 NTU/hour respectively.

*Matrix Tests*

In the Monoparin®-containing samples, the largest change over time was seen in the L/L sample, which decreased by 0.121 NTU. It can be seen from Figure 8.5 that all readings were within the same range.
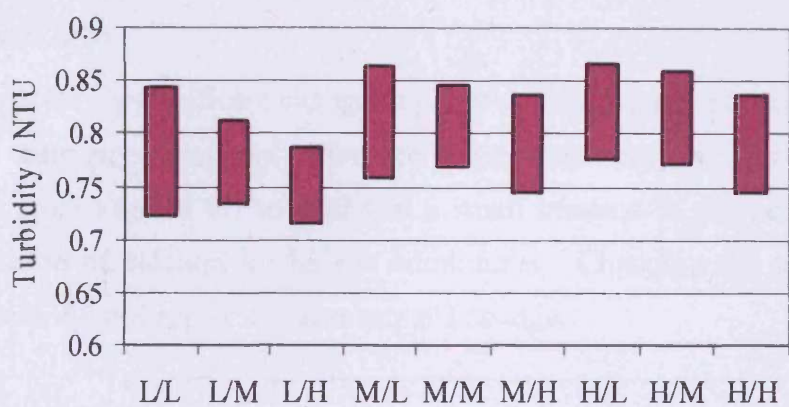


*Figure 8.5 Turbidity Ranges of Monoparin®-Containing Admixtures*

For the Fragmin®-containing samples, the largest decrease over time was 0.127 NTU, seen in the M/M sample. Again, all readings were within a narrow range (see Figure 8.6).
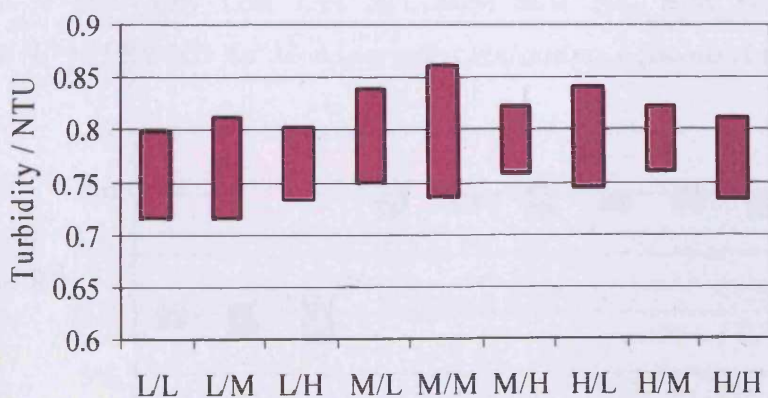


*Figure 8.6 Turbidity Ranges of Fragmin®-Containing Admixtures*

### 8.3.5 pH MEASUREMENT

The pH values for the lipid control samples ranged from 5.89 to 5.95, and were very similar to those of both Monoparin® (5.90 to 5.95) and Fragmin® (5.89 to 5.95).

For the aqueous control samples, the range measured was 5.86 to 5.90. Values measured for Fragmin®-containing samples ranged from 5.86 to 5.89. Monoparin®-containing aqueous samples had a slightly larger range, between 5.84 and 5.92, but this was still within acceptable limits.

*Matrix Tests*

There was no significant change in pH over time for any of the admixtures, nor was there any significant difference between the samples. However, it can be seen from Figures 8.7 to 8.10 that a small increase in pH occurred upon the inclusion of calcium in the test admixtures. Changing the concentration of heparin did not appear to cause any pH change.
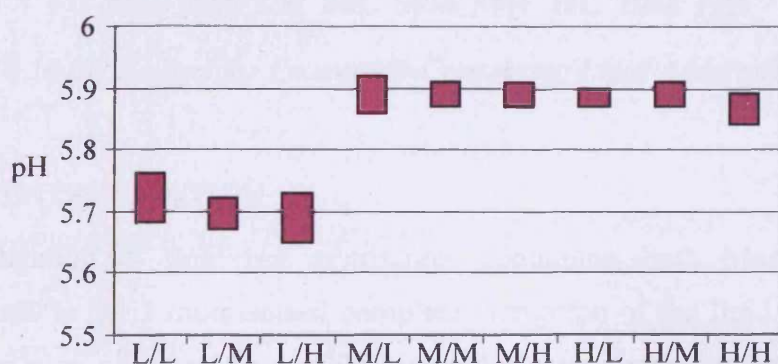


*Figure 8.7 pH Ranges for Monoparin®-Containing Aqueous Admixtures*
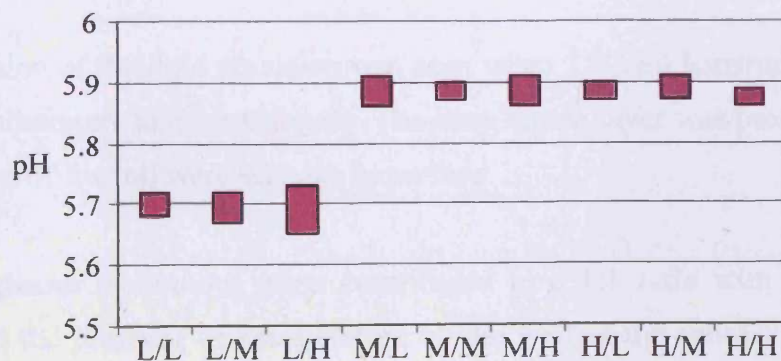


*Figure 8.8 pH Ranges for Fragmin®-Containing Aqueous Admixtures*

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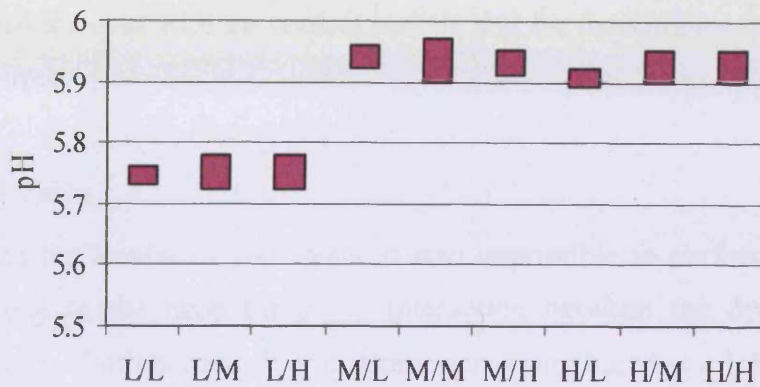
*Figure 8.9 pH Ranges for Monoparin®-Containing Lipid Admixtures*
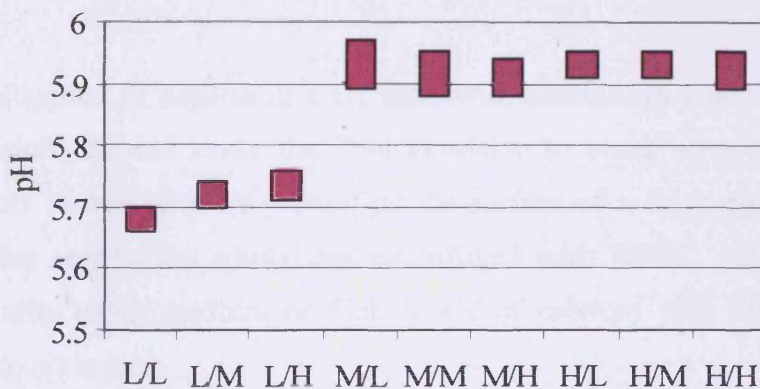


*Figure 8.10 pH Ranges for Fragmin®-Containing Lipid Admixtures*

## 8.3.6 CENTRIFUGATION

Centrifugation of lipid test admixtures containing both Monoparin® and Fragmin® in a 1:1 ratio caused complete disruption of the lipid emulsion and the formation of an oil layer. The control sample separated into an aqueous layer and a deep cream layer, with no evidence of free oil.

Disruption of the lipid emulsion was seen when 1 IU/ml heparin was included in the admixture and centrifuged. The deep cream layer was present, but large globules of free oil were seen on its surface.

The aqueous admixture, when centrifuged in a 1:1 ratio with each heparin, showed the presence of precipitation on the wall of the centrifuge tube. This

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was also the case with the control sample and for the samples including heparin at 1 IU/ml.

*Matrix Tests*

As with the Neubauer slide tests, it was impossible to perform all the matrix tests, due to the need for a 1:1 interaction between the drug and the test admixture. Both heparin formulations were centrifuged at a 1:1 ratio with each of the three calcium concentrations and compared to WFIs combined with the test admixtures.

