PROBLEMS ASSOCIATED WITH THE PROVISION OF MICRONUTRIENTS TO PARENTERAL NUTRITION PATIENTS

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In loving memory of my beloved father, Haji Mohd Said bin Ibrahim

SUMMARY

Parenteral Nutrition (PN) provides nutrition to patients through the intravenous route, and is comprised of carbohydrates, fats, proteins, vitamins, trace elements, electrolytes and fluids. The vitamins are a major limiting factor in the shelf life determination of a PN formulation and are generally added just before administration. This thesis reports on some stability studies of a multivitamin infusion for the possible development of a minibag which may be stored before administration to PN patients.

The physicochemical stability of the water-soluble vitamins contained in Solivito® N Adult Injection (Fresenius Kabi), the fat-soluble vitamins contained in Vitlipid® N Adult Injection (Fresenius Kabi) and the combined admixture of the water-soluble and fat-soluble vitamins, prepared in 100 ml 0.9% sodium chloride in Freeflex® (Fresenius Kabi) minibags was investigated. Physical tests include nephelometry, pH, dissolved oxygen, microscopy and particle size determination by laser diffraction, while the chemical tests used validated stability indicating reversed phase HPLC methods. The vitamins investigated were pyridoxine, nicotinamide, thiamine, folic acid, riboflavin sodium phosphate, Vitamin A and Vitamin E.

The water-soluble vitamins and the fat-soluble vitamins were found to be stable, with more than 90% remaining, at 4°C for 168 hours. For samples stored at 25°C, they remained stable for 168 hours only if light-protected. During simulated clinical conditions, the water-soluble and fat-soluble vitamins, and the combined vitamin admixture were found to be stable for up to 30 days storage at 4°C, and remained stable for a further 24 hours at 25°C if light protected. The light protective effects of different coloured plastic covers to be used during delivery of the vitamin minibags were also investigated.

The stability data showed promising results with the vitamins in the minibag remaining stable when assessed under simulated clinical conditions and these longer shelf-life vitamin minibags would benefit PN patients in the hospital and home settings.

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LIST OF ABBREVIATIONS

μg	Microgram
μL	Microliter
μm	Micrometer
30d25C	30 days storage in the refrigerator followed by 25°C for 24 hours
30d40C	30 days storage in the refrigerator followed by 40°C for 24 hours
7d25C	7 days storage in the refrigerator followed by 25°C for 24 hours
7d40C	7 days storage in the refrigerator followed by 40°C for 24 hours
A.S.P.E.N.	American Society for Parenteral and Enteral Nutrition
Al25C	Freeflex® minibag wrapped with aluminium foil, stored at 25°C
Al40C	Freeflex® minibag wrapped with aluminium foil, stored at 40°C
AMA	American Medical Association
ANOVA	Analysis of variance
BAPEN	British Association for Parenteral and Enteral Nutrition
Black25C	Freeflex® minibag wrapped with black plastic cover, stored at 25°C
Black40C	Freeflex® minibag wrapped with black plastic cover, stored at 40°C
BNF	British National Formulary
BP	British Pharmacopoeia
CB	Combined fat-soluble and water-soluble vitamin admixture
coA	Coenzyme A
Cr	Chromium
Cu	Copper
CVC	Central venous catheter

D[4,3]	Volume mean diameter
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
Eur Ph	European Pharmacopoiea
FA	Folic acid
FAD	Flavine-adenine dinucleotide
FAL25	Freeflex® minibag, air added, light exposed, stored at 25°C
FAL30	Freeflex® minibag, air added, light exposed, stored at 30°C
FAL40	Freeflex® minibag, air added, light exposed, stored at 40°C
FAN25	Freeflex® minibag, air added, light protected, stored at 25°C
FAN30	Freeflex® minibag air added, light protected, stored at 30°C
FAN4	Freeflex® minibag, air added, light protected, stored at 4°C
FAN40	Freeflex® minibag, air added, light protected, stored at 40°C
Fe	Iron
FF25	Clear Freeflex® minibag stored at 25°C
FF25C	Clear Freeflex® minibag stored at 25°C
FF30	Clear Freeflex® minibag stored at 30°C
FF4	Clear Freeflex® minibag stored at 4°C
FF40	Clear Freeflex® minibag stored at 40°C
FF40C	Clear Freeflex® minibag stored at 40°C
FFlgt	Clear Freeflex® minibag exposed to artificial fluorescent light
FFuv	Clear Freeflex® minibag irradiated with UVA
FFW25	Clear Freeflex® minibag exposed to light at 25°C
FFW30	Clear Freeflex® minibag exposed to light at 30°C

FFW40	Clear Freeflex® minibag exposed to light at 40°C
FH_4	Tetrahydrofolic acid
FMN	Flavine mononucleotide (riboflavine-5'-phosphate)
FNL25	Freeflex® bags, no air added, light exposed, stored at 25°C
FNL30	Freeflex® bags, no air added, light exposed, stored at 30°C
FNL40	Freeflex® bags, no air added, light exposed, stored at 40°C
FNN25	Freeflex® bags, no air added, light protected, stored at 25°C
FNN30	Freeflex® bags, no air added, light protected, stored at 30°C
FNN4	Freeflex® bags, no air added, light protected, stored at 4°C
FNN40	Freeflex® bags, no air added, light protected, stored at 40°C
FPL	Freeflex® minibag protected from light
FPL25	Dark Freeflex® minibag stored at 25°C
FPL30	Dark Freeflex® minibag stored at 30°C
FPL4	Dark Freeflex® minibag stored at 4°C
FPL40	Dark Freeflex® minibag stored at 40°C
FPLW25	Dark Freeflex® minibag exposed to light at 25°C
FPLW30	Dark Freeflex® minibags exposed to light at 30°C
FPLW40	Dark Freeflex® minibags exposed to light at 40°C
FPT	6-Formylpterin
g	Gram
GAL25	Glass bottles, air added, light exposed, stored at 25°C
GAL30	Glass bottles, air added, light exposed, stored at 30°C
GAL40	Glass bottles, air added, light exposed, stored at 40°C
GAN25	Glass bottles, air added, light protected, stored at 25°C

GAN30	Glass bottles, air added, light protected, stored at 30°C
GAN4	Glass bottles, air added, light protected, stored at 4°C
GAN40	Glass bottles, air added, light protected, stored at 40°C
GNL25	Glass bottles, no air added, light exposed, stored at 25°C
GNL30	Glass bottles, no air added, light exposed, stored at 30°C
GNL40	Glass bottles, no air added, light exposed, stored at 40°C
GNN25	Glass bottles, no air added, light protected, stored at 25°C
GNN30	Glass bottles, no air added, light protected, stored at 30°C
GNN4	Glass bottles, no air added, light protected, stored at 4°C
GNN40	Glass bottles, no air added, light protected, stored at 40°C
h	Hour
HEPA	High efficiency particulate air
HPLC	High Performance Liquid Chromatography
HPN	Home parenteral nutrition
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IU	International unit
kg	Kilogram
klux	Kilolux
L	Liter
LCT	Long-chain triglycerides
MAG	Malnutrition Advisory Group
MCT	Medium-chain triglycerides
mg	Milligram

ml	Milliliter
mmol	Millimol
Mn	Manganese
Mo	Molybdenum
MUFA	Monounsaturated fatty acids
MUST	Malnutrition Universal Screening Tool
N	Nicotinamide
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NICE	National Institute for Health and Clinical Excellence
nm	Nanometer
NTU	Nephelometric turbidity unit
°C	Degree Celcius
P	Pyridoxine
PABA	Para-amino benzoic acid
$PFAT_5$	Lipid globule size greater than 5 μm
PICC	Peripherally inserted central catheter
PN	Parenteral nutrition
PSC	Pharmaceutical stability chamber
PteGlu	Pteroylglutamic acid
PUFA	Polyunsaturated fatty acids
r^2	Correlation coefficient
Red25C	Freeflex® minibag wrapped with red plastic cover, stored at 25°C

Red40C	Freeflex® minibag wrapped with red plastic cover, stored at 40°C
rsd	Relative standard deviation
RSP	Riboflavin sodium phosphate
rt	Retention time
sd	Standard deviation
Se	Selenium
SV	Solivito® N Adult in 0.9% sodium chloride infusion
T	Thiamine
TPP	Thiamine pyrophosphate
USP	United States Pharmacopoeia
UV	Ultraviolet
UVA	Ultraviolet A
VL	Vitlipid® N Adult in 0.9% sodium chloride infusion
W/m^2	Watts per square metre
Zn	Zinc

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CHAPTER ONE

INTRODUCTION

1.1. Parenteral Nutrition

Parenteral Nutrition (PN) is an alternative method of providing nutritional support for patients via the intravenous route, the other methods being by the oral or enteral route. This intravenous route of administering artificial nutrition was pioneered by Dudrick et al (1968), who demonstrated for the first time, the success of administering concentrated glucose, amino acids and other essential nutrients towards growth development in both animals and man. Comprehensive history and development of PN have been described by many researchers (Driscoll 2003; Hardy and Sizer 2004; Vinnars and Wilmore 2003; Wretlind 1981).

Patients selected for PN are malnourished patients or patients who have the potential to become malnourished. In hospital settings, malnutrition remains an under-recognised problem. The prevalence of malnutrition of hospital patients on admission was demonstrated to be up to 40% with even more becoming malnourished during their hospital stay (Campbell et al. 2002; McWhirter and Pennington 1994). Awareness of malnutrition in hospital patients is now increasing and recently, the National Institute for Health and Clinical Excellence (NICE) has issued a guideline that all hospital patients should be screened to identify those who are malnourished or at risk of malnutrition, and that nutrition support should be considered in these patients (NICE 2006a).

Therefore, evaluation of nutritional status helps to determine patients who are at risk of developing nutrition related complications. Nutritional assessment includes body mass index, clinical history, dietary and biochemical measurements. Many screening tools evaluating nutritional status have been

developed to detect malnutrition, an example is the 'malnutrition universal screening tool' ('MUST') (Malnutrition Advisory Group 2003), developed by the British Association for Parenteral and Enteral Nutrition (BAPEN).

1.2. Indications for PN

PN is indicated for patients who are unable to tolerate enteral feedings, such as in patients with ileus or bowel obstruction, patients with non-functioning gastrointestinal tract, for example after massive small bowel resection, or when the risk of enteral nutrition related complications such as aspiration is unacceptably high (A.S.P.E.N. Board of Directors 1986; Hall 1983; Payne-James et al. 1989).

Provision of PN is a component of a specialised service provided to ward inpatient areas. With the setting up of nutrition support teams in the hospitals where patient care is provided by a multidisciplinary team, the PN service has also expanded to outpatient settings at home, also known as home parenteral nutrition (HPN) (NICE 2006b).

HPN is indicated for patients with irreversible intestinal failure and those awaiting surgery to restore gut functionality, of which Crohn's disease is the most common (Jones 2003; Mughal and Irving 1986). Long-term HPN has markedly improved the quality of life and the survival of these patients with severe intestinal obstruction or malabsorption, such as short bowel syndrome (Gottrand et al. 2005; Messing 1995).

1.3. Routes of Administration of PN

PN can be administered either via central or the peripheral routes (Palmer and MacFie 2001).

1.3.1. Central Route

The central venous route is indicated for patients who require long-term PN, patients who have poor peripheral venous access or those who require high calories with fluid restrictions. This route is also appropriate for patients who already have an indwelling central venous catheter (CVC), such as patients in intensive care units (Palmer and MacFie 2001).

1.3.2. Peripheral Route

The peripheral route is suitable in patients who require PN for up to two weeks and do not require high caloric requirements (Michel et al. 1981). The use of peripheral PN is said to be increasing with new research developments favouring this route, such as the availability of iso-osmolar lipid emulsions, and the development of PN admixtures containing lipids. The increased use of the peripheral route is also due to the fact that patients actually require much lower energy requirements than formerly considered necessary (Palmer and MacFie 2001; Payne-James and Khawaja 1993).

1.3.3. Peripherally Inserted Central Catheter

Typically, a CVC is inserted into the superior vena cava via the subclavian or internal jugular vein. Another type of CVC which is gaining interest is the peripherally inserted central catheter (PICC). PICC lines are inserted via peripheral access into the basilic or cephalic vein in the cubital fossa, with the tip running up into the larger veins into the superior vena cava. PICC lines have the advantage of avoiding direct puncture of the central veins (Palmer and MacFie 2001).

1.4. Complications of Administration

Complications of administration via the central route are CVC-related, and include problems such as catheter insertion, catheter occlusion, inadvertent

catheter removal, venous thrombosis and catheter-related sepsis. These complications could be minimised by enhancing professional skills in CVC placements and development of care guidelines (Grant 2002; Wolfe et al. 1986). The main limitation with peripheral PN is the incidence of thrombophlebitis (Everitt and McMahon 1994).

Besides catheter-related complications, another complication of PN is metabolic complications, such as plasma electrolyte abnormalities, fluid overload and those related to glucose metabolism, which are hypoglycaemia or hyperglycaemia (Maroulis and Kalfarentzos 2000).

1.5. Components of PN Admixtures

The composition of a PN formulation consists of three macronutrients: carbohydrates in the form of glucose, proteins as amino acid solutions, and fats in the form of lipid emulsions. Carbohydrates and fats provide the patients energy requirements. Also included are the major electrolytes such as sodium, potassium, calcium, magnesium, chloride and phosphate. The addition of micronutrients, consisting of the vitamins and trace elements, make a PN complete. PN regimens may be compounded as either 'two-in-one' admixtures, which do not contain lipid emulsion, or 'all-in-one' admixtures, in which the lipid emulsion is included (Allwood 2001). PN administration has developed from separate infusions of the macronutrients in bottles to administration of an all-in-one bag.

1.5.1. Proteins

The amino acids provide a source of amino acids which are utilised by the body as proteins. Traditionally, they are classified into essential and non-essential amino acids. Essential amino acids in adults are leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, whilst

some of the non-essential amino acids are alanine, arginine, glutamate, glutamine, glycine, serine and taurine (Cuthbertson and Rennie 2001).

The new approach recognises the fact that the non-essential amino acids may become 'indispensable' or 'conditionally indispensable' based on dietary needs and dependent on clinical conditions. For example, in infants, histidine, cysteine and tyrosine are also required. Arginine may be of significance in the critically ill where it is thought to enhance immunity in patients suffering from injury, trauma or sepsis. Another example is glutamine, the most abundant amino acid in the body which serves as nitrogen transporter between tissues, and provides metabolic fuel for the cells of the gastrointestinal tract. It has found usefulness after major surgery, burns and infections. Until recently, pharmaceutical problems with glutamine were its solubility and instability to heat, therefore the synthetic dipeptides were developed for parenteral solutions (Furst et al. 2001).

1.5.2. Carbohydrates

Carbohydrates are supplied in the form of glucose solutions, the specific glucose solutions used depending on the patient's fluid limitation. Adult glucose requirement is about 145-160 g per day (Furst et al. 2001) and should not exceed 7 g/kg/day (A.S.P.E.N. Board of Directors 2002a). To prevent metabolic complications, the optimal amount can be set at 4-5 g/kg/day (Sauerwein and Romijn 2001). Management of glucose abnormalities in PN patients have been described (Knapke et al. 1989), the two common abnormalities being hyperglycaemia and hypoglycaemia .

1.5.3. Lipids

Lipids serve to provide caloric requirements and have the advantage of providing more calories at a lower osmolarity than glucose. They also serve as a source of essential fatty acids, such as linoleic acid and linolenic acid, which

are necessary for the functioning of all tissues. In addition, lipids have numerous roles in physiologic functions and immune status (Dupont and Carpentier 1999). Lipid requirement is about 1-1.5 g/kg/day (Sauerwein and Romijn 2001).

Conventional lipid emulsions are typically made from soybean or safflower oil. These are long-chain triglycerides (LCT), rich in omega-6 polyunsaturated fatty acids (PUFA). The excessive intake of PUFA was later associated with interference of immune function. New lipid emulsions from medium-chain triglycerides (MCT) derived from palm oil or coconut oil, and LCT combination mixture (MCT/LCT) were then developed to decrease the PUFA content. More recently, lipid emulsion development has also included olive oil, rich in monounsaturated fatty acids (MUFA) and fish oil, rich in omega-3 fatty acids, and these have been shown to have anti-inflammatory and immunomodulatory effects (Adolph 2001; Furst et al. 2001). Mixed lipid emulsions made of soybean, MCT, olive oil and fish oil have been designed to reduce the amount of omega-6 fatty acids and to increase the amount of omega-3 fatty acids (Waitzberg 2005).

1.5.4. Micronutrients

The term 'micronutrients' includes two main classes of nutrient substances that are essentially required in the diet in very small amounts. These are the vitamins or the essential organic micronutrients, and trace elements or the essential inorganic micronutrients. The essential trace elements include zinc, iron, copper, chromium, selenium, manganese, molybdenum, fluoride and iodide. There are other dietary micronutrients, such as co-enzyme Q10, lipoic acid and carnitine but these can be synthesised in the body, and are therefore not recognised as being essential (Shenkin 2001).

Generally, there are two main functions of micronutrients. They either act as cofactors which activate enzyme activity, or as coenzymes as part of the

enzyme catalysed reactions. Another role they perform is to act as free-radical scavengers, protecting tissues from oxidative damage due to the generation of reactive oxygen species such as the superoxide free radicals (Shenkin 2003; Shenkin 2006). The micronutrients will be discussed in detail under Vitamins (see 1.6) and Trace elements (see 1.7).

1.5.5. Fluids and Electrolytes

Electrolytes are important for the body's physiologic functions. Sodium, potassium, calcium, magnesium and phosphorus are some of the major electrolytes required. Electrolyte abnormalities are associated with many disease states (Driscoll 1989; Solomon and Kirby 1990), therefore routine monitoring is essential to prevent any biochemical abnormalities.

The standard adult and paediatric electrolytes doses are as tabulated in Table 1.1.

Table 1.1: Daily parenteral electrolyte requirements for adults and paediatrics

Electrolyte	*Units*	*Adult (A.S.P.E.N. Board of Directors 2002a)*	*Paediatric (A.S.P.E.N. Board of Directors 2002b)*
Sodium	mmol/kg	1-2	3
Potassium	mmol/kg	1-2	2
Chloride	mmol/kg	as needed to maintain acid-base balance with acetate	5
Calcium	mmol	5-7.5	depends on age
Magnesium	mmol	4-10	
Phosphorus	mmol	20-40	

Fluid requirements are individualised, for adults they are generally met with volumes of 30 to 40 ml/kg/day (A.S.P.E.N. Board of Directors 2002a).

1.6. Vitamins

Vitamins differ from the other nutrients in that they do not build body tissues but are components of certain enzymes and hormone systems, which are essential for the normal life processes. Vitamins are required for growth, maintenance, reproduction and lactation.

Vitamins originally come from the term 'vitamine', coined by the Polish biochemist named Casimir Funk. Funk isolated the antiberi-beri factor in rice husks, and, having evidenced it was an amine, chose the term 'vitamine', from the words vital and amine, because it was vital to life. Four different vitamins were proposed by Funk in his 'vitamine theory', the other three vitamins were anti-rickets vitamine, anti-scurvy vitamine, and anti-pellagra vitamine. Since not all the factors later proved to be amines, the term 'vitamine' was changed to 'vitamin' to avoid chemical confusion (Combs Jr. 1998).

The vitamins are organic, low molecular weight substances. They are classified broadly according to their physical properties. Vitamins are classified into two groups by their solubility. The water-soluble vitamins consist of members of the vitamin B group and ascorbic acid or vitamin C. They generally function to assist the activity of important enzymes such as those involved in the production of energy from carbohydrates and fats, thus are often referred to as coenzymes. They are not stored to a great extent in the body and are excreted in the urine when present in excess of the body's needs; therefore they are considered relatively non-toxic. The water-soluble vitamins are appreciably soluble in polar solvents.

The fat-soluble vitamins include vitamins A, D, E, and K, these are appreciably soluble in non-polar solvents. They have the potential for storage in the body, and therefore for toxicity.

1.6.1. Water-soluble Vitamins

1.6.1.1. B-vitamins

The B-vitamins were grouped together in a single class, although they differ in chemical structure and metabolic function, because they were originally isolated from the same sources, liver and yeast.

The B-vitamins include thiamine (Vitamin B_1), riboflavin (Vitamin B_2), niacin (Vitamin B_3), pyridoxine (Vitamin B_6), folic acid, Vitamin B_{12} (cobalamins), pantothenic acid and biotin, which will be discussed.

1.6.1.1.1. Thiamine (Vitamin B_1)

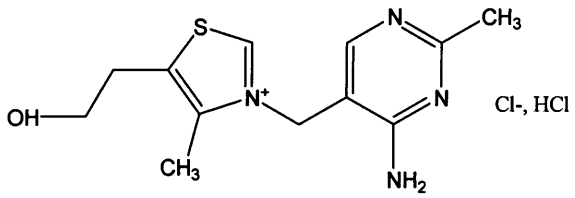


Figure 1.1: Chemical structure of thiamine hydrochloride

Thiamine, or Vitamin B_1, was the first member of the vitamin B-group to be identified and was originally named as aneurine, the antineuritic vitamin, because of its function in curing polyneuritis in deficient pigeon. Thiamine, which in Greek means a vitamin containing sulphur (Carpenter 2003), contains a pyrimidine and a thiazole nucleus linked by a methylene bridge. The free thiamine base is unstable and two derivatives are commonly used in food and pharmaceutical preparations, which are thiamine mononitrate and thiamine hydrochloride (Figure 1.1).

The thiamine molecule is not biologically active (Basu and Dickerson 1996), however thiamine functions in the body in the form of the active coenzyme thiamine diphosphate, also known as thiamine pyrophosphate (TPP). TPP is the coenzyme for the decarboxylases, the group of enzymes that remove carboxyl groups. One function of TPP is in the oxidative decarboxylation of pyruvate

dehydrogenase. This is the key enzyme required for the conversion of pyruvate, the product of carbohydrate metabolism, to acetyl coenzyme A. Impaired entry of pyruvate into the citric acid cycle can lead to high concentrations of lactate and pyruvate, and to lactic acidosis.

Thiamine deficiency can result in chronic peripheral neuritis called beri-beri, characterised by anorexia, cardiac enlargement and neurologic involvement, which can be associated with heart failure and oedema. Deficiency also can result in Wernicke-Korsakoff syndrome, a condition affecting the central rather than the peripheral nervous system (Combs Jr. 1998; Indraccolo et al. 2005). Cases of lactic acidosis have been reported in patients given PN therapy, which were traced to thiamine deficiency (Madl et al. 1993; MMWR 1997; Nakasaki et al. 1997).

Thiamine is degraded by heat and by ultraviolet irradiation (Basu and Dickerson 1996). It is incompatible when mixed with reducing agents such as sulphites, in particular by sodium metabisulphite, used as an antioxidant in some amino acids infusions, where the thiamine molecule is cleaved into pyrimidine and thiazole moieties (Allwood and Kearney 1998). The degradation pathway is shown in Figure 1.2.

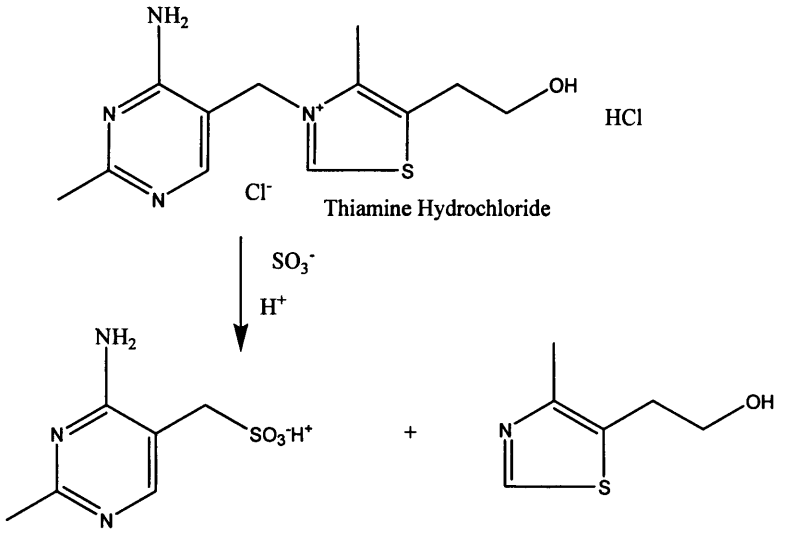


Figure 1.2: Thiamine degradation pathway (Allwood and Kearney 1998)

1.6.1.1.2. Riboflavin

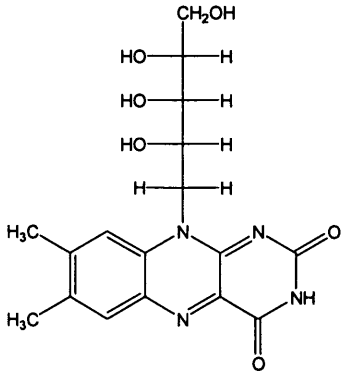


Figure 1.3: Chemical structure of riboflavin

Riboflavin, or Vitamin B_2, consists of an isoalloxazine derivative with a sugar alcohol ribityl side chain (Figure 1.3). Riboflavin is converted to two main coenzymes, flavin mononucleotide (FMN) or riboflavin-5-phosphate and flavin-adenine dinucleotide (FAD). FMN and FAD function as coenzymes for enzymes called flavoproteins or flavoenzymes, these are oxidases that function aerobically, and dehydrogenases that function anaerobically. The flavin coenzymes act as electron carriers in a wide variety of oxidation-reduction reactions, including the mitochondrial electron transport chain (Hardman et al. 1996), thus supporting energy production by aiding in the metabolism of fats, carbohydrates and proteins. Flavoproteins are also essential for the conversion of pyridoxine and folate to their respective coenzyme forms. Riboflavin is also required for red blood cell formation and respiration, antibody production, and for regulating human growth and reproduction (Basu and Dickerson 1996).

The clinical signs of riboflavin deficiency occur mostly in the mucocutaneous surfaces of the mouth. These include angular stomatitis (cracks at the corners of the mouth), cheilosis (inflammation of the lips), glossitis (inflammation of the tongue), magenta tongue and corneal vascularisation. Riboflavin deficiency often coexists with nicotinic acid deficiency and can be complicated by pyridoxine deficiency (Basu and Dickerson 1996).

Riboflavin is heat stable, stable in acids but not in alkaline solutions, and unstable to UV light with irreversible decomposition (Basu and Dickerson

1996). Photolysis of riboflavin leads to the formation of lumiflavin (in alkaline solution) and lumichrome (in acidic or neutral solution) which has a greenish-yellow fluorescence. It is noted that exposure of milk in clear glass to sunlight or fluorescent light (with a peak wavelength of 400-500 nm) can result in the loss of significant amounts of riboflavin as a result of photolysis. The resultant lumiflavin and lumichrome catalyse the oxidation of vitamin C, so that even relatively brief exposure to light, causing little loss of riboflavin, can lead to a considerable loss of vitamin C. Lumiflavin and lumichrome also catalyse oxidation of lipids to lipid peroxides (Bender 1992).