Centrifugation of heparin in a 1:1 ratio with admixtures containing low levels of calcium did not cause the lipid emulsion to break completely, but small amounts of free oil were present on the surface of a thick cream layer. This was also seen in the admixtures centrifuged with WFIs. All the admixtures containing either medium or high levels of calcium with heparin formed a discrete oil layer.

Centrifugation of the low calcium admixture with heparin included at 1 IU/ml did not cause disruption of the lipid emulsion. M/H samples contained a small amount of free oil on the surface of the cream layer, and H/H samples contained larger oil globules, again on the surface of the cream layer.

All aqueous samples containing medium or high levels of calcium showed precipitation on the wall of the tube, irrespective of heparin content. No precipitation was observed in the admixtures with low levels of calcium.

## 8.3.7 OSMOLALITY

The osmolality of lipid control samples ranged from 1695 to 1714 mOsm/kg. For admixtures containing Monoparin®, values of 1669 to 1699 mOsm/kg were obtained, and for Fragmin® the values were 1730 to 1733 mOsm/kg.

The osmolality values for aqueous samples were higher than those for the lipid-

containing admixtures. Control samples ranged from 1877 to 1908 mOsm/kg, Monoparin®-containing samples from 1902 to 1912 mOsm/kg and Fragmin®-containing samples from 1902 to 1905 mOsm/kg.

*Matrix Tests*

A clear difference was observed between the osmolality of the lipid and aqueous admixtures in all samples (see Figure 8.11). Samples containing low levels of calcium consistently gave lower readings than those containing medium or high levels. The inclusion of heparin did not appear to affect the osmolality of the samples.
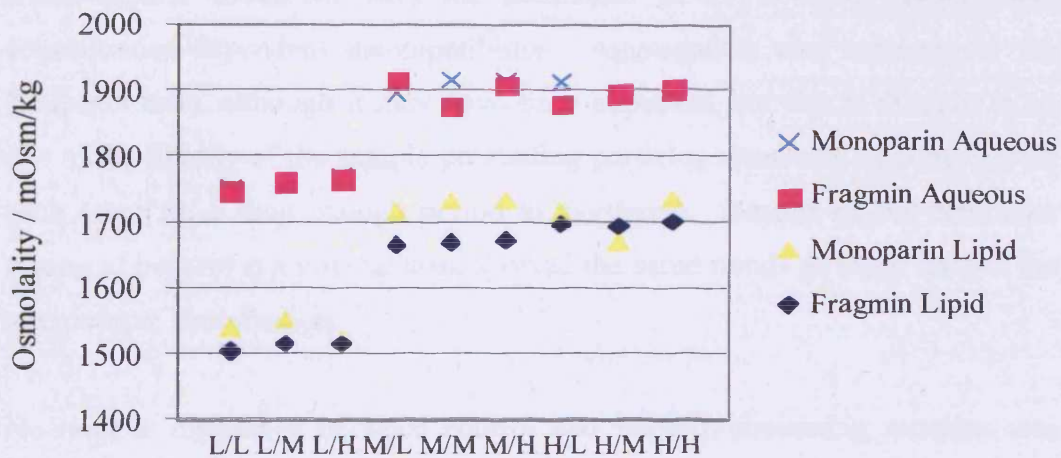


*Figure 8.11 Osmolality of Heparin-Containing Admixtures*

## 8.4 DISCUSSION

Visual examination did not reveal any differences between control or heparin-containing admixtures, nor between the matrix test admixtures and no significant change over time in turbidity readings was seen for any sample.

At 0 hours all samples were microscopically acceptable, however, the development of differing degrees of flocculation with the addition of heparin indicates that an incompatibility with the admixture occurred. Flocculation was observed in admixtures at every level of calcium, when Monoparin® was included. Low calcium admixtures contained 0 mmol/ml calcium and may

have been expected not to flocculate (see Section 8.1.2.1) but it is thought that the other multivalent ions present (magnesium and zinc) could have acted in a similar way to calcium in these admixtures, producing the bridges described in Section 8.1.2.1. The presence of large crystalline aggregates in the aqueous admixtures also demonstrates a potential stability issue. These are likely to have developed as a consequence of an incompatibility between the test admixtures and the heparin formulations, as these aggregates were not observed in the control admixtures.

The extensive lipid damage observed in the Neubauer and centrifuge tests, when heparin interacted with the admixture in a 1:1 ratio, shows clear concentration-dependent incompatibility. Aggregation was not seen in the Neubauer tests, although it may have been expected, but this is thought to be due to the fluidity of the sample preventing particles remaining in contact with each other for a long enough period to aggregate. Results of the Neubauer testing of heparin at a clinical dose showed the same trends as those seen in the microscopic examination.

No notable difference between control and heparin-containing samples was seen in the laser diffraction results until 48 hours, when the control sample remained at 4.30 $\mu$m, but the heparin-containing samples increased to 5.29 $\mu$m. In the matrix tests, a maximum diameter of 5.29 $\mu$m was obtained only in the H/H samples.

It can been seen from Figures 8.7 to 8.10 that the addition of calcium to the matrix admixtures caused an increase in pH, but the change was not greater than 0.5 units. The ranges seen for control versus heparin-containing admixtures were also smaller than 0.5 units. The possible increase in admixture pH following addition of heparin (see Section 8.1.2.2) was not observed.

Osmolality readings were not changed from those of the controls by the

addition of heparin, in either lipid or aqueous, but the inclusion of calcium in the matrix tests caused an increase in both lipid and aqueous admixture osmolality, in the presence and absence of heparin.

Due to the lack of chemical stability testing on heparin conclusions must be based on literature review and physical test results. This suggests that exposure to factors that may be potentially detrimental to heparin, such as air, should be minimised, particularly as the dose included in the admixture is very low, and even a small loss may constitute a significant proportion of the original dose.

## 8.5 CONCLUSION

No one analytical method was able to prove or disprove the compatibility of heparin with the test admixtures and, as the combined results were not conclusive, the addition of either Monoparin® or Fragmin® to the test admixture cannot be recommended. Although the well-documented interaction between heparin, calcium and lipid emulsion may explain the reason for avoiding the addition of heparin to this lipid admixture, the interaction between heparin and the aqueous admixture was unexpected. Although crystal formation has been seen upon addition of other drugs to the test admixture, the tendency of the crystals to aggregate has not and as such, this phenomenon requires further investigation.

Summaries of the results for the matrix tests are shown in Tables 8.3 to 8.6. Where the results of the physical tests were not conclusive, the combination has been classed as unstable. It is important to consider that the low concentration of both calcium and heparin is zero. Examination of Table 8.3 shows that the stability of the lipid admixture cannot be assured when any Monoparin® is added. Aqueous admixtures containing low and medium concentrations of calcium were able to tolerate the addition of up to 1 IU/ml of Monoparin®, but when the calcium concentration was at its highest, no Monoparin® could be added (Table 8.4). It can be seen in Table 8.5 that, for

the low calcium concentration lipid admixtures, Fragmin® could be included at up to 1 IU/ml with no detrimental effects seen over the test period, and up to 0.5 IU/ml for the medium calcium concentration. However, at the high calcium concentration, the addition of Fragmin® was seen to destabilise the admixture. Results for Fragmin®-containing aqueous admixtures were the same as those for Monoparin® (Table 8.6).

By comparing the data in the tables, it can be seen that the addition of Monoparin® to the lipid test admixture was generally more detrimental than Fragmin®, despite Fragmins® higher number of units per millilitre of injection, but that both heparins had similar interactions with the aqueous admixture. Furthermore, it is apparent that the addition of heparin is more destabilising for the lipid than the aqueous test admixture.

The dose of heparin used in PN admixtures, though appropriate for catheter maintenance, is sub-therapeutic and as such, no suitable chemical assay was found. The decision as to whether to add heparin to the test admixture must therefore be made solely on the basis of the physical compatibility results, and the clinical response of the patient monitored. Whilst chemical analysis would have been informative, it was unnecessary in determining whether heparin was an appropriate drug to add to the test admixture; it is clear from the results of the physical compatibility testing that it is not.