1.6.1.1.3. Niacin

The term niacin (Vitamin B_3) is the generic descriptor for nicotinic acid and nicotinamide (Figure 1.4) (Bender 1992). Nicotinamide is the active form which functions in the body as either nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP). NAD and NADP serve as coenzymes for a wide variety of reactions in the metabolism of carbohydrates, fatty acids and amino acids in which there is a transfer of hydrogen ion (Basu and Dickerson 1996).

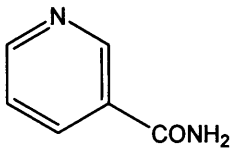


Figure 1.4: Chemical structure of nicotinamide

Niacin can be obtained from the diet or synthesised from the amino acid tryptophan. The equivalence of dietary tryptophan and preformed niacin is relatively low, the generally accepted ratio being 60 mg tryptophan as equivalent to 1 mg of preformed niacin (Bender 1992). Although niacin is synthesised in the body, it is categorised as a vitamin because its precursor, tryptophan, is an essential amino acid, and thus the synthesis of niacin is dependent upon diets. The conversion of tryptophan to niacin is dependent on pyridoxine supplies.

Deficiency of niacin leads to the clinical condition known as pellagra which involves changes in the skin (dermatitis), gastrointestinal tract (diarrhoea) and nervous system (dementia).

Both nicotinic acid and nicotinamide are very stable in dry form but in solution, nicotinamide is hydrolysed to nicotinic acid by acids and bases (Combs Jr. 1998).

1.6.1.1.4. Pyridoxine

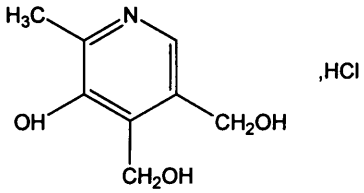


Figure 1.5: Chemical structure of pyridoxine hydrochloride

Pyridoxine (Vitamin B_6) is used as a name to cover the 'Vitamin B_6 group' or 3-hydroxy-2-methyl-pyridine derivatives (Figure 1.5). These are pyridoxine (new terminology pyridoxol, the alcohol), its aldehyde (pyridoxal), and its amine (pyridoxamine). All three forms (or vitamers) are interchangeable and are therefore comparably active. Plant tissues contain Vitamin B_6 mostly as pyridoxine while animal tissues contain mostly pyridoxal and pyridoxamine. Pyridoxine hydrochloride is the commonly available synthetic form (Basu and Dickerson 1996).

The vitamers are converted in the liver mainly to pyridoxal phosphate and to some extent to pyridoxamine phosphate. Pyridoxal phosphate acts as a coenzyme, and is involved in many reactions of amino acid metabolism and synthesis, in glycogen phosphorylase for the utilisation of glycogen in muscle, and in steroid hormone action (Bender 1992). An example of amino acid reaction utilising pyridoxal phosphate is biosynthesis of the neurotransmitters (Table 1. 2), derived from decarboxylation of amino acids.

Table 1.2: Some amines derived from amino acids under the action of vitamin B_6-dependent decarboxylases in the body (adapted from Basu 1996)

Amino acid/derivative	*Amine*
Histidine	Histamine
5-Hydroxytryptophan	5-Hydroxytryptomine (serotonin)
Aspartic acid	β - Alanine
Glutamic acid	γ – Aminobutyric acid (GABA)
Tyrosine	Noradrenaline / adrenaline Tyramine / dopamine
Cysteic	Taurine

Deficiency of Vitamin B_6 is rare in man due to its substantial stores in the body and wide distribution in foods. Symptoms of Vitamin B_6 deficiency include seborrhoea-like skin lesions about the eyes, nose and mouth accompanied by glossitis and stomatitis; convulsive seizures and peripheral neuritis; and rarely anaemia (Hardman et al. 1996).

Vitamin B_6 is stable under acidic conditions but rapidly destroyed by light (Basu and Dickerson 1996).

1.6.1.1.5. Folic Acid

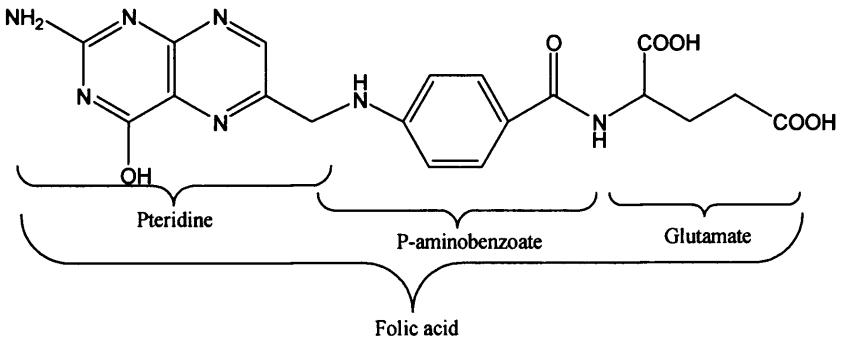


Figure 1.6: Chemical structure of folic acid

Folic acid, also known as pteroylglutamic acid (PteGlu), consists of a pteridine nucleus coupled to para-aminobenzoic acid (PABA), to become pteroic acid molecule, and conjugated with one to seven molecules of L-glutamic acid (Figure 1.6). The pteridine nucleus is composed of two rings, pyrimidine and

pyrizine. Different substituent groups can be attached to the pyrizine ring, resulting in over 150 different forms existing in nature. All biologically active forms of folic acid are known as folacins or folates. Folates are stored in tissues primarily as the polyglutamates (Basu and Dickerson 1996).

Following absorption, folic acid is rapidly reduced to the active coenzyme, tetrahydrofolic acid (FH_4). Tetrahydrofolate plays a vital role in metabolic reactions, both in catabolism and biosynthetic reactions such as in DNA synthesis and amino acid synthesis (Bender 1992).

Deficiency of folic acid affects DNA replication, notably in cells with the most rapid rates of multiplication. These include the gastrointestinal tract, liver and the bone marrow. Signs and symptoms of deficiency related to gastrointestinal tract include soreness of tongue, cheilosis, loss of appetite, abdominal pain and diarrhoea. Folate deficiency leads to macrocytic or megaloblastic anemia, with signs such as tiredness, breathlessness, pallor of the skin and mucous membranes, and systolic murmurs (Basu and Dickerson 1996; Mason 1999).

Folic acid is light sensitive, following ultraviolet (UV) exposure, folic acid is degraded to photoproducts, para-aminobenzoyl-L-glutamic acid and 6-formylpterin (FPT). FPT is degraded further to pterin-6-carboxylic acid upon continued irradiation (Off et al. 2005). The rate of degradation varies with pH, with a decrease in the degradation rate from acid to alkaline medium, and optimum stability at pH 6-7 (Akhtar et al. 1999).

Crystalline folic acid is moderately stable to heat, with significant degradation occurring at 200°C leading to an amorphous product. Thermal decomposition shows firstly, the loss of glutamic acid moiety from the folic acid structure, then the degradation of pterin and PABA, leaving a black carbon residue (Vora et al. 2002).

1.6.1.1.6. Vitamin B_{12}

Vitamin B_{12} is different from other vitamins in that it contains cobalt, which is a mineral. Vitamin B_{12} consists of a family of compounds called cobalamins amongst which one of the major forms is cyanocobalamin (with a cyanide group; Figure 1.7).

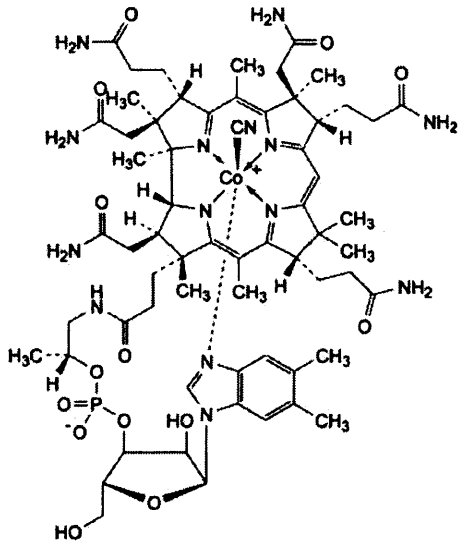


Figure 1.7: Chemical structure of cyanocobalamin

In the body, Vitamin B_{12} is mostly stored in the liver and has a half-life of about 400 days, and has a considerable enterohepatic circulation.

There are three vitamin B_{12}–dependent enzymes in the tissues, these are methionine synthetase, methylmalonyl-CoA mutase and leucine mutase (Basu and Dickerson 1996). One of the enzymes, methionine synthetase, is used to generate methionine from homocysteine, a reaction dependent on Vitamin B_{12}.

$$CH_3 - FH_4 + Homocysteine \xrightarrow{Methioninesynthetase} FH_4 + Methionine$$

In the above reaction, both Vitamin B_{12} and folic acid are required for the conversion of homocysteine into methionine.

When the above reaction is blocked due to deficiency of Vitamin B_{12}, the FH_4 needed for other folate requiring reactions is not formed, causing functional

folate deficiency, thereby releasing immature red blood cells into the circulation resulting in pernicious anaemia. Signs and symptoms of Vitamin B_{12} deficiency are similar to folate deficiency. In addition, Vitamin B_{12} deficiency may cause neurological disorders, characterised by peripheral neuropathy. However, Vitamin B_{12} deficiency is less common than folate deficiency because it is more stable, the body store is less easily depleted and excretion is reduced by enterohepatic circulation.

Vitamin B_{12} is stable to heat at 100°C in aqueous solutions at pH 4-7. However, it is very sensitive to light and alkaline, and in the presence of reducing agents (Basu and Dickerson 1996).

1.6.1.1.7. Pantothenic Acid

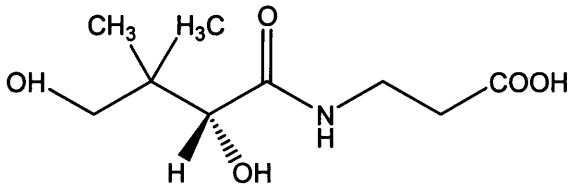


Figure 1.8: Chemical structure of pantothenic acid

Pantothenic acid is a component of a much larger coenzyme, coenzyme A (CoA) which is required for many acyl group transfer reaction pathways. It is also part of acyl carrier protein which is involved in fatty acid synthesis. Thus it is involved in lipid metabolism and the synthesis of cholesterols and steroids. As a component of acetyl CoA, it is involved in many acetylations, such as the conversion of choline to acetylcholine.

Pantothenic acid is widely distributed in the body. Free pantothenic acid is available as hygroscopic oil and is chemically unstable. It is hydrolysed in acid and alkaline media to pentoic acid and alanine. It is used mainly in the form of calcium and sodium salts which are readily soluble in water (Basu and Dickerson 1996).

1.6.1.1.8. Biotin

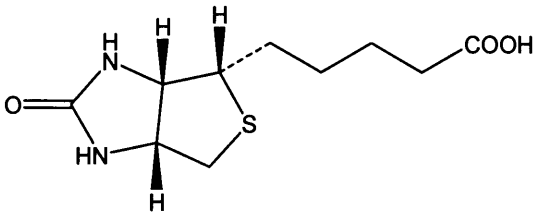


Figure 1.9: Chemical structure of biotin

Biotin (Figure 1.9) is present in almost all foods, the best sources are from organ meats, egg yolks and yeast. Raw egg whites contain the glycoprotein avidin which can bind to biotin forming a complex that cannot be absorbed by the body. The complex is broken down by heat or irradiation.

Biotin-dependent enzymes are involved in gluconeogenesis, biosynthesis of fatty acids, propionate metabolism and catabolism of leucine. Biotin is sensitive to heat, especially when conditions support lipid peroxidation (Basu and Dickerson 1996).

1.6.1.2. Ascorbic Acid (Vitamin C)

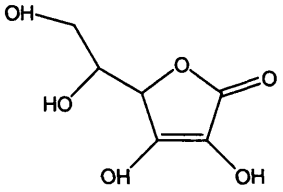


Figure 1.10: Chemical structure of ascorbic acid

Vitamin C is the generic descriptor for all compounds that exhibit the biological activity of ascorbic acid (Combs Jr. 1998). The chemical structure of ascorbic acid is shown in Figure 1.10.

Ascorbic acid or Vitamin C is isolated mostly from sources different from the vitamins B-complex, mainly from citrus fruits. The functions of ascorbic acid include synthesis of collagen, synthesis of carnitine which is required for transporting fatty acids into mitochondria for oxidation to provide energy for

the cell, and metabolism of neurotransmitters such as tyrosine and the conversion of dopamine to noradrenaline. It is a strong reducing agent, and hence plays an important role as an antioxidant and free radical scavenger. It is also involved in the utilisation of other nutrients, such as iron, and for the function of the immune system (Combs Jr. 1998).

Deficiency of Vitamin C leads to a condition called scurvy, due to inhibition of collagen synthesis. Some of the clinical signs include swollen, bleeding and sensitive gums, hardening and roughness around hair follicles, and delayed wound healing.

The human body cannot synthesise ascorbic acid, therefore the body depends on dietary intake to maintain its vital biological functions. In vivo, ascorbic acid is metabolised by reversible oxidation to dehydroascorbic acid, which subsequently undergoes hydrolysis to 2,3-diketo gulonic acid. 2,3-diketo gulonic acid then undergoes either decarboxylation to carbon dioxide and 5-C fragments (xylose, xylonic acid, lyxonic acid), or oxidation to oxalic acid and 4-C fragments (e.g. threonic acid) (Combs Jr. 1992).

In PN mixtures, ascorbic acid is the least stable of all the vitamins (Allwood and Kearney 1998). It is degraded in the presence of oxygen, where it is oxidised reversibly to dehydroascorbic acid, and further degraded irreversibly to gulonic acid, threonic acid and oxalic acid (Figure 1.11).

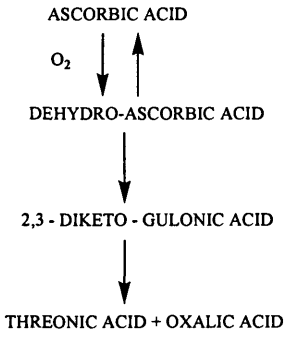


Figure 1.11: Degradation pathway of ascorbic acid (Allwood and Kearney 1998)

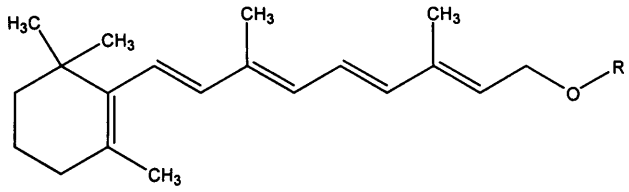
Degradation of ascorbic acid is enhanced by heat and catalysed by trace metals such as copper ions (Allwood 1982b). Degradation is also enhanced by neutral to alkaline pH conditions. Ascorbic acid complexes with disulfides such as cystine (Allwood 1984b; Allwood and Kearney 1998; Combs Jr. 1998).

1.6.2. Fat-soluble Vitamins

1.6.2.1. Vitamin A

Vitamin A refers to compounds with similar structures possessing similar biological activity of retinol. 0.3 micrograms all-trans-retinol is equivalent to 1 international unit (IU) Vitamin A activity (Combs Jr. 1998).

Figure 1.12 shows the chemical structures of the vitamin A group, or the retinoids, which consist of three essential features. These are: (i) a beta-ionone ring, or the hydrophobic head; (ii) a side chain composed of two isoprenoid units joined together. These conjugated polyenes absorb light and are subject to isomerisation in the presence of light; (iii) a polar group R which determines the chemical reactivity. It can either be an ester as in retinol palmitate, an aldehyde as in retinal, or be oxidised to retinoic acid.



Substance	R	Molecular formula	M
All-(*E*)-retinol	R = H	$C_{26}H_{30}O$	286.5
All-(*E*)-retinol acetate	CO-CH_3	$C_{22}H_{32}O_2$	328.5
All-(*E*)-retinol propionate	CO-C_2H_5	$C_{23}H_{34}O_2$	342.5
All-(*E*)-retinol palmitate	CO-$C_{15}H_{31}$	$C_{36}H_{66}O_2$	524.9

Figure 1.12: Chemical structures of vitamin A groups (BP 2005)

Vitamin A is usually used in the form of esters such as the acetate, propionate and palmitate. In the British Pharmacopoeia (BP), the term 'retinol' is used for

preparations containing synthetic esters and 'vitamin A' is used for preparations containing naturally occurring compounds. In this thesis, the Vitamin A ester being studied is retinol palmitate.

Vitamin A is necessary for vision, for normal differentiation of epithelial cells, and for reproduction. Retinol and retinal are active in maintaining normal vision and reproduction as well as normal growth and development, while the retinoic acid is active in epithelial cell differentiation for normal growth and development (Basu and Dickerson 1996).

Deficiency of Vitamin A can lead to night blindness and a condition called xerophthalmia, or dryness of the eye. Deficiency also leads to keratinisation of the epithelial cells, thus promoting colonisation by bacteria and infection.

Vitamin A is generally stable to heat, acid and alkali. Vitamin A is very sensitive to oxygen in air, especially in the presence of light and heat, although the retinoid esters are relatively stable. Exclusion of air can be obtained by sparging with an inert gas and addition of a protective antioxidant e.g. α-tocopherol (Combs Jr. 1998).

1.6.2.2. Vitamin D

Vitamin D refers to the group of steroids that exhibit the biological activity of cholecalciferol (Combs Jr. 1998). Ergocalciferol or Vitamin D_2 is mainly present in plants and fungi while cholecalciferol or Vitamin D_3 is mainly present in animals. Besides food sources, vitamin D is also synthesised by the action of sunlight on the skin. Ultraviolet B penetrates the skin and photolyses 7-dehydrocholesterol, a provitamin D_3, to form cholecalciferol or vitamin D_3.

The BP specifies that if calciferol or Vitamin D is prescribed, then ergocalciferol (Figure 1.13) or cholecalciferol (Figure 1.14) should be dispensed (British Pharmacopoeia Commission 2005).

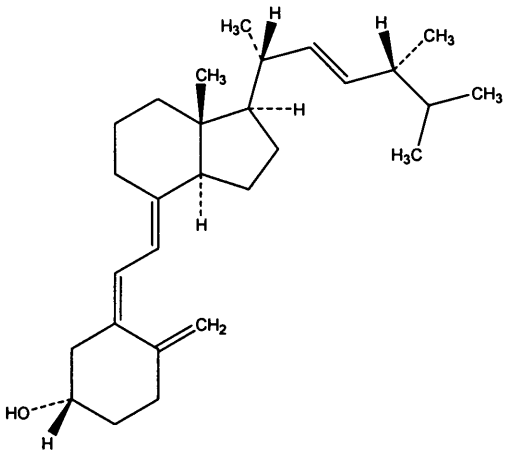


Figure 1.13: Chemical structure of ergocalciferol

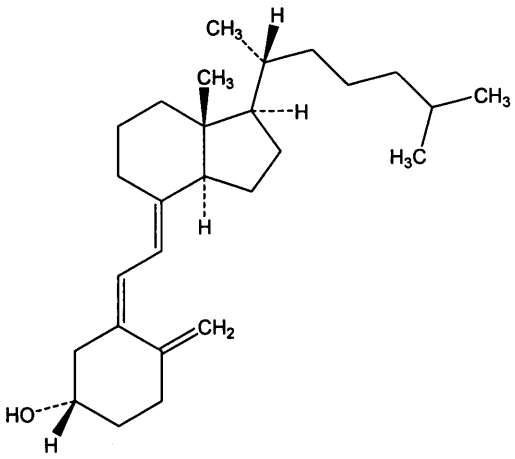


Figure 1.14: Chemical structure of cholecalciferol

Vitamin D functions mainly as a steroid hormone. To be biologically active, vitamin D is hydroxylated, first in the liver to form 25-hydroxy-vitamin D_3, and then in the kidney to form the hormone, 1, 25-dihydroxyvitamin D_3. 1,25-dihydroxyvitamin D_3 regulates serum calcium and phosphorus by stimulating intestinal absorption and mobilising calcium stores from bone, and mobilising calcium reabsorption in the kidneys. The hormone is regulated by parathyroid hormone through a feedback mechanism.

1.6.2.3. Vitamin E

Vitamin E refers to two classes of compounds, tocopherols and tocotrienols, which exhibit the biological activity of α-tocopherol. There are eight possible stereoisomers for tocopherols. Synthetic preparations of vitamin E presently available are mixtures of all eight stereoisomers, which are designated with the prefix all-racemic-. All-racemic-alpha-tocopherol was formerly called dl-alpha-tocopherol and has equivalent biopotency of 1.1 IU/mg (Combs Jr. 1998).

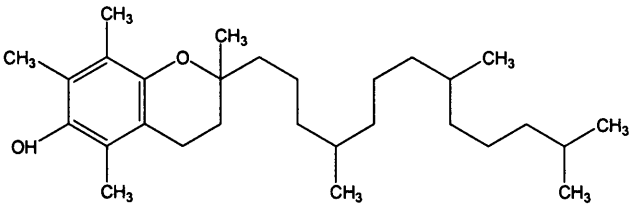


Figure 1.15: Chemical structure of all-racemic-alpha-tocopherol

Vitamin E is a good quencher of free radicals, thus it serves as an antioxidant. Vitamin E exerts its antioxidant function through its oxidation to α-tocopherolquinone (Basu and Dickerson 1996). Its major function is protection of PUFA of cell membrane phospholipids from oxidation by free radicals to hydroperoxides. Because of its antioxidative properties, Vitamin E is believed to prevent diseases that are associated with oxidative stress such as cardiovascular disease, cancer and neurologic disorders (Bartels et al. 2004; Brigelius-Flohe et al. 2002). Selenium complements Vitamin E to scavenge and reduce free radicals generated from PUFA.

Vitamin E is destroyed under oxidising conditions such as exposure to air and light, accelerated by heat and the presence of copper (Basu and Dickerson 1996).

1.6.2.4. Vitamin K

Vitamin K consists of a group of fat-soluble vitamins containing 2-methyl-1,4-naphthaquinone derivatives. They are phylloquinone, the naturally occurring vitamin synthesised from plants and designated as vitamin K_1; the menaquinones, which are synthesised by bacteria and designated as K_2; the synthetic menadione or vitamin K_3, and menadiol or vitamin K_4 (Combs Jr. 1998). The chemical structure of phytomenadione is shown in Figure 1.16.

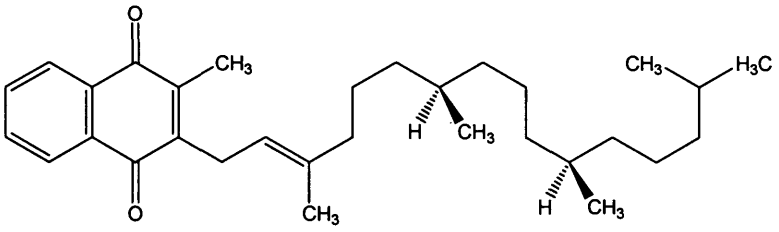


Figure 1.16. Chemical structure of phytomenadione

Vitamin K plays an important role in blood coagulation. In newborn infants, there is a deficiency of Vitamin K and therefore it is given to the baby immediately after birth to prevent hemorrhagic disease of the newborn. There have been controversies on the use of vitamin K in newborn in that it was associated with incidence of leukaemia, however this risk was found to be unproven (American Academy of Pediatrics Vitamin K Ad Hoc Task Force 1993).

1.7. Trace Elements

The benefits of adding trace elements to PN solutions are now widely recognised. They are essential for rehabilitation and maintenance of good nutritional status of patients (AMA Department of Foods and Nutrition 1979a). Trace elements either function as co-factors, activating the enzyme activity, or they form an essential component of the enzymes (Shenkin 2001). Many act as antioxidants, by protecting the tissues from oxygen free radicals (Leung 1998). The inorganic micronutrients considered essential include zinc, copper, selenium, manganese, chromium, molybdenum, iron, and iodine (Hardy and Reilly 1999) and will be discussed. Other trace elements considered probably essential are fluorine, boron, cobalt and vanadium.

Trace element contaminants such as aluminium have been detected in PN solutions, the potential sources of contamination coming from packaging materials, manufacturing methods, and raw materials for production of the component solutions (Borg et al. 1994; Pluhator-Murton et al. 1999).

1.7.1. Zinc

There are over 300 zinc-containing enzymes. Zinc (Zn) is essential for the activity of many enzymes, such as those involved in protein synthesis, in protein and carbohydrate metabolism, and DNA synthesis and gene transcription (Scheplyagina 2005; Shenkin 2006).

Zn deficiency results in many abnormalities such as skin lesions, impaired immunity, and impaired wound healing. In PN therapy, acute Zn deficiency occurs because of increased demand for Zn as a result of increasing anabolism or protein synthesis (Shenkin 2006; Takagi et al. 1986).

Historically, Zn has been found as a contaminant in amino acid solutions and other intravenous solutions, due to Zn leaching out of the rubber stoppers used in glass bottles (Van Caillie et al. 1978).

Parenteral Zn for PN supplementation is available as zinc sulphate, zinc chloride or zinc gluconate (Hardy and Reilly 1999).

1.7.2. Copper

Copper (Cu) is a component of many enzymes, mainly oxidative enzymes, hydroxylases and superoxide dismutases. It is a cofactor for ceruloplasmin, which oxidises ferrous ion to ferric ion, therefore maintaining iron utilisation as well as production of haemoglobin and red blood cells. Cu deficiency therefore is manifested by anaemia, leucopenia and neutropenia (Hardy and Reilly 1999) and has been reported in patients on prolonged PN (Tokuda et al. 1986).

Parenteral Cu is available as copper sulphate, copper chloride and copper gluconate (Hardy and Reilly 1999).

1.7.3. Selenium

Selenium (Se) plays a structural role as a constituent of selenoproteins, and enzymic role as part of many key antioxidant enzymes such as in glutathione peroxidase, as selenocysteine. Selenocysteine breaks down hydrogen peroxide, the end-product of superoxide dismutase reaction, into hydrogen and water. The role of Se parallels that of vitamin E, with each supporting their respective antioxidant functions where Se is hydrophilic while vitamin E is lipophilic. Known roles of selenoproteins are tabulated in Appendix 1. (Hardy and Hardy 2003; Leung 1998; Rayman 2000).

Parenteral Se for PN supplementation is available as selenomethionine, selenious acid and sodium selenite (Hardy and Reilly 1999).

1.7.4. Manganese

Manganese (Mn) is involved in numerous organ functions such as for normal immune function, energy production and defence against free radicals. Mn is a co-factor in enzymatic systems such as Mn-superoxide dismutase and plays a role in antioxidant protection, and in pyruvate carboxylase which has a role in energy metabolism (Aschner and Aschner 2005).