*Table 8.3 Stability of FK Regimen 5 (Lipid) containing Monoparin®*

| Calcium Concentration | Monoparin® Concentration | | |
|---|---|---|---|
| | Low | Medium | High |
| Low | Stable 48 hrs | Unstable | Unstable |
| Medium | Stable 48 hrs | Unstable | Unstable |
| High | Stable 48 hrs | Unstable | Unstable |

*Table 8.4 Stability of FK Regimen 5 (Aqueous) containing Monoparin®*

| Calcium Concentration | Monoparin® Concentration | | |
|---|---|---|---|
| | Low | Medium | High |
| Low | Stable 48 hrs | Stable 48 hrs | Stable 48 hrs |
| Medium | Stable 48 hrs | Stable 48 hrs | Stable 48 hrs |
| High | Stable 48 hrs | Unstable | Unstable |

*Table 8.5 Stability of FK Regimen 5 (Lipid) containing Fragmin®*

| Calcium Concentration | Fragmin® Concentration | | |
|---|---|---|---|
| | Low | Medium | High |
| Low | Stable 48 hrs | Stable 48 hrs | Stable 48 hrs |
| Medium | Stable 48 hrs | Stable 48 hrs | Unstable |
| High | Stable 48 hrs | Unstable | Unstable |

*Table 8.6 Stability of FK Regimen 5 (Aqueous) containing Fragmin®*

| Calcium Concentration | Fragmin® Concentration | | |
|---|---|---|---|
| | Low | Medium | High |
| Low | Stable 48 hrs | Stable 48 hrs | Stable 48 hrs |
| Medium | Stable 48 hrs | Stable 48 hrs | Stable 48 hrs |
| High | Stable 48 hrs | Unstable | Unstable |

# CHAPTER 9

# GENERAL DISCUSSION

# CHAPTER 9 – GENERAL DISCUSSION

## 9.1 DISCUSSION

Detailed discussion and conclusions on the addition of individual drugs have been made in the appropriate chapters and will not be reiterated in this section.

Although it has been recommended that drugs are not added to PN unless absolutely necessary (Lee and Allwood 2001), many benefits of doing so have been proposed (see Section 1.3). The possibility of adding drugs to PN admixtures appealed to many of the members of the pharmacy profession surveyed in Chapter 2. The interest displayed, and the paucity of available information, validated the implementation of this research. The drugs chosen for testing were some of those suggested by the respondents to the questionnaire, and the positive results for the stability and compatibility of many of the drugs investigated suggest that further work and the possible construction of a stability database may be possible and worthwhile. Additionally, this work has shown that, with the use of the appropriate stability assessment methods, the main reason cited for not adding drugs to PN ("Lack of information", see Figure 2.2) may be overcome and the benefits safely obtained.

The assurance of physical and chemical stability of a drug-containing admixture is not enough. In order to supply a safe and appropriate treatment to a patient, clinical and microbiological factors must also be considered. All questionnaire respondents indicated that, in their hospitals, the drug additions were made in an aseptic unit. Pharmacy-based additive services should be preferred for several reasons:

- The pharmacist is available to assess stability information;
- A dispensing check will be carried out on each addition, thus reducing the risk of error;
- The risk of microbiological contamination can be minimised;

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- Comprehensive documentation is produced and retained;
- Drug wastage can be minimised.

It is generally accepted that it is necessary to employ a number of methods to adequately assess PN stability and compatibility and that it is not possible to extrapolate data between different regimens. Both of these views have been upheld by the experiments carried out. Further to this, concentration-dependent incompatibilities have been shown for some drugs, which demonstrates that, not only must the drug and formulation be specified but also the concentration to be included in the admixture.

Several external factors may impact on the stability of a drug in a PN admixture, such as light exposure and composition of the infusion bag. Experiments were designed to test the effects of these factors and the protocols of these experiments may be followed when testing other drugs. It must be noted that although light mediated degradation occurs both within the infusion bag and the administration set (Allwood 1982), the degree of degradation within the latter was not quantified in this work, but must be considered in practice.

The use of inline filters to protect the patient from the adverse effects of the infusion of particles or microbes (Section 1.2.1) has been recommended by the BPNG (Bethune et al. 2001), but at the current time their use is not universal. The presence of enlarged particles in some PN admixtures supports this recommendation.

## 9.2 LIMITATIONS

### 9.2.1 QUESTIONNAIRE

Analysis of the questionnaire results indicated the presence of polarised views from some respondents, particularly over whether or not drugs should be added to PN (see Section 2.3.2.2). It was not possible to determine from the

responses whether the pharmacists' role or level of experience influenced their attitude.

The small sample size was acknowledged as a limitation of this study. However, as an exploratory survey intended to investigate current practice, the questionnaire fulfilled its objective.

All additions of drugs to PN were made in the light of available stability information (Figures 2.4 and 2.8). The questionnaire investigated the sources of information used by respondents, but not its quality.

## 9.2.2 LIPID-FREE ADMIXTURE

In order to assess the interactions of the drugs with the aqueous phase of the admixture, admixtures were compounded which contained all of the components of the original regimen, with the exception of the lipid emulsion. The omission of the emulsion resulted in an increased concentration of solutes within the admixture which may have had an effect on the drug-PN interactions. Following the example of many other studies of this type, a volume of WFIs equal to that of the lipid emulsion was added to aqueous admixtures in an attempt to counteract this effect, however, it can be seen from Figure 7.12 that it was not an entirely successful model. This may be due to the fact that the lipid emulsion contains numerous additives besides the oil and water, such as glycerol and phospholipids, which may have influenced admixture and/or drug stability via mechanisms other than the dilution effect.

Attempted removal of the lipid by a centrifugation method, and subsequent analysis of the resultant aqueous phase, was also unsuccessful as it was only possible under the experimental conditions to cause the admixture to cream heavily and not to crack whilst trials of solid phase extraction were also attempted, but did not lead to the development of an assay which consistently and repeatably obtained all of the drug from the admixture.

Drugs are known to partition between lipid and aqueous phases and may behave differently within each one. This should be investigated when considering bioavailability in future work (see Section 9.5.1).

## 9.2.3 INTERACTIONS

The nature of the chemical interactions occurring between the various components of the admixtures was not investigated in this study. While such work would be difficult owing to the complexities of the admixture, analysis of reaction mechanisms may improve understanding of the factors governing stability.

## 9.3 THE RESPONSIBILITIES OF THE PHARMACIST

In considering the pharmaceutical implications of adding drugs to PN, it is necessary to establish the wide range of factors that a pharmacist must be aware of when supplying or preparing a medicinal product.

The safety, quality and efficacy of a medicinal product are paramount. It is stated within "Medicines, Ethics & Practice: A Guide for Pharmacists" (Anon. 2004b) that:

> *"The public is entitled to expect that medicines...will be safe, effective and appropriate for the condition to be treated and the intended recipient."*

> *"The public is entitled to expect that products extemporaneously prepared in a pharmacy will be prepared accurately, suitable for use and meet the accepted standards for quality assurance."*

and that:

> *"Pharmacists must be satisfied as to the safety and appropriateness of the formula for the product."*

Further to this, Appelbe and Wingfield (2001) explain that:

*"No medicinal product may be placed on the market [or] be distributed by way of wholesale dealing unless a marketing authorisation has been issued by the Medicines Control Agency or the European Agency for the Evaluation of Medicinal Products."*

and:

*"In dealing with an application for a marketing authorisation, the licensing authority must give particular consideration to the safety, quality and efficacy of the products."*

Although pharmacists preparing parenteral nutrition admixtures in accordance with a prescription are exempt from requiring a marketing authorisation for the product (Appelbe and Wingfield 2001), it is clear from the statements above that there is an expectation that consideration for its safety, quality and efficacy will be upheld by the pharmacist. In addition to assuring these factors, clinical considerations, such as the appropriateness and dosage of the medicinal product, and principles of Good Manufacturing Practice also lie within the remit of the pharmacist.

When supplying an unlicensed medicine or a "special" such as a PN admixture, a pharmacist, along with the prescriber, will, under civil law, assume liability for it, and the Royal Pharmaceutical Society of Great Britain (Anon. 2004a) states that:

*"The pharmacist must ensure that the supply [of an unlicensed medicine] is made in the best interests of the patient and the potential risk to the patient of making the supply has to be weighed against the detriment to the patient of not making the supply."*

The Code of Ethics (Anon. 2004b) states that pharmacists must, at all times, act in the interests of the patient and if the principles discussed above are adhered to, the needs of the patient may be safely met.

## 9.4 SUGGESTED STABILITY TESTING PROTOCOL

Trissel (1983) commented that:

> *"It is unfortunate when dedicated individuals work hard on a project, making a sincere effort, only to have their papers go unpublished because of serious flaws in the design or conduct of the study."*

The proposal of a stability testing protocol gives structure to the analysis of drug-PN combinations, and may be valuable in guiding those who wish to analyse the combinations.

When first considering whether to add a drug to a PN admixture it is important to ascertain whether the drug is a suitable candidate for inclusion in a PN admixture. According to Driscoll et al. (1991) the necessary characteristics are:

- Stable drug dosage regimen over 24 hours;
- Appropriate pharmacokinetics for continuous infusion;
- Chemically stable for the duration of infusion;
- Stable infusion rate.

If these criteria are not met, it may not be appropriate to include the drug in an admixture. If necessary, an alternative drug from the same therapeutic group may be considered.