Mn can be found as a contaminant in PN solutions. There have been reports of Mn toxicity where excess intake can lead to deposition of Mn in the brain resulting in Mn-induced neurotoxicity. Hypermanganesia can result from a high amount of infused Mn, causing persistent inflammation and PN-related cholestasis. Excess intake has also been associated with cholestatic disease and nervous system disorders (Aschner and Aschner 2005; Fell et al. 1996; Reimund et al. 2000).

For PN supplementation, Mn is available as manganese sulphate, manganese chloride or manganese gluconate (Hardy and Reilly 1999).

1.7.5. Chromium

Chromium (Cr) has a major role in the maintenance of normal glucose metabolism, where it is a component of glucose tolerance factor, required in improving insulin action (Leung 1995). Cr deficiency in PN patients is rare because of its presence as a contaminant in amino acid solutions (Leung et al. 1995). Serum Cr has been found to be high in children on PN even though intake followed the recommended guideline (Moukarzel et al. 1992). However Cr deficiency has been reported in a patient on long-term home PN, with signs of increased serum glucose concentration with glycosuria (Tsuda et al. 1998).

Parenteral Cr is available in the form of chromic chloride (Hardy and Reilly 1999).

1.7.6. Molybdenum

Molybdenum (Mo) is involved in oxidation/reduction reactions, such as in xanthine oxidase, for metabolism of purines, and sulphite oxidase for oxidation of sulphites formed from methionine and cysteine metabolism. Parenteral Mo is available as ammonium molybdate tetrahydrate (Hardy and Reilly 1999).

1.7.7. Iron

Iron (Fe) is essential for haemoglobin formation and for oxidative processes. The main function is to transport oxygen within the blood and muscle. In the body, Fe is stored in two principal forms, ferritin and haemosiderin. In aqueous form, iron exists in two oxidation states, the ferrous form Fe^{2+}, and the ferric form Fe^{3+}. This property enables Fe to act as a catalyst in redox reactions by accepting or donating electrons (Parfitt 1999; Yip and Dallman 1996).

Fe deficiency develops quite slowly in patients with normal Fe turnover as the body stores of Fe can suffice for up to one year, therefore Fe supplementation is not necessary during short-term PN (Leung 1995). However, patients on

long-term PN have been shown to have Fe deficiency anaemia due to chronic blood loss (Khaodhiar et al. 2002).

The compatibility of Fe with PN solutions has not been established. Small amounts of Fe of 10 to 15 mg dose could be compatible with amino acid-glucose PN solutions but incompatible with All-in-One admixture, causing cracking and creaming of the lipid component (Khaodhiar et al. 2002).

Parenteral Fe for PN supplementation is available as ferric chloride. Other sources of Fe include ferrous citrate or ferrous gluconate solutions (Hardy and Reilly 1999).

1.7.8. Iodine

Iodine is an essential component of the thyroid hormones, thyroxine and triiodotyronine. Iodine deficiency disorders include goitre, cretinism, impaired mental function, increased incidence of stillbirths, and perinatal and infant mortality (Parfitt 1999).

Parenteral iodine can be administered as either the sodium or potassium iodide, which also can be found in trace element preparations (Hardy and Reilly 1999).

1.8. Micronutrient Requirements

1.8.1. Vitamin Requirements

Micronutrient requirements as recommended by the American Medical Association (AMA) Nutrition Advisory Group (AMA Department of Foods and Nutrition 1979b) are generally used at present.

Vitamin requirements are increased in patients receiving PN therapy. The daily parenteral vitamin requirements recommended by A.S.P.E.N are listed in Table 1.3. Parenteral vitamin requirements differ from enteral nutrition requirements,

where enteral nutrition requirements are designed for healthy individuals to prevent deficiencies and to minimise the risk from nutrition-related chronic disease, but dosing guidelines for parenteral vitamins on the other hand are approximates to the needs of patients with increased requirements in acute and chronic disease.

Table 1.3: Daily parenteral vitamin requirements in adults and infants (ASPEN 2002a, ASPEN 2002b)

Vitamins	*Adult (A.S.P.E.N. Board of Directors 2002a)*	*Infants (A.S.P.E.N. Board of Directors 2002b)*
Thiamine	3 mg	1.2 mg
Riboflavin	3.6 mg	1.4 mg
Niacin	16 mg	17 mg
Folic acid	400 µg	140 µg
Pantothenic acid	5 mg	5 mg
Vitamin B_6	1.7 mg	1 mg
Vitamin B_{12}	5 µg	1 µg
Biotin	60 µg	20 µg
Ascorbic acid	90 mg	80 mg
Vitamin A	1000 µg	700 µg (retinol equivalent)
Vitamin D	5 µg	10 µg
Vitamin E	15 mg	7 mg
Vitamin K	120 µg	200 µg
Carnitine	-	2-10 mg/kg

Commercial vitamins for PN are commonly available as multivitamin formulations such as Solivito® N (Fresenius Kabi), Cernevit® (Baxter), Vitlipid® N Adult (Fresenius Kabi) and Vitlipid® N Infant (Fresenius Kabi) injections (Table 1.4). Commercial products for the US market have been summarised by Kelly (2002). Individual vitamin injections are also available especially of the water-soluble formulations like the B vitamins, folic acid and ascorbic acid where additional requirements may be required.

Table 1.4: Vitamin content in parenteral multivitamin preparations (BNF Joint Formulary Committee 2006)

Vitamin	*Units*	*Solivito N*	*Cernevit*	*Vitlipid N Adult*	*Vitlipid N Infant*
Ascorbic acid	mg	100	125	-	-
Thiamine	mg	2.5	3.51	-	-
Riboflavin	mg	3.6	4.14	-	-
Pyridoxine	mg	4	5.5	-	-
Nicotinamide	mg	40	46	-	-
Vitamin B_{12}	µg	5	6	-	-
Pantothenic acid	mg	15	17.25	-	-
Biotin	µg	60	69	-	-
Folic acid	µg	400	414	-	-
Vitamin A	IU	-	3500	3300	2300
Ergocalciferol	IU	-	220	200	400
α-Tocopherol	mg	-	11.2	10	7
Vitamin K	µg	-	-	150	200

1.8.2. Trace Element Requirements

The first guideline for the requirement of trace elements in PN which was introduced by the AMA Nutrition Advisory Group was concerned with four trace elements i.e. zinc, copper, chromium and manganese, where the committee recommended that the trace elements be made in single-entity IV solutions (AMA Department of Foods and Nutrition 1979a). However, multiple trace elements are now available in a formulation such as Additrace® (Fresenius Kabi) and Decan® (Baxter) for adults, and Peditrace® (Fresenius Kabi) for paediatrics.

1.9. Problems Associated with Micronutrients Provision

The provision of micronutrients to PN patients is complicated by many factors. Firstly, the majority of PN patients are supplemented with their micronutrients orally, which may explain why serious micronutrient deficiencies are uncommon. However, the proportion that is absorbed orally depends on the remaining absorptive capacity of the gastrointestinal tract. When given intravenously, the potential for micronutrients toxicity is greater as this route

bypasses the safe method of controlling absorption by the gastrointestinal tract. Therefore care has to be taken in situations where the excretory mechanism is not effective, such as fat-soluble vitamins, or where the therapeutic and toxicity margin is narrow, such as selenium (Shenkin 1986).

Secondly, a deficiency of micronutrient is difficult to determine by serum or laboratory values since these do not reflect tissue concentrations and body stores. Only extreme deficiencies that lead to clinical symptoms are recognised (Forbes and Forbes 1997; Gallitelli 1995; Shenkin 1997). Having said this, there are many studies that have been performed to determine the prevalence and deficiencies of micronutrients in patients, such as vitamins A, D, E and K deficiencies (Clark et al. 1993; DeVito et al. 1986; Jamieson et al. 1999; Pettei et al. 1993; Shenkin et al. 1987; Steephen et al. 1991; Vandewoude et al. 1986), and thiamine, riboflavin and pyridoxine deficiencies (Jamieson et al. 1999).

Another problem with the provision is with respect to compatibility and stability in PN formulations. A formulation is regarded as unstable when the component drug contains less than 90% of its stated content. These physical chemical stability problems concerning the provision of micronutrients in PN formulations, with particular emphasis on the vitamins shall be discussed in this thesis. This thesis will not address the microbiological and sterility issues which are also determinants of a parenteral product's shelf life.

1.10. Stability Issues Concerning Micronutrients in PN

Many issues concerning the stability of PN towards improving its safety and efficacy, and its practical considerations have been addressed (Allwood 1984a, 1999; Allwood 2000b, 2002; Allwood and Kearney 1998; Barnett et al. 1990; DeRitter 1982; Manning and Washington 1992; Niemiec Jr. and Vanderveen 1984; Rey et al. 2005). DeRitter (1982) outlined the stability characteristics of most vitamins, as listed in Appendix 2, and classified the labile vitamins which

can cause problems of instability in pharmaceutical dosage forms as vitamin A, vitamin K, ascorbic acid, cyanocobalamin, folic acid, pantothenic acid, panthenol, and thiamine.

PN formulations are the most complex extemporaneous admixtures to be prepared, where individual patient requirements such as energy requirements, electrolyte requirements and clinical conditions are taken into consideration. At the same time, PN admixtures can consist of different proportions and types of glucose, amino acids, lipid emulsions and electrolytes, as well as the inclusion of micronutrients. Because of the many components in a PN formulation, pharmaceutical problems of instability and incompatibility of components exist, thus leading to major concerns such as reduction in potency, risk of precipitation and breakdown of the lipid emulsion.

Many factors can affect the physical and chemical stability of micronutrients in PN formulations. These include the component solutions such as the electrolytes, storage temperatures, the presence of oxygen, the presence of trace elements, and the effects of light. PN stability issues are also compounded by other factors such as types of containers used and sequence of mixing.

Some examples of the effects of electrolytes, oxygen and light are discussed under 1.10.1, 1.10.2 and 1.10.3.

1.10.1. Electrolytes

Normal daily requirements of electrolytes are generally tolerated in PN admixtures. However, physical incompatibilities may arise when greater needs are required and the final PN volume is small, especially for neonates. The common electrolytes having problems of incompatibility are calcium and phosphate ions leading to calcium phosphate precipitation (see Figure 1.17).

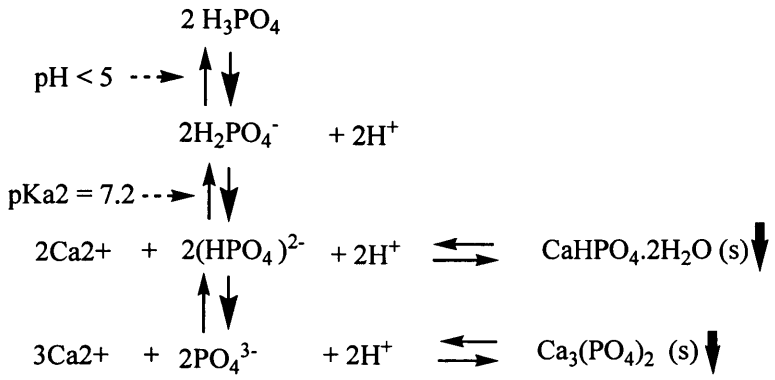


Figure 1.17: Speciation of inorganic phosphate in PN admixture (Allwood and Kearney 1998)

The factors influencing this solubility are the final pH of the PN admixture, the types of amino acids used, the types of calcium and phosphates used and temperature (Allwood and Kearney 1998; Poole 1983). Calcium phosphate precipitates were of concern when the American Food and Drug Administration reported two deaths in 1994, thought to be associated with calcium phosphate precipitates in an all-in-one PN admixture (Food and Drug Administration 1994).

1.10.2. Oxygen

Dissolved oxygen can originate from the component infusions, from the compounding process, from infusion delivery and from oxygen permeation through the plastic container. An example of an oxidative reaction is the degradation of ascorbic acid. The main cause of ascorbic acid loss in PN admixtures is due to reaction with dissolved oxygen. In the presence of dissolved oxygen and catalysed by copper, ascorbic acid is rapidly oxidised to dehydroascorbic acid (see Figure 1.11) (Allwood 1984a; Allwood et al. 1996; Gibbons et al. 2001).

PN admixtures can also peroxidise to potentially harmful peroxides by reaction with dissolved oxygen, in particular the lipid emulsions (Allwood et al. 1996; Helbock et al. 1993; Muhlebach and Steger 1998; Steger and Muhlebach

1997). PUFAs in lipid emulsions react with hydroxyl radicals, autocatalyse and finally producing lipid hydroperoxides (Figure 1.18).

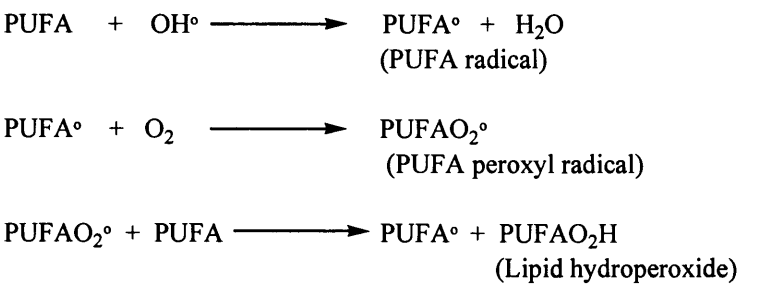


Figure 1.18: Lipid peroxidation (adapted from Muhlebach and Steger 1998)

Peroxides are toxic products and the maximum permissible peroxide values are given for some lipid emulsions by the European Pharmacopoiea. These peroxides diffuse into cells forming reactive species such as free radicals, causing tissue and cell damage. Potential toxicity of peroxides is high in patients with immature or compromised antioxidant system. Peroxides have been associated with hypoischaemic encephalopathy, bronchopulmonary dysplasia, retinopathy and necrotising enterocolitis in premature infants (Laborie et al. 1999; Pitkanen et al. 1991).

1.10.3. Light

Light is a form of electromagnetic radiation, when a molecule absorbs a photon in the UV-Visible spectrum, it becomes electronically excited and major photophysical events take place, as shown by the Jablonski diagram (Figure 1.19). The mechanism of photodegradation involves absorption of a photon by the vitamin molecule that proceeds to an electronically-excited state, and transformation into a photodegraded product. These events have been described in many references on the photostability of drugs (Moore 1987; Tonnesen 2004).

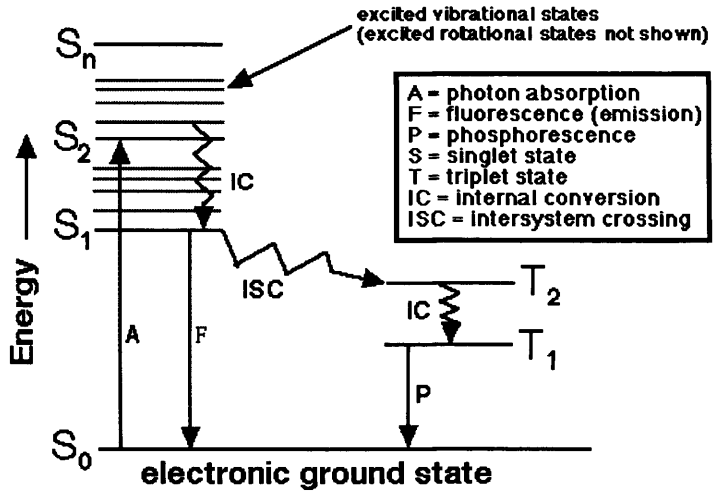


Figure 1.19: Jablonski diagram, adapted from Chasteen (1995)

Light exposure has also been reported to induce the generation of peroxides in PN admixtures (Lavoie et al. 1997; Neuzil et al. 1995; Silvers et al. 2001; Steger and Muhlebach 1997), the potential harm has been described previously.

It has been reported that lipid emulsions provide partial protection of vitamins to light, especially for Vitamin A, probably due to the opacity of the lipid emulsions (Shenkin 2003). Smith et al (1988) attributed the protection as being due to Vitamin A dissolving in the lipid emulsion.

Many micronutrients in PN are sensitive to light, the most sensitive are found in the vitamin group, in particular Vitamin A, Vitamin E, Vitamin K and riboflavin (Allwood 1982a; Allwood 2000a). These vitamins have been discussed individually above. Vitamin A is degraded by photolysis while Vitamin E is degraded by photooxidation (Allwood and Martin 2000). UV light has been implicated to be the main light source in the degradation of vitamin A (Allwood 1982a). In addition, many reviews have reported that vitamins are affected only by the ultraviolet component, and not by fluorescent light or other artificial light sources since they emit insignificant amount of UV light (Allwood 2000a; Allwood and Kearney 1998; Dahl et al. 1986).

The wavelength of UVA radiation is between 320 – 380 nm. Some multivitamin studies in PN use phototherapy light. The wavelength of phototherapy light is between 420 – 470 nm of the visible spectrum (Bhatia et al. 1980). Therefore, it should be noted that phototherapy light studies use a different set of light wavelengths under examination.

1.10.4. Other Influencing Factors

Vitamin losses may depend on the chemical composition of the amino acid solutions. For example, thiamine is degraded by the reducing agent bisulphite, present in certain amino acid infusions such as Freamine® (Kearney et al. 1995; Scheiner et al. 1981). Freamine®, however, is no longer available on the market.

Other factors include the type of plastic used (Balet et al. 2004; Henton and Merritt 1990; Lee et al. 1980) and the methods of preparation (Ball and Barnett 1996).

1.11. Problems Associated with Provision of Vitamins to PN Patients

Patients receiving PN require regular administration of vitamins and a number of trace elements. Once a PN is prepared it may be stored for a period of time before it is delivered to the patient. During storage, some vitamins are known to be degraded much quicker and, during PN administration, vitamins may also be degraded by effects of artificial light on the wards, or by sunlight, in particular the UV light (Allwood and Martin 2000).

Vitamins therefore are generally not considered to be stable enough for prolonged storage in PN solutions, and in general are advised to be added to PN shortly before use (Das Gupta et al. 1986).

1.12. Aims of Project

There are differing data on the stability of vitamins in PN admixtures because of the wide variety of PN compositions possible and also studies that have been carried out have different experimental regimes. For example, thiamine in PN electrolyte solution was found to be stable with no significant degradation observed at room temperature at 24 hours (Baumgartner et al. 1997), but in another study (Scheiner et al. 1981) the concentration of thiamine remaining at 24 hours was 89%.

Many vitamin stability studies carried out by other investigators looked at the ad-hoc stability in the recommended base regimen only, together with the other nutrient components, looking into factors such as the amino acid solutions used, as in the case for thiamine and ascorbic acid (Allwood 1982b; Kearney et al. 1998) and the presence of trace elements (Allwood 1982b).

In addition, generally, the water-soluble vitamins are recommended to be diluted in aqueous solutions while the fat-soluble vitamins are recommended to be diluted in lipid emulsions. So far, no studies have been conducted to look into the fat-soluble vitamins stability in an aqueous solution, nor the stability of the combined water-soluble and fat-soluble vitamins admixture in an aqueous solution.

In view of the above and of the fact that vitamins are recommended to be added shortly before use to PN, a simple minibag vitamin formulation which need not require reconstitution and further handling was considered for development. These multivitamin minibag formulations would be ready-to-use preparations which could be infused separately or together with the PN big bags containing macronutrients only, either pre-prepared from the triple chamber bags obtained commercially, or from those prepared centrally by the hospital pharmacy departments.

Therefore, the stability of water-soluble and fat-soluble multivitamins in 100 ml Freeflex® (Fresenius Kabi) minibags was examined. 0.9% sodium chloride (normal saline) was chosen as the aqueous base vehicle because it is a commonly used intravenous infusion with a relatively neutral pH between pH 5.35 to pH 5.40.

The aims of this thesis were:

1. To assess the dissolved oxygen content of the base vehicle in the Freeflex® minibag and the permeability of the minibag plastic container to oxygen.
2. To look into the stability of the water-soluble B vitamins (pyridoxine, nicotinamide, thiamine, folic acid and riboflavin sodium phosphate) contained in Solivito® N Adult injection (Fresenius Kabi), and the fat-soluble vitamins A and E contained in Vitlipid® N Adult (Fresenius Kabi) injection in an aqueous normal saline minibag formulation. In particular, to study the losses of these vitamins over time, storage temperatures and upon exposure to artificial fluorescent light.
3. To study the stability of these vitamins in minibag formulation upon simulation of a real clinical set-up, with lighting conditions mimicking hospital lighting conditions and general daylight UV exposure.
4. To study the stability of the vitamins under investigation upon delivery conditions and upon different protective covers used.

The results of these vitamin studies would be practically useful for use by ward patients and by home PN patients. These results would be useful too for tropical countries where PN solutions would be subjected to temperatures much higher than room temperature.

CHAPTER TWO

GENERAL METHODS

2.1. Introduction

This chapter describes the chemical assays of the vitamins analysed in the thesis project by High Performance Liquid Chromatography (HPLC). Nowadays, HPLC methods are increasingly used in the analysis of vitamins. HPLC methods have relatively high precision and accurate retention times and peak area measurement. Bakshi and Singh (2002) presented the steps towards the development of stability indicating assay methods for regulatory requirements and reported that 85-95% of the methods in the literature are by HPLC methods.

This chapter also describes the statistical analysis and the instruments used for the physical tests throughout the thesis.

2.2. Statistical Analysis

Microsoft Excel 2002 software for Windows® and SPSS statistical software for Windows® version 12 were used for data analysis. All results throughout the project were expressed as the mean ± standard deviation (sd) from triplicate samples, unless stated otherwise.

For all experimental chapters, differences between groups were analysed using repeated measures analysis of variances (ANOVA), and the Bonferroni test was used to determine where the differences lay if there was any statistical difference. A p value of <0.05 was considered to be statistically significant.

2.3. Chemical Assays by HPLC

HPLC uses the technique of separation of analytes, achieved by partitioning of compounds between a stationary phase and a mobile phase. The mobile phase acts as a carrier for the sample solution. When the sample solution is injected and flows through the column with the mobile phase, the solution resolves into its components, the degree of resolution being dependent upon the interaction between the component and the column and/or the stationary phase. Following separation, the peaks are monitored using detectors (Groves and Murty 1995; Snyder et al. 1997).

In developing the methods for separating these multivitamin injections into their component vitamins, the initial step was searching for previously published literature on vitamin assays by HPLC methods. Current official methods for vitamin assays, for example the BP and the United States Pharmacopoeia (USP) are for the determination of single component vitamins only.

Some of the literature revealed HPLC methods for determination of water-soluble vitamin component from plasma (Chamberlain 1985), in foods (Kall 2003), and pharmaceutical formulations (Amin and Reusch 1987; Holler et al. 2003; Ivanovic et al. 1999; King et al. 1998; Kothari and Taylor 1982; Sadlej-Sosnowska et al. 1986; Williams et al. 1973). Some of the literature that revealed HPLC methods for determination of fat-soluble vitamin components included determinations in serum (Mata-Granados et al. 2004), in pharmaceuticals (Allwood and Martin 2000; Billion-Rey et al. 1993; Doughty et al. 1996; Supelco 1999), in plasma (Sau Man Po et al. 1997), and in foods (Rizzolo and Polesello 1992).

Several methods were initially investigated, the methods that described the determination of multiple component vitamins in one assay, and using a similar HPLC system and column available in the laboratory were carried out.

With regards to determining the ultraviolet (UV) detection, for the water-soluble vitamins, the maximum ultraviolet (UV) absorbance of the individual vitamin standards was measured with a spectrophotometer (Model U-3300, Hitachi). The maximum absorbance for the vitamins was found to be in the ranges of 200-220 nm and 250-280 nm. The wavelength between 250-280 nm was preferred because most interferences will absorb much less above 240 nm (Snyder et al. 1997), therefore, the methods that used UV detection between 250-280 nm were carried out.

For the water-soluble vitamins, a simple method with good separation was adapted from Supelco® Application Note 148 (Supelco 2000) vitamin analysis method, which used a C18 column on gradient run, UV detection at 220 nm and using phosphate buffer 0.05 M pH7 and methanol as the mobile phase. The UV detection was changed to 265 nm in this method to avoid interference peaks.

For the fat-soluble vitamins, the UV absorbance maxima were obtained from referenced data. The UV absorbances found to be mostly used were 254 nm for vitamin K (Billion-Rey et al. 1993), 265 nm for ergocalciferol (United States Pharmacopeia Council of Experts 2002), 280 nm and 292 nm for vitamin E (Allwood and Martin 2000; Billion-Rey et al. 1993; Mata-Granados et al. 2004), and 325 nm for vitamin A (Allwood and Martin 2000). Therefore, different methods that use UV detection between 325nm and 265nm were carried out.

Modified methods performed by Allwood (1982a) and Allwood and Martin (2000) were adapted for the determination of the fat-soluble vitamins.

The following sections describe the HPLC methods for determining the component vitamins of the water-soluble multivitamin injection, Solivito® N Adult (Fresenius Kabi) (see 2.3.3), and the fat-soluble multivitamin injection, Vitlipid® N Adult (Fresenius Kabi) (see 2.3.4), obtained by single runs, the

reasons for selecting the vitamins analysed, and their validation and stability-indicating tests.

2.3.1. HPLC Instrument System

The HPLC Spectra System® from Thermo Finnigan (Thermo Electron Corporation, San Jose, California) was used in the vitamin analysis. The system consisted of SCM1000 vacuum membrane degasser, P2000 high pressure pump, AS 1000 autosampler, UV 1000 UV detector, and Chromquest™ Chromatography workstation. The column used was Varian® C18 stainless steel column, 150 mm x 3 mm ID, 5 um particle size, fitted with a ChromSep® C18 stainless steel guard column, 10 mm x 2 mm ID.

2.3.2. General Materials for HPLC

The materials and instruments used for HPLC analysis are as listed in Table 2.1.