If the drug complies with these requirements, a thorough literature search should be performed, which may provide evidence of stability in the required medium. Stability data for the drug in fluids or admixtures other than the test

admixture cannot be extrapolated but may indicate where problems are likely to occur because, while it is not safe to make the assumption that chemically similar compounds will have the same effect on, or be affected in the same way by a PN admixture, knowledge of a drug's physicochemical characteristics will provide information on factors that may be relevant. Any differences in drug formulation from those used in published studies must be considered, particularly the nature of any excipients.

At this time physical stability testing can begin with the use of the Neubauer slide technique as a screening tool which has been shown to be beneficial in rapidly identifying concentration-dependent incompatibilities between the drug and the test PN admixture (Price and Cosslett 2001). Following the Neubauer technique, the remaining physical tests can be performed, to fully assess physicochemical compatibility. Time zero readings must be taken in order to analyse changes over time.

If the addition of the drug to the test admixture does not damage the stability of the admixture, chemical analysis of the drug may be performed using an appropriate stability indicating technique. High performance liquid chromatography (HPLC) is a widely used technique that has been useful in the analysis of many (but not all) of the drugs tested in these studies. Identification of a suitable technique and assay may be possible from the literature, however, it is usually necessary to adjust the assay due to the presence of PN in the sample. If no suitable assay is found in the literature, it will be necessary to develop one, and advice should be sought from specialised texts on the subject.

Before embarking upon full chemical analysis using an HPLC method, dilution, degradation and freezing studies are necessary. Dilution studies involve ascertaining an appropriate dilution factor to ensure the column is not overloaded, but that the drug concentration is high enough to be detected. Degradation analysis involves subjecting the drug to various conditions with the intention of damaging it and then ensuring that the chosen assay can detect

the difference between the parent compound and the degraded drug. Freezing studies are necessary if the drug and admixture are to be stored frozen. For these studies the drug is frozen for at least the same period as it will be frozen in the actual study, then defrosted, analysed and its chromatogram compared to that of a control sample.

Problems highlighted in the literature search should be controlled for when designing the experiment, and the admixture containing the drug must be stored under the necessary conditions for a specified length of time. As with the physicochemical analysis, it is important to analyse the samples at time zero, as a baseline with which to compare the results.

Any drug additions must be thoroughly analysed in the proposed PN admixture before the combination is administered to patients. Amphotericin B is a water-insoluble antifungal which was formulated as a liposomal preparation to reduce its dose-limiting nephrotoxicity. The high cost of the product led to trials involving the direct addition of the drug to a lipid emulsion, which initially also seemed to reduce the drug's side-effects (Chavanet et al. 1992). However, it became apparent on further investigation that the drug was not being incorporated into the lipid as first thought, but was forming a precipitate in the aqueous phase, which was being masked by the opacity of the lipid (Ericsson et al. 1996; Trissel 1995; Washington 1993). The instability led to a decrease in the dose being received by the patient and hence an apparent decrease in toxicity (Ericsson et al. 1996; Trissel 1995; Washington 1993). Potentially hazardous particles were also infused into the patient. This example demonstrates the need for full assessment of drug-PN combinations.

Once all the physical and chemical results have been obtained and analysed, it may be possible to decide if the drug is suitable for addition to the test admixture. A flow chart has been constructed to assist in the decision making process (see Appendix 7).

## 9.5 FUTURE WORK

### 9.5.1 BIOAVAILABILITY

Early studies of drugs added to PN admixtures relied on visual examination as evidence of physical compatibility. This information, though useful, was far from complete and gave no indication of the chemical stability of the compounds involved. The introduction of stability indicating assays such as HPLC into this field has allowed more complete evaluations of admixtures to be made, but even this is not comprehensive. A further factor to be considered in the development of a complete analysis would be the bioavailability of the test drug.

In order for a drug to be absorbed and used by the body, it must first be in solution, which may already be the case for many formulations potentially suitable for inclusion in PN. Body fluids are aqueous and therefore lipid formulations may be expected to take longer to be absorbed by the body, but in the case of intravenous administration, absorption is bypassed. If a drug is administered as a suspension, the particles must dissolve either in the admixture or within the body. Even if the drug is formulated as a solution for injection, it must remain so when added to the PN admixture and throughout infusion. Because most drugs are weak acids or bases (York 1988), changes in pH can affect their solubility, but this may be detected during stability testing.

Environmental pH changes affect the degree of ionisation of a drug, depending upon its pKa, thus influencing membrane permeability. Biological membranes are more permeable to unionised compounds due to their relatively higher lipid solubility and the potential for interaction between the charged cell membrane and an ionised drug.

The administration of hydrophobic drugs may be improved by the use of a lipid emulsion as a drug delivery vehicle, in which the drug is partitioned into the lipid phase of the emulsion, for example in the case for propofol, an anaesthetic agent. However, the incorporation of a drug into the oil phase of an emulsion

may affect the emulsion's or the drug's physicochemical characteristics. The use of phase separation techniques to separate the oil and water phases may allow analysis of the chemical characteristics of the drug in each phase, which may inform bioavailability studies.

At this time stability assessments are carried out under the premise that the amount of drug leaving the infusion bag in the admixture is the amount of drug entering the body and reaching the physiological target. However, the solubility, ionisation and partitioning properties of a drug may affect its bioavailability. When a drug is added to a lipid emulsion, it will partition between the two immiscible phases in a ratio dependent upon its solubility in each phase. Drugs also partition between aqueous and lipid environments within the body, for example between the blood and cell membranes. This behaviour may be measured *in vitro* and a partition coefficient, which may be used to predict a drug's biological activity (Martin 1993), determined. Investigations of this nature may be carried out in future as an indicator of the influence of the PN admixture on the bioavailability of drugs within.

### 9.5.2 GUIDELINES

As mentioned in Chapter 1, there are currently no national guidelines relating to drugs in PN. Following recommendations by White (2001), guidelines on the administration of drugs via enteral feeding tubes were released by BAPEN in 2003. The interest shown by healthcare professionals to those guidelines and by the respondents to the questionnaire in Chapter 2 suggests that guidelines on drugs in PN would be a welcome addition to the literature. Appendix 7 shows a stability assessment flow chart which may be used as the basis for future guidelines.

### 9.5.3 STABILITY DATABASE

In continuing this work it is hoped that a stability database of drugs in PN may be constructed. Such a database would provide information on the compatibility and stability of drug-PN combinations and perhaps offer

suggestions for alternative drugs or administration options for those combinations deemed unsuitable for use. Although the work may be somewhat limited by the variation in PN admixtures used in practice, the increased use of MCBs may maximise the usefulness of data produced.

### 9.5.4 CHEMICAL ASSAY FOR HEPARIN

The development of a suitable chemical assay for heparin at the low dose required in PN would allow a more complete evaluation of its addition to PN. Potentially detrimental stability factors that have been proposed could be examined and their influence assessed, for example, the effects of air and glucose and the extent of sorption and pooling.

### 9.5.5 DRUG-FILTER INTERACTIONS

There have been reports in the literature of some drugs being filtered out or adsorbed by inline filters, for example digoxin, diazepam, dopamine and several cytotoxic drugs (Butler et al. 1980; De Muynck et al. 1988; De Vroe et al. 1990; Rusmin et al. 1976). It seems that this is most likely to be a problem for drugs administered in low concentrations or formulated as suspensions or colloids. If a drug-containing admixture is to be infused through an inline filter, as recommended by the BPNG (Bethune et al. 2001), analysis of drug concentration post-filter should be undertaken.

## 9.6 CONCLUDING REMARKS

Despite the apparent axiom that drugs should not be added to PN admixtures, this work has shown that, with proper planning and thorough analysis, the practice may be safely performed, to the ultimate benefit of the patient.

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# APPENDICES

# APPENDIX 1 – PILOT QUESTIONNAIRE

1) Are any drugs added into parenteral nutrition (PN) in your hospital?

<div align="center">YES/NO</div>

If YES, please state why they are added

………………………………………………………………………………..

Please go to QUESTION 2

If NO, please state why not

………………………………………………………………………………...

What drugs, if any, would you like to be able to add?

………………………………………………………………………………...

Please go to QUESTION 7

2) Are the drugs added to aqueous, lipid-containing or both types of PN?

<div align="center">AQUEOUS / LIPID-CONTAINING / BOTH</div>

3) How do you decide whether a drug can be added to PN?

………………………………………………………………………………...

4a) What drugs are added to PN in your hospital and at what doses?

| Drug | Dose |
|---|---|
|  |  |
|  |  |
|  |  |

4b) Over what period of time are these infusions run?

………………………………………………………………………………...