Table 2.1: Instruments and materials used in HPLC analysis

Materials	*Supplier / Manufacturer*
Solivito® N Adult injections	Fresenius Kabi Limited, Birchwood, Warrington
Vitlipid® N Adult Injections	Fresenius Kabi Limited, Birchwood, Warrington
Water for Injections 500ml	Fresenius Kabi Limited, Birchwood, Warrington
0.9% Sodium Chloride Intravenous infusion BP in Freeflex® bags, 100ml	Fresenius Kabi Limited, Birchwood, Warrington
Barnstead® Nanopure Ultrafiltered deionised water, type 1	Barnstead Thermolyne Corporation, Dubuque, Iowa
diPotassium hydrogen orthophosphate trihydrate $K_2HPO_4.3H_2O$ (Analytical Reagent grade)	Fisher Scientific UK, Loughborough, Leicestershire
Orthophosphoric acid (Analytical Reagent grade)	Fisher Scientific UK, Loughborough, Leicestershire
Methanol (HPLC grade)	Fisher Scientific UK, Loughborough, Leicestershire
Absolute ethanol (Analytical Reagent grade)	Fisher Scientific UK, Loughborough, Leicestershire
Vitamins reference standards (USP and Analytical Reagent grades)	Sigma Chemical Co., St. Louis, Missouri

Materials	*Supplier / Manufacturer*
Autosampler 2.0 ml amber vials Autosampler PTFE/silicon vial seals	Fisher Scientific UK, Loughborough, Leicestershire
Sterile plastic syringes, disposable 10ml, 20ml, 50ml BD Plastipak®	Becton Dickinson, Drogheda, Ireland
Sterile disposable needles, 19G /21G BD Microlance®	Becton Dickinson, Drogheda, Ireland
Disposable pasteur pipettes, 3 ml Liquippettes®	Elkay Laboratory Products Ltd, Basingstoke, Hampshire
Microcentrifuge tubes with caps, 1.5 ml Fisherbrand®	Fisher Scientific UK, Loughborough, Leicestershire
Magnetic stirrer	Fisher Scientific UK, Loughborough, Leicestershire
Micropipettes 200 µL, 1000 µL	Fisher Scientific UK, Loughborough, Leicestershire
Weighing balance Sartorius® Model A02, RC2100	Sartorius AG, Goettingen, Germany
Ultra-low temperature freezer	Sanyo Electric Biomedical Co., Moriguci City, Osaka
Pharmaceutical refrigerator	Lec Refrigeration, Prescot, Merseyside
pH meter	Orion Research, Inc., Beverly, Massachusetts

2.3.3. The Water-soluble Vitamins

Solivito® N Adult is an injectable multivitamin, in the form of a yellow powder for reconstitution. It contains nicotinamide 40 mg, pyridoxine hydrochloride 4.9 mg (corresponds to 4 mg vitamin B_6), riboflavin sodium phosphate 4.9 mg (corresponds to 3.6 mg vitamin B_2), sodium ascorbate 113 mg (corresponds to 100 mg vitamin C), sodium pantothenate 16.5 mg (corresponds to 15 mg pantothenic acid), thiamine mononitrate 3.1 mg (corresponds to 2.5 mg vitamin B_1), biotin 60 micrograms, cyanocobalamin (vitamin B_{12}) 5 micrograms and folic acid 400 micrograms. The inactive ingredients consist of methyl parahydroxybenzoate 0.5 mg, glycine 300 mg and disodium edetate 0.5 mg.

2.3.3.1. Reference Standards for Water-soluble Vitamins Assay

The reference standards used are as listed in Table 2.2.

Table 2.2: Reference standards for water-soluble vitamins

Water-soluble Vitamins	*Grade*
l- Ascorbic acid A-2218	USP
p- Aminobenzoic acid A-9878	Analytical Reagent
d- Biotin B-4501	Analytical Reagent
Cyanocobalamin C-3607	USP
Folic acid F-7876	Analytical Reagent
Niacinamide (Nicotinamide) N-3376	Analytical Reagent
Calcium pantothenic acid P-2250	Analytical Reagent
Pyridoxine hydrochloride P-9755	Analytical Reagent
Riboflavin R-4500	Analytical Reagent
Sodium riboflavin 5-phosphate R-7774	Analytical Reagent
Thiamine hydrochloride T-4562	USP

2.3.3.2. HPLC Analysis for Water-soluble Vitamins Assay

All HPLC assays were carried out at ambient temperature. A reversed phase HPLC analysis with a gradient elution method was used. The mobile phase was a mixture of phosphate buffer 0.05 M pH 7.00 and methanol. The following gradient run was employed:

1% methanol from 1-5 minutes,

1% to 30% methanol from 5-20 minutes, and

30% methanol from 20-25 minutes.

An equilibration time of 5 minutes was set before the next sample run. The flow rate was set at 1 ml/min with UV detection at 265 nm. 20 µL sample was injected via the autosampler.

2.3.3.3. Preparation of Phosphate Buffer

The mobile phase consisted of methanol and phosphate buffer. Fresh solution of 0.05 M phosphate buffer was prepared by dissolving 0.05 moles $K_2HPO_4.3H_2O$ in ultrafiltered deionised water, stirred with a magnetic stirrer in a beaker. To make a 2 litre buffer solution, the amount of $K_2HPO_4.3H_2O$ (formula mass FM= 228.22) to be weighed was:

0.05 M x 228.22 x 2 = 22.82 g (±0.05 g)

The solution was adjusted to pH 7.00 ± 0.05 units with orthophosphoric acid, made up to volume in a 2 L volumetric flask and finally filtered under vacuum through a 0.2 μm polyamide filter.

2.3.3.4. Preparation of Water-soluble Vitamins Standard Solution

All preparations were carried out in diffused light. Stock solutions and standard solutions were protected from light to minimise photodegradation by covering the volumetic flasks with aluminium foil.

Stock standard solutions were prepared by dissolving in ultrafiltered deionised water in volumetric flasks, in each case an amount equivalent to about 20 times the content of the respective vitamins as in Solivito® N Adult solution. For folic acid which is only slightly soluble in water (0.016 mg/ml (O'Neil 2001)), about 0.2 ml NaOH 0.02% (0.05 M) solution was added to aid with its solubilisation.

For identification of the peaks, the stock solutions were further diluted to an amount equivalent to about twice the content of the respective vitamins as in Solivito N® solution. The standard solutions were analysed individually, as well as mixed with the Solivito N® solution and analysed by reversed phase HPLC.

2.3.3.5. Preparation of Water-soluble Vitamins Sample Solution

One vial of Solivito N® Adult was reconstituted with 10 ml ultrafiltered deionised water. 10 ml of the reconstituted solution was syringed out, transferred to a volumetric flask and made up to 100 ml with deionised water.

2.3.3.6. Identification, Calibration and Stability-Indicating Tests for Water-soluble Vitamins

The peaks were identified by injecting the standard solutions individually and by the spiking method, i.e. mixing the sample with the individual standards. To validate the method of analysis, calibration curves of each individual vitamin to be analysed and separated from the gradient run were determined. Five standard solutions at concentrations of 40, 60, 80, 100 and 120% of Solivito® N Adult solution were prepared and triplicate analyses were carried out at each concentration level.

Separate samples of Solivito N® solutions were also subjected to the following conditions to cause forced degradation, to examine if the degradation peaks interfered with the vitamin peaks:

a. with hydrochloric acid to pH of less than 2
b. with sodium hydroxide solution to pH 12 or higher
c. heated at 80°C in a water bath for 3 hours
d. exposed to sunlight, by placing samples in clear vials near a window sill
e. with hydrogen peroxide 37%

Sample solution was also kept in the freezer at -80°C for up to 132 nights to check its stability.

2.3.3.7. Results and Discussion for Water-soluble Vitamins Assay

The chromatograms of the Solivito N® solution are illustrated in Figures 2.1 and 2.2. The chromatogram peaks with the approximate retention times (rt) identified were:

Ascorbic acid	approximate rt of 0.76 minutes
Pyridoxine hydrochloride (P)	approximate rt of 3.7 minutes
Nicotinamide (N)	approximate rt of4.8 minutes

Thiamine hydrochloride (T)	approximate rt of 6.3 minutes
Folic acid (FA)	approximate rt of 11.1 minutes
Riboflavin sodium phosphate (RSP)	approximate rt of 12.4, 14.9, 16.9, 17.3, 20.5 minutes
Pantothenic acid	approximate rt of 22.47 minutes

From the chromatogram, 5 peaks were identified for riboflavin sodium phosphate (Figure 2.3), as confirmed by the chromatogram of its standard solution. These peaks may be the isomers of riboflavin phosphate, riboflavin diphosphate and that of free riboflavin but tests were not carried out to ascertain this. For the purpose and ease of reporting, the highest peak identified at about 17.3 minutes was used and further evaluated for method validation.

Ascorbic acid was eluted very early in the gradient run at less than one minute. It would have been thought that ascorbic acid would be eluted much later if the pH of the buffer was made acidic as ascorbic acid has a pKa of 4.2, therefore ascorbic acid would be in the unionised form. A test run using phosphate buffer of pH 4.5 instead of pH 7.0 however, showed similar retention time whereby the ascorbic acid was eluted at less than one minute.

Peaks for panthothenic acid and cyanocobalamin were also identified, where the peaks were detected after 20 minutes gradient run. The concentration of cyanocobalamin in the multivitamin solution was too minute, such that when run at the concentration found in the solution, there was hardly a noticeable peak. The biotin peak could not be identified in this gradient run.

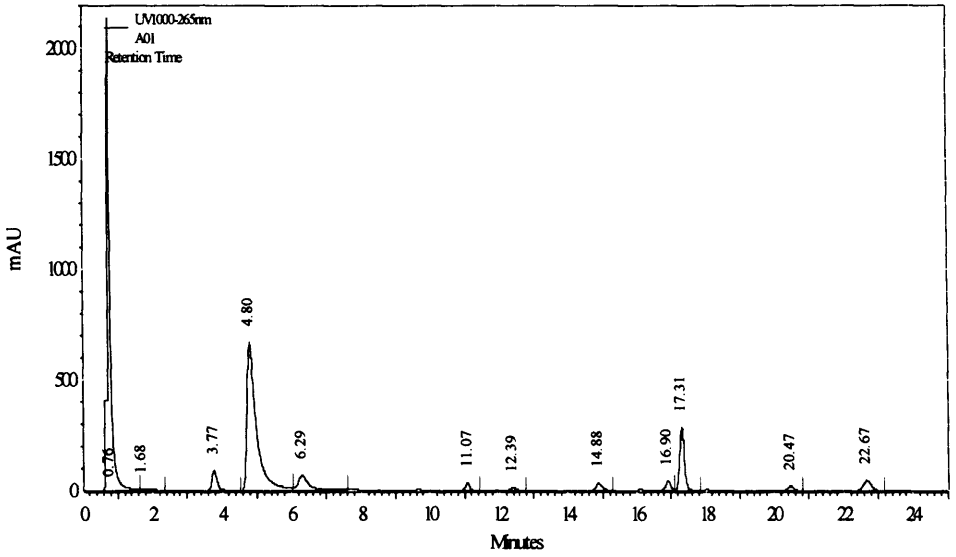


Figure 2.1: Chromatogram of Solivito® N Adult solution (full scale)

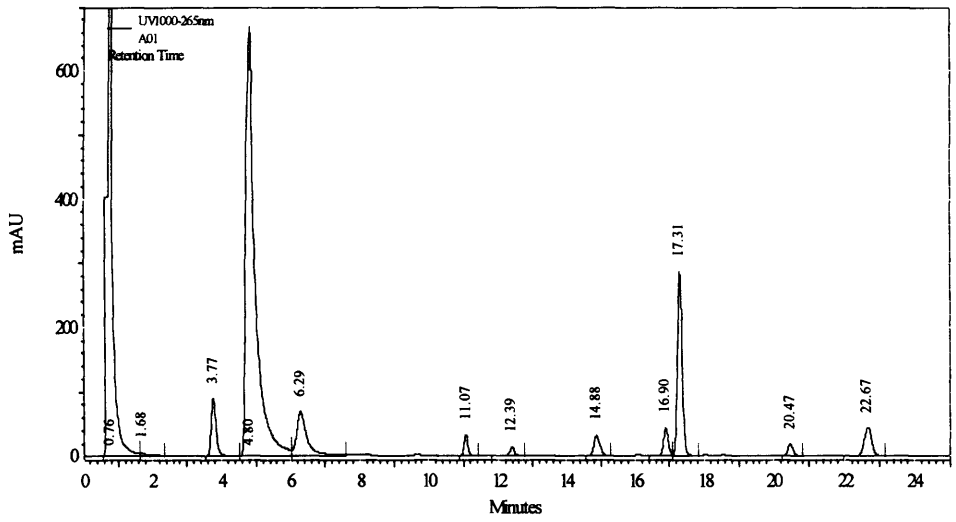


Figure 2.2: Chromatogram of Solivito® N Adult solution. Separation of pyridoxine (rt 3.77 min), nicotinamide (rt 4.80 min), thiamine (rt 6.29 min), folic acid (rt 11.07 min), and riboflavin sodium phosphate (rt 12.4 min, 14.9 min, 16.9 min, 17.31 min, 20.5 min) from Solivito® N Adult solution

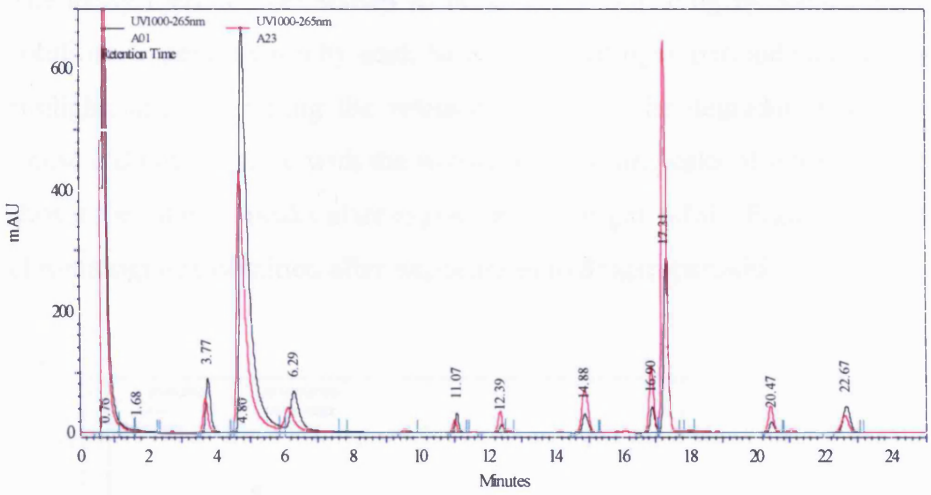


Figure 2.3: Chromatogram of Solivito N® solution (black) and riboflavin sodium phosphate standard solution with Solivito N® mixture (red)

There were slight variations in the retention times of the peaks identified depending on the temperature conditions and column life. However, the sequence of the peaks identified remained the same throughout every HPLC run.