4c) Do you have stability information for these combinations?

<div align="center">YES/NO</div>

If YES, where was the information obtained?

MANUFACTURER / LITERATURE / OTHER (please specify)

5) Who adds the drugs to the PN?

………………………………………………………………………………...

6) Where are the additions made?

ASEPTIC UNIT / PHARMACY / OPEN WARD / OTHER (please specify)

7) Are any drugs Y-sited with PN in your hospital?

<div align="center">YES/NO</div>

If YES, please state why they are Y-sited
..................................................................................................
Please go to QUESTION 8

If NO, please state why not
..................................................................................................

What drugs, if any, would you like to be able to Y-site?
..................................................................................................
Please go to QUESTION 11

8) Are the drugs Y-sited with aqueous, lipid-containing or both types of PN?

<div align="center">AQUEOUS / LIPID-CONTAINING / BOTH</div>

9) How do you decide whether a drug can be Y-sited with PN?
..................................................................................................

10a) What drugs are Y-sited with PN in your hospital and at what doses?

| Drug | Dose |
|------|------|
|      |      |
|      |      |
|      |      |

10b) Over what period of time are these infusions run?
..................................................................................................

10c) Do you have stability information for these combinations?

<div align="center">YES/NO</div>

If YES, where was the information obtained?

<div align="center">MANUFACTURER / LITERATURE / OTHER (please specify)</div>

11) Have you experienced any problems with your practice?

<div align="center">YES/NO</div>

If YES, what happened and how was it overcome?
..................................................................................................
..................................................................................................

12) Does your trust have any guidelines relating to the addition/Y-siting of drugs to PN admixtures?

YES/NO

If NO, are you aware of any plans to write them?

YES/NO

If there are plans to write guidelines, who will be involved in writing them?

PHARMACIST / NURSE / CLINICIAN /
OTHER (please specify) / DON'T KNOW

Please include a copy of your guidelines if possible. Many thanks for completing this questionnaire.

# APPENDIX 2 – QUESTIONNAIRE COVERING LETTER

Dear Sir/Madam,

I am a pharmacist and PhD student at the Welsh School of Pharmacy, Cardiff University. My PhD is entitled "Pharmaceutical implications of drug additions to parenteral nutrition admixtures". In conjunction with the BPNG and as part of my work I would like to find out about current practices with regards addition/Y-siting of drugs with parenteral nutrition. I would be very grateful if you could spare some time to complete the attached questionnaire and return it by Friday 2nd May 2003. All responses will be treated as confidential.

You may fill in the attached Word document and return it as an attachment, or if you would prefer to print out the attachment and fax or post it, please do so and use the contact details below. If you do not have access to Word and would like a paper copy posted or faxed to you, please contact me.

Thank you for your help, your input is much appreciated.

Rebecca Price

pricer@cf.ac.uk
Tel/Fax: 029 20874987

Welsh School of Pharmacy
Cardiff University
PO Box 13
Redwood Building
King Edward VII Avenue
Cardiff
CF10 3XF

# APPENDIX 3 – QUESTIONNAIRE

## DRUGS AND PARENTERAL NUTRITION

NB - for questions where more than one answer applies to your practice, please tick all that apply.

## SECTION A - ADDITION OF DRUGS TO PARENTERAL NUTRITION ADMIXTURES

1) Are any drugs added into parenteral nutrition (PN) in your hospital?

Yes ☐

No ☐

**If NO**, please indicate the reason(s):

Insufficient information ☐

Other (please specify) ☐

..................................................................................

What drugs, if any, would you like to be able to add?

..................................................................................

Please go to **SECTION B**

**If YES**, please state why they are added:

Save nursing time ☐

Save pharmacy time ☐

Decrease fluid and/or electrolyte load ☐

Reduce access sites ☐

Reduce costs ☐

Decrease catheter manipulations ☐

Decrease infection risk ☐

Other (please specify) ☐

..................................................................................

..................................................................................

2) How, generally, do you decide whether a drug can be added to PN?

..............................................................

..............................................................

3) Please fill in the table below, detailing what drugs you use, the doses used, whether the drug is added to aqueous, lipid or both types of PN and the duration of infusion.

| DRUG | DOSE AND / OR CONCENTRATION | AQUEOUS / LIPID / BOTH | DURATION OF INFUSION |
|------|------|------|------|
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

(if insufficient space, please add to end of questionnaire)

4) Do you have stability information for these combinations?

Yes      ☐

No      ☐

**If YES**, where was the information obtained?:

Drug manufacturer      ☐

Parenteral nutrition manufacturer      ☐

Hospital drug information department      ☐

QC / In-house testing      ☐

Literature      ☐

Other (please specify)      ☐

..............................................................

5) Who adds the drugs to the PN?:

    Nurse ☐

    Aseptic pharmacist ☐

    Clinician ☐

    Other (please specify) ☐

    ...........................................................................

6) Where are the additions made?:

    Pharmacy aseptic unit ☐

    Satellite aseptic unit ☐

    Open ward ☐

    Other (please specify) ☐

    ...........................................................................

Please go to **SECTION B**

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## SECTION B - Y-SITING DRUGS WITH PARENTERAL NUTRITION ADMIXTURES

1) Are any drugs Y-sited with PN in your hospital?

    Yes ☐

    No ☐

    **If NO**, please indicate the reason(s):

        Insufficient information ☐

        Other (please specify) ☐

    ...........................................................................

    What drugs, if any, would you like to be able to Y-site?

    ...........................................................................

Please go to **SECTION C**

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**If YES**, please state why they are added:

| | |
|---|---|
| Save nursing time | ☐ |
| Save pharmacy time | ☐ |
| Decrease fluid and/or electrolyte load | ☐ |
| Reduce access sites | ☐ |
| Reduce costs | ☐ |
| Decrease catheter manipulations | ☐ |
| Decrease infection risk | ☐ |
| Other (please specify) | ☐ |

...............................................................................................

2) How, generally, do you decide whether a drug can be Y-sited with PN?

...............................................................................................

...............................................................................................

3) Please fill in the table below, detailing what drugs you use, the doses used, whether the drug is Y-sited with aqueous, lipid or both types of PN and the duration of infusion.

| DRUG | DOSE AND / OR CONCENTRATION | AQUEOUS / LIPID / BOTH | DURATION OF INFUSION |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

(if insufficient space, please add to end of questionnaire)

4) Do you have stability information for these combinations?

| | |
|---|---|
| Yes | ☐ |
| No | ☐ |

**If YES**, where was the information obtained?:

Drug manufacturer ☐

Parenteral nutrition manufacturer ☐

Hospital drug information department ☐

QC / In-house testing ☐

Literature ☐

Other (please specify) ☐

..............................................................................................

Please go to **SECTION C**

------------------------------------------------------------------------------------

**SECTION C - PRACTICAL ISSUES**

1) Have you experienced any problems with your practice?

    Yes ☐

    No ☐

    **If NO**, please go to **SECTION D**

    **If YES**, what happened?:

    Line blockage ☐

    Loss of potency of drug ☐

    Parenteral nutrition instability ☐

    Other (please specify) ☐

    ..............................................................................

2) How was the problem overcome?:

    Separate line used for drug ☐

    Multilumen catheter used ☐

    Changed to different drug ☐

                     (options continue overleaf)

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Drug omitted completely ☐

Other (please specify) ☐

............................................................................

Please go to **SECTION D**

--------------------------------------------------------------------------------

## SECTION D - GUIDELINES

1) Does your trust have any guidelines relating to the addition/Y-siting of drugs to PN admixtures?

Yes ☐

No ☐

**If NO**, are you aware of any plans to write them?

Yes ☐

No ☐

**If there are plans to write guidelines**, who will be involved in writing them?:

Pharmacist ☐

Nurse ☐

Clinician ☐

Dietician ☐

Don't know ☐

Other (please specify) ☐

............................................................................

Please could you attach or post a copy of the guidelines if possible.

Many thanks for your time. If you have any further comments please make them below.

# APPENDIX 4 – BATCH NUMBERS & EXPIRY DATES

| Preparation | Batch Numbers | Expiry Dates |
|---|---|---|
| Vamin® 14 | 96387A01 | 04/01 |
| | 97064B01 | 01/02 |
| | 98918A01 | 09/04 |
| | 99085A01 | 01/05 |
| | 10079A01 | 03/06 |
| Additrace® | 000998 | 09/02 |
| | 010814 | 08/03 |
| | 030526 | 05/05 |
| Glucose 10% | 99J14BZ | 09/01 |
| | 02576007 | 09/04 |
| | 03576008B | 10/05 |
| Glucose 50% | 00E04BR | 04/02 |
| | 01558002 | 05/03 |
| | 03558011A | 11/05 |
| Addiphos® | 19831-01 | 03/02 |
| | 00DV12 | 04/03 |
| | 03HV13 | 08/06 |
| Intralipid® 20% | 15540-51 | 11/01 |
| | 22887-51 | 06/00 |
| | 28163-51 | 01/03 |
| | 1006638 | 03/04 |
| | 1015910 | 07/05 |
| Intralipid® 10% | 1003111 | 06/03 |
| | 0105691 | 06/05 |
| | 0122113 | 04/06 |
| Vitlipid® N Adult | 20871-02 | 06/01 |
| | 1022405 | 04/06 |
| Solivito® N | GK0846 | 04/02 |
| | IC1684 | 07/03 |
| | LB0939 | 07/06 |
| Sodium chloride 30% | 168643 | 10/03 |
| Potassium chloride 15% | R068 | 03/04 |
| Calcium chloride 1 mmol/ml | 132963 | 07/02 |
| | 180243 | 05/04 |
| Magnesium sulphate 50% | 902124 | 02/02 |
| | 20492 | 11/05 |
| Zinc sulphate 50 µmol/ml | 1290236 | 12/01 |
| | 0602009A | 06/04 |
| Water for Injections | 99C24G50 | 02/01 |
| | 02W213 | 05/07 |
| Zantac™ | B4037NB | 12/00 |
| | B4648HD | 07/01 |
| | C058198 | 12/04 |
| Hypnovel® | B1104 | 04/05 |

|  | B1105 | 06/05 |
|  | B1109 | 04/07 |
| Zydol™ | 166B | 07/05 |
|  | 199B | 02/06 |
| Solu-Cortef® | GJ0418 | 08/04 |
|  | HF1238 | 05/05 |
|  | MB0017 | 10/09 |
| Efcortesol™ | B04484NA | 11/00 |
|  | C028293 | 11/02 |
| Monoparin® | 15654 | 04/00 |
|  | 11815 | 04/05 |
| Fragmin® | 98703A01 | 03/05 |
| Acetonitrile | 0415367 |  |
|  | 0450034 |  |
| Ammonium acetate | 9738988 |  |
| Hexane | 0555603 |  |
| Hydrogen Peroxide | 0553531 |  |
| Methanol | 0561949 |  |
|  | 0563739 |  |
| Monobasic potassium dihydrogen orthophosphate | 0380151 |  |
| Monobasic sodium phosphate | 0113243 |  |
| Orthophosphoric acid | 0245624 |  |
| Sudan Red III | 25H0344 |  |
| Toluidine Blue O | 37H3734 |  |
| Triethylamine | 0127604 |  |

NOTE:

All PN components used had a minimum of one year shelf-life remaining at the time of compounding.

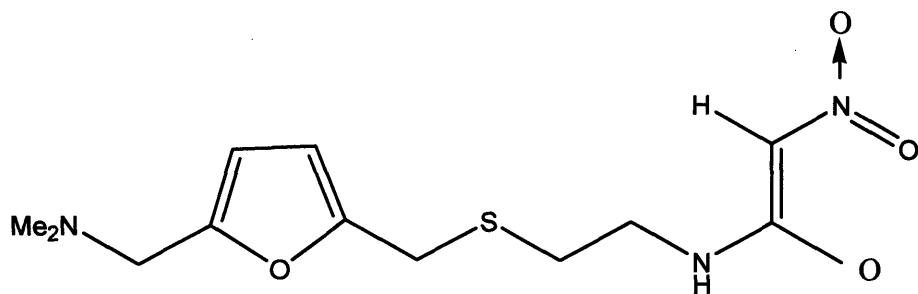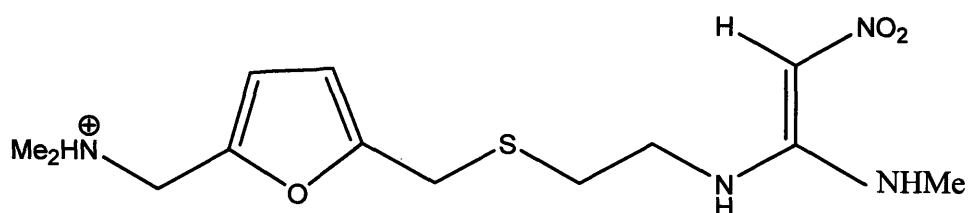# APPENDIX 5 – DRUG DEGRADATION PRODUCTS

RANITIDINE
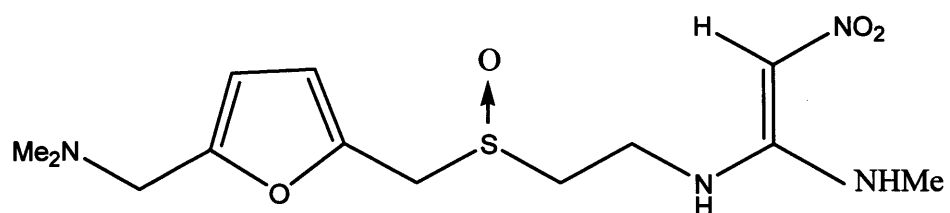


Oxidation product (Carey et al. 1981)
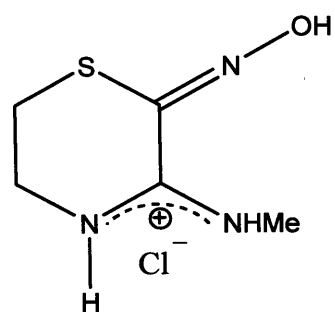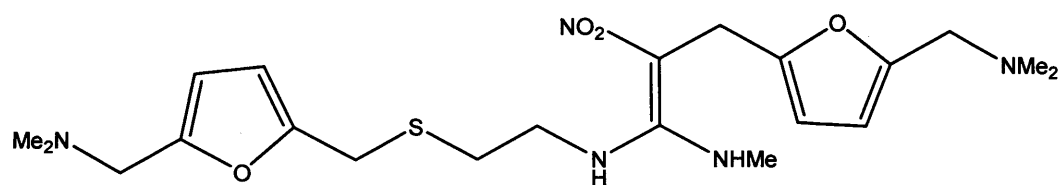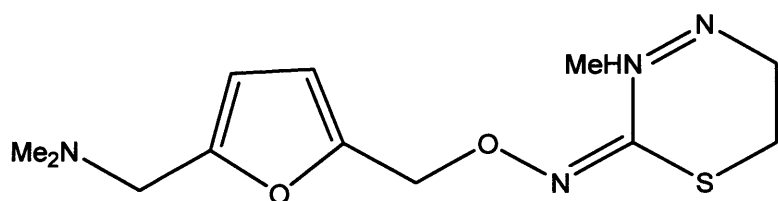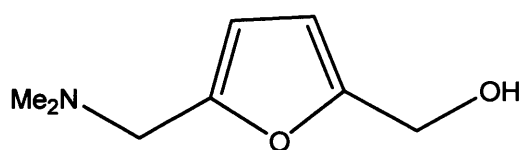








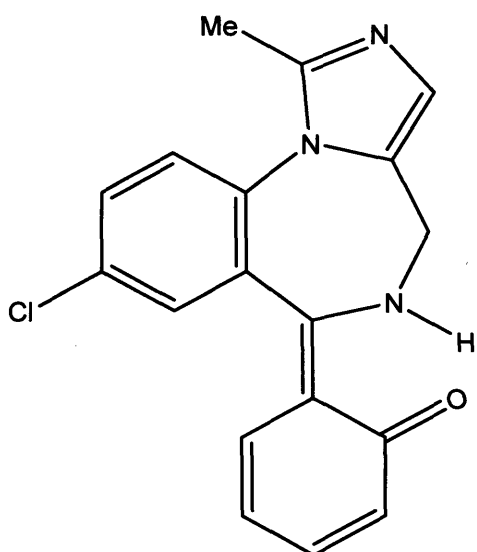Acid hydrolysis products (Inagaki et al. 1993)
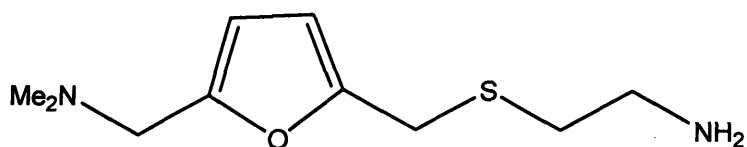
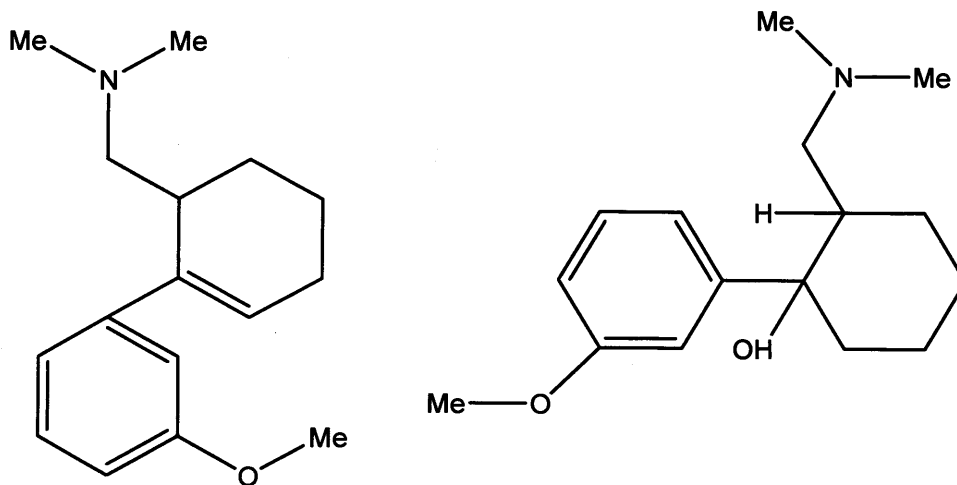Alkaline hydrolysis products (Inagaki et al. 1993)



Photolysis product (Latch et al. 2003)
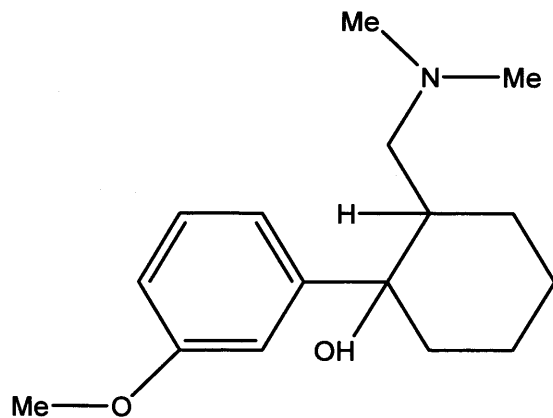
MIDAZOLAM



Main photolysis product (Andersin et al. 1994)
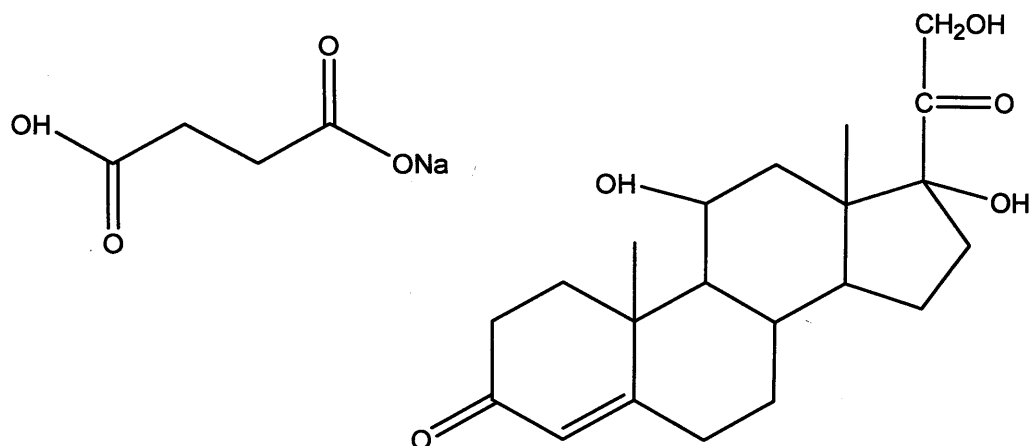
TRAMADOL



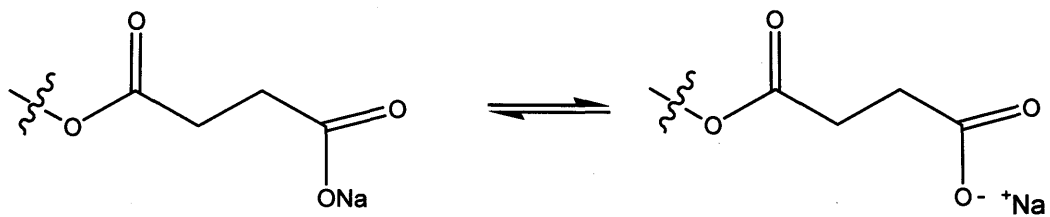Acid degradation products (Krzek and Starek 2004)



Alkaline degradation product (Krzek and Starek 2004)
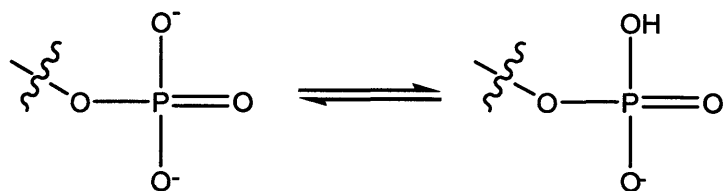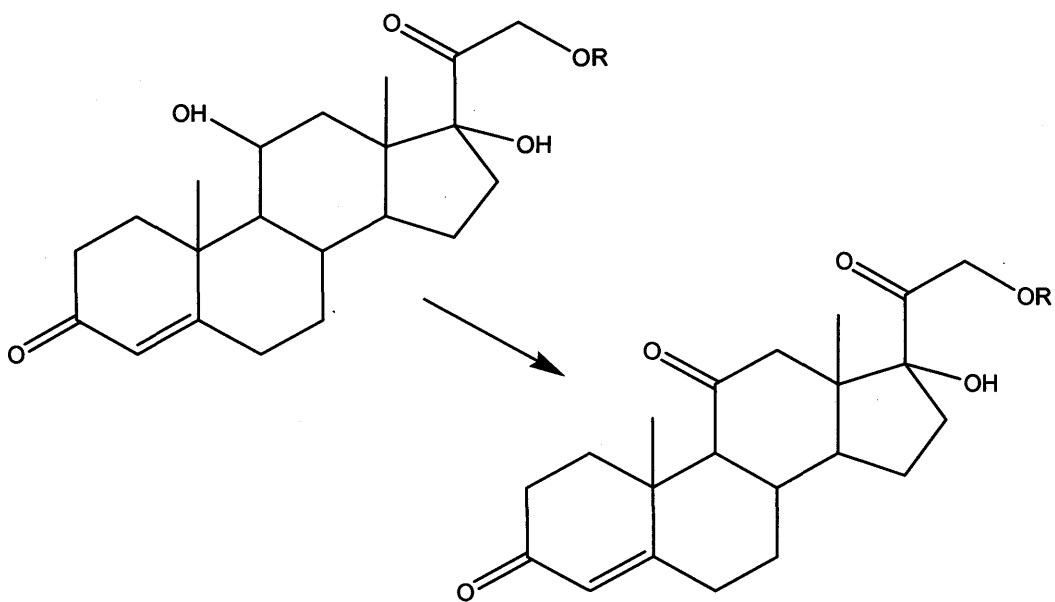
HYDROCORTISONE



Hydrolysis products of HSS (Rigge and Jones 2005)

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pH dependent equilibrium of HSS (Garrett 1962)
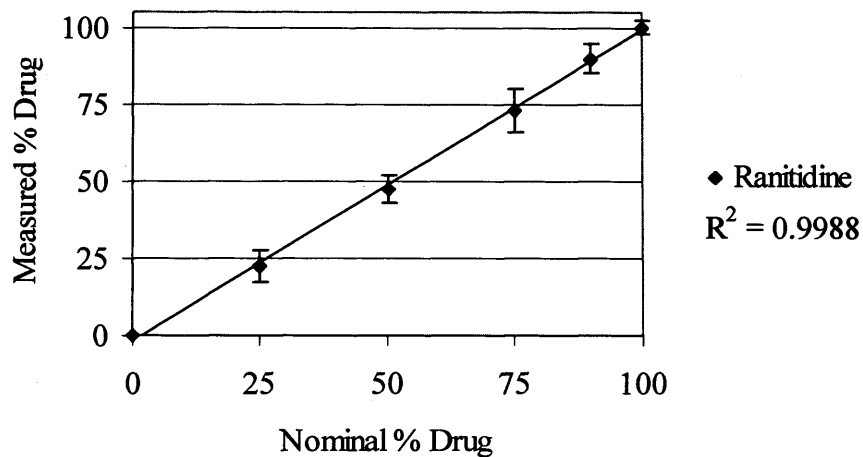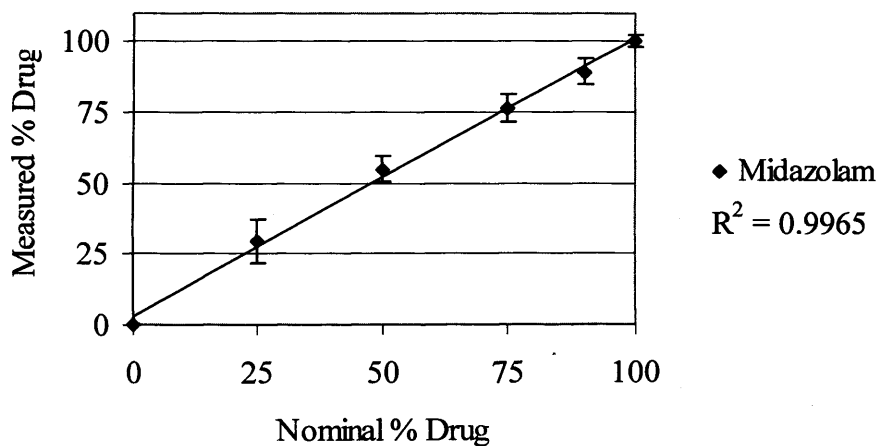


pH dependent equilibrium of HSP (Garrett 1962)



Steroid oxidation products (Byrn and Kessler 1987)

# APPENDIX 6 – HPLC CALIBRATION CURVES
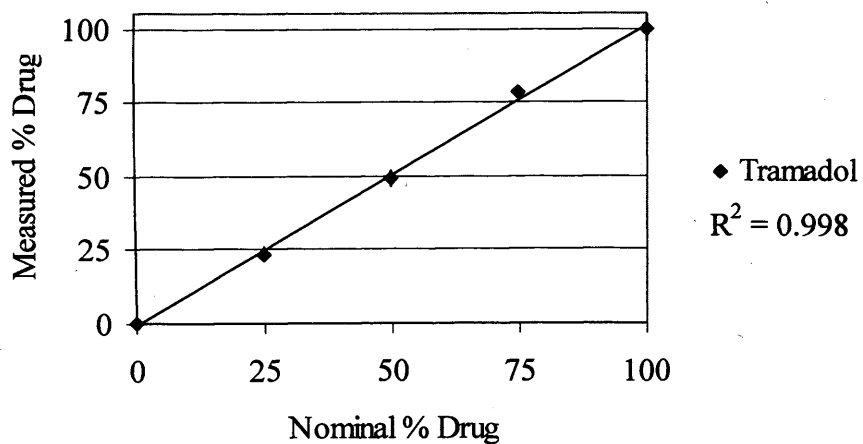
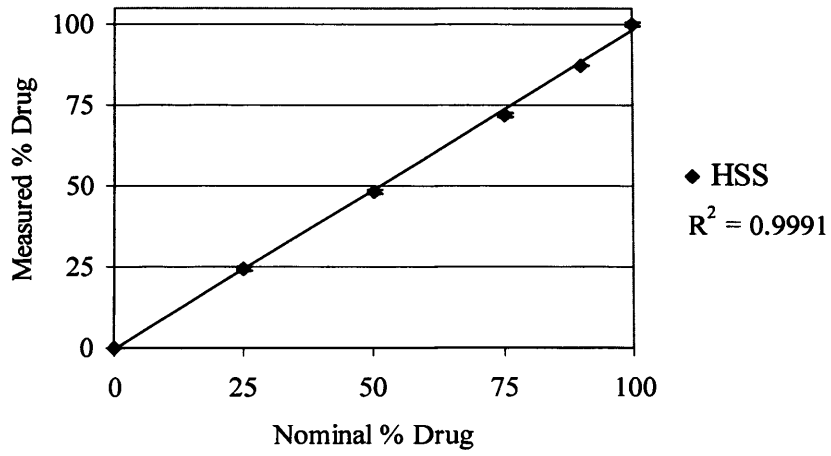Ranitidine



◆ Ranitidine
$R^2 = 0.9988$

Midazolam



◆ Midazolam
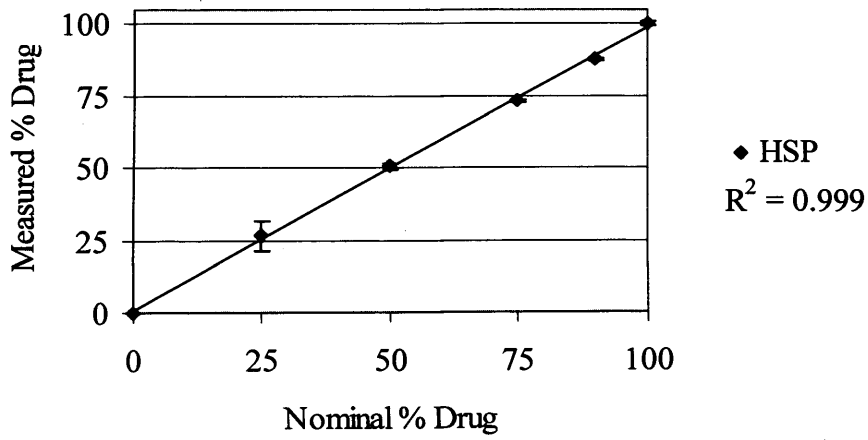$R^2 = 0.9965$

Tramadol



◆ Tramadol
$R^2 = 0.998$

## Hydrocortisone Sodium Succinate



## Hydrocortisone Sodium Phosphate



NOTE: Error bars represent ±RSD.

# APPENDIX 7 – STABILITY ASSESSMENT FLOW CHART



Does the drug have a stable dosage regimen over 24 hrs?

Is it appropriate to infuse the drug continuously?

Is the drug given at a stable infusion rate?

Is the drug likely to be chemically stable for 24 hrs?

This drug is not suitable for addition to PN

Search the literature for information on drug in required diluent

Is the drug physically compatible with the required diluent?

Does the data encompass the dose required?

Is the dose tested suitable?

Does the data encompass the period required?

Is the period tested suitable?

Is the drug chemically stable in the required diluent?

Physical compatibility testing is necessary

Consider stability issued highlighted in literature and storage conditions

Analyse drug in diluent using standard techniques. Is combination compatible?

Does the data encompass the dose required?

Is the dose tested suitable?

Does the data encompass the period required?

Is the period tested suitable?

Chemical stability testing is necessary

Consider stability issued highlighted in literature and storage conditions

Analyse drug in diluent using HPLC. Is the drug stable?

The drug can be added aseptically to the specified diluent. Ensure doses and time periods are not exceeded.

This combination is not appropriate. Consider alternative diluent or drug, or screen lower dose using Neubauer technique.

→ Yes
→ Unknown
→ No

# APPENDIX 8 – PUBLICATIONS

Cosslett, A. G. and Price, R. 2003. Stability of Ranitidine in Parenteral Nutrition Admixtures (PNAS). *Clinical Nutrition* 22(S1), pp. S104. Poster presented at ESPEN Conference, Cannes, France.

Cosslett, A. G. and Price, R. 2004. Stability of Drug Additions to Parenteral Nutrition (PN) Admixtures. *Clinical Nutrition* 22(4), pp. 935. Poster presented at ESPEN Conference, Lisbon, Portugal.

Price, R. 2001. Parenteral Nutrition Support for Intestinal Failure – Gut Wrenching Solutions. *Feeding Times* 1, pp. 1-3. A meeting report for the BPNG Summer Symposium.

Price, R. and Cosslett, A. G. 2001. Validation of a drug-parenteral nutrition (PN) stability screening method. *Clinical Nutrition* 20(S3), pp. 27. Poster presented at ESPEN Conference, Munich, Germany.

Price, R. and Cosslett, A. G. 2002. Validation of a Drug-Parenteral Nutrition (PN) Stability Screening Method. Poster presented at Welsh School of Pharmacy Postgraduate Research Conference.

Price, R. and Cosslett, A. G. 2002. Stability of Hydrocortisone in Parenteral Nutrition (PN) Admixtures. *Clinical Nutrition* 22(S1), pp. 45. Poster presented at ESPEN Conference, Glasgow, UK.

Price, R. and Cosslett, A. G. 2002. Comparison of a New Screening Tool for the Determination of Drug-Parenteral Nutrition (PN) Admixture Stability with Standard PN Stability Techniques. Recipient of the Mike Tarr Award, at the Guild of Hospital Pharmacists Annual Conference, Manchester, UK.

Price, R. and Cosslett, A. G. 2003. Stability of Ranitidine in Parenteral Nutrition Admixtures. Poster presented at Welsh School of Pharmacy Postgraduate Research Conference.

Price, R. and Cosslett, A. G. 2004. Stability of Drug Additions to Parenteral Nutrition (PN) Admixtures. Winner of the poster prize, BPNG Conference, Liverpool, UK.