Peaks of degraded products, such as those from exposure to sunlight (Figure 2.4), were found mostly at retention times of less than 2 minutes, therefore it was decided that this method would not be appropriate to analyse ascorbic acid as the peaks of the degraded products would interfere with the ascorbic acid peak. Peaks of degraded products were also detected after 20 minutes, therefore making this assay unsuitable for the analysis of pantothenic acid.

Therefore five vitamins were found to be suitable for assay by this method, which are pyridoxine, nicotinamide, thiamine, folic acid and riboflavin sodium phosphate. The vitamins chosen were based on convenience as they could be assayed in a single run, hence the ease of their analysis. These vitamins were further evaluated for validation and stability-indicating.

The assay method was shown to be stability-indicating by subjecting sample solutions to degradation by acid, base, heat, hydrogen peroxide and exposure to sunlight, and examining the retention times of the degradation components. These did not interfere with the rest of the vitamin peaks of interest. Figure 2.4 shows the vitamin peaks after exposure to sunlight, whilst Figure 2.5 shows the chromatograms obtained after exposure to hydrogen peroxide.

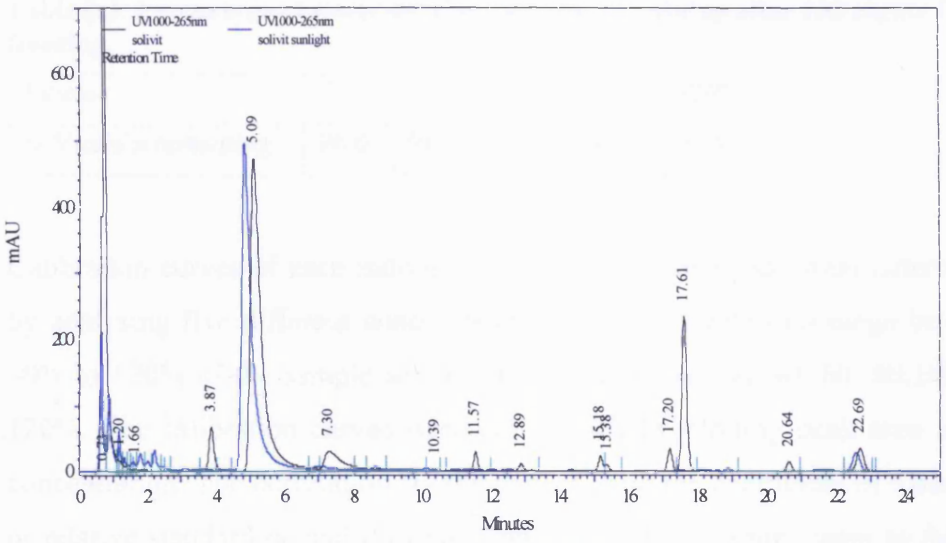


Figure 2.4: Chromatogram of Solivito® N Adult solution (black) and Solivito® N Adult solution exposed to sunlight (blue)

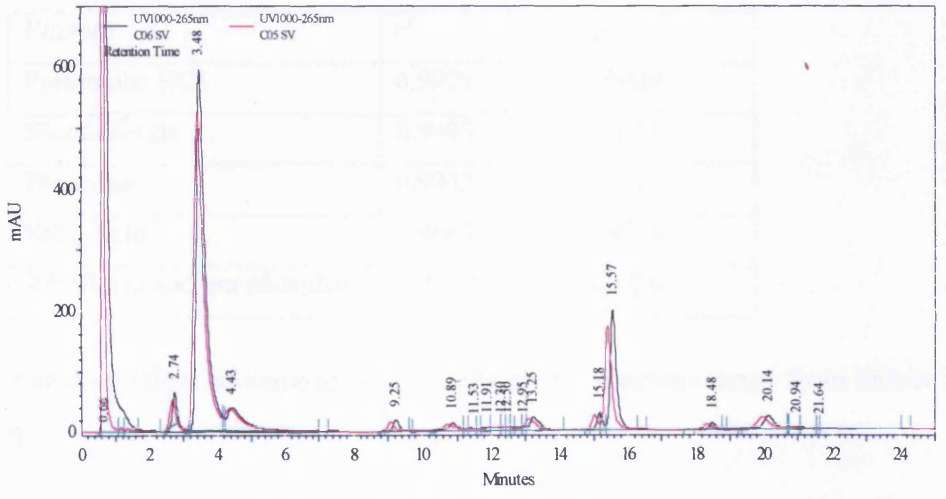


Figure 2.5: Chromatogram of Solivito® N Adult solution (black) and Solivito® N Adult solution degraded with hydrogen peroxide (magenta)

It was noted that there were variations in the retention times of the vitamin peaks of Figure 2.5 compared with those from Figure 2.4. This was due to the fact that the assay of the chromatogram in Figure 2.5 was carried out on a different column and at a different time period.

All the vitamins analysed were also stable when stored in the freezer, as shown in Table 2.3.

Table 2.3: Percentage of water-soluble vitamins remaining after 132 nights from freezing

Vitamin	*P*	*N*	*T*	*FA*	*RSP*
% Vitamin remaining	96.0	98.7	96.2	94.4	97.9

Calibration curves of each individual vitamin to be analysed were determined by analysing five different concentrations at the concentration range between 40% to 120% of the sample solution in triplicate, namely 40, 60, 80,100 and 120%. The calibration curves were constructed by plotting peak area versus concentration. The correlation coefficients (r^2) and the coefficient of variations or relative standard deviations (rsd) obtained for the vitamins were as follows (Table 2.4):

Table 2.4: Correlation coefficients (r^2) and relative standard deviations (rsd) of the water-soluble vitamin samples

Vitamin	r^2	*rsd*
Pyridoxine HCl	0.9929	0.0029
Nicotinamide	0.9993	0.0013
Thiamine	0.9942	0.0351
Folic Acid	0.9665	0.0024
Riboflavin sodium phosphate	0.9990	0.0029

The coefficient of variations for all the above vitamins range from below 1% to 4%.

The sample vial analysed was left in the autosampler overnight for 20 hours and reanalysed. The chromatograms were found to have similar peak areas of within ± 3% indicating stability during analysis.

The validated stability-indicating method described was able to determine five vitamins present in Solivito® N Adult injection diluted with 0.9% sodium chloride, namely pyridoxine, nicotinamide, thiamine, folic acid and riboflavin sodium phosphate, in a single run. A test run of this method was also carried out with Solivito® N Adult injection diluted with glucose 5% intravenous solution, and the test run showed that similar peaks were obtained. This method may be used for analysis of Solivito® N Adult when diluted in glucose 5% solution, if further tests on forced degradation were conducted to ensure that degradation peaks would not interfere with the vitamin peaks of interest.

2.3.4. The Fat-soluble Vitamins

Vitlipid® N Adult is a fat-soluble multivitamin injection. A 10 ml ampoule of Vitlipid® N Adult contains retinol palmitate 1940 µg, corresponding to vitamin A 990 µg (3300 IU), ergocalciferol 5 µg (200 IU), dl-alpha-tocopherol 9.1 mg (10 IU), and phytomenadione 150 µg. The inactive ingredients consist of fractionated soybean oil 1000 mg, fractionated egg phospholipids 120 mg and glycerol anhydrous 220 mg.

2.3.4.1. Reference Standards for Fat-soluble Vitamins Assay

The reference standards used are listed in Table 2.5.

Table 2.5: Reference standards for fat-soluble vitamins

Fat-soluble Vitamins	*Grade*
all trans-retinol palmitate (vitamin A) ~1,800,000 USP units/g R-1512	Analytical Reagent
Ergocalciferol (vitamin D_2) ~40,000,000 USP units/g E-5750	Analytical Reagent
dl-alpha- tocopherol ~95% HPLC T-3251	HPLC
Vitamin K_1 V-3501	Analytical Reagent

2.3.4.2. HPLC Analysis for Fat-soluble Vitamins Assay

A reversed phase HPLC analysis using two different UV detections in a single run was employed. The mobile phase was methanol HPLC grade, set at a flow rate of 1.5 ml/min. The wavelengths used were 292 nm from 0-7 minutes, for the detection of vitamins D, E and K, and wavelength 325 nm from 7-15 minutes for the detection of vitamin A. A 20 μL sample was injected via the autosampler.

2.3.4.3. Preparation of Fat-soluble Vitamins Standard Solutions

Stock and standard solution volumetric flasks and containers were protected from light by wrapping with aluminium foil and preparations were carried out in diffused light. A 100 ml stock standard solution of each vitamin equivalent to about 20 times the amount in one vial Vitlipid® N Adult was prepared. Vitamin A standard was mixed with about 50 ml absolute ethanol to aid with its dissolution, and then made up to 100 ml with methanol. Vitamin D and vitamin E were respectively mixed and made up to volume with methanol. The stock solutions were stored in the freezer at -80°C.

Freshly prepared standard solutions of concentrations equivalent to 40, 60, 80, 100, and 120% of the sample solution were prepared from the stock solutions in volumetric flasks and diluted with methanol.

2.3.4.4. Preparation of Fat-soluble Vitamins Sample Emulsion

10 ml of Vitlipid® N Adult injection was syringed out, transferred to a 100 ml flask and made up to volume with ultrafiltered deionised water.

2.3.4.5. Identification, Calibration and Stability-Indicating Tests for Fat-soluble Vitamins Assay

The sample emulsion was injected into the HPLC system to determine the chromatogram peaks. The peaks and retention times of vitamins A and E were identified by individually injecting the standard solutions equivalent to 100% of sample emulsion, and by spiking, as a mixture, with an equal amount of the standard solutions. Identification of Vitamin K and Vitamin D peaks was achieved by spiking with ten times the concentration of those in the sample emulsions, in order for the peaks to show as the concentrations in the original sample were too small.

Validation of the HPLC method was determined by running the vitamin assays in triplicate at five different concentrations equivalent to 40, 60, 80, 100 and 120% of the sample emulsion, and plotting calibration curves of area versus concentration.

Forced degradation of the sample emulsion was also carried out at conditions similar to those performed with the water-soluble vitamins (see 2.4.6.) to determine if the degradation products interfered with the peaks of interest.

2.3.4.6. Results and Discussion for Fat-soluble Vitamins Assay

A typical chromatogram of the sample Vitlipid® N Adult emulsion is shown in Figure 2.6. Peaks were observed at 2.07 minutes for Vitamin D, 2.37 minutes for Vitamin E, 6.09 minutes for Vitamin K and 8.82 minutes for Vitamin A.

At the time of determining the assay method for the fat-soluble vitamins, it was believed that the Vitamin K peak was too small with lack of sensitivity, to be clearly assayed in this HPLC run, therefore it was omitted in further analysis. With new computer software and expertise now available, this would allow the data to be looked at in the future.

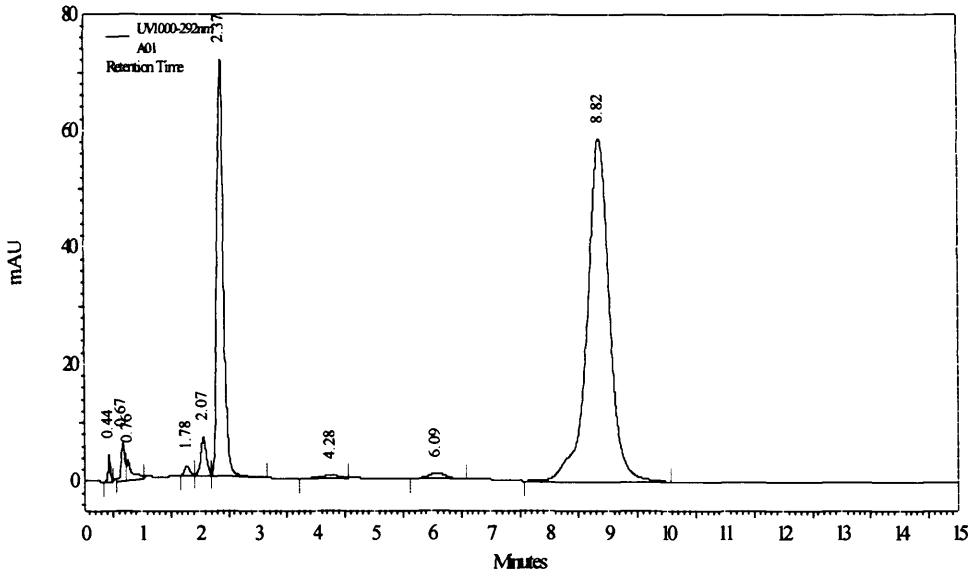


Figure 2.6: Typical chromatogram of Vitlipid® N Adult emulsion. Vitamin D peak at 2.07 min, vitamin E peak at 2.37 min and vitamin A peak at 8.82 min

Depending on the temperature condition and column life, a slight shift of the retention time was observed.

The relative standard deviations (rsd) and correlation coefficients (r^2) obtained from plots of the peak areas versus concentrations of the standards for the vitamins were as follows (Table 2.6):

Table 2.6: Correlation coefficients (r^2) and relative standard deviations (rsd) of the fat-soluble vitamin samples

Vitamin	r^2	*rsd*
Ergocalciferol (Vitamin D)	0.8036	0.0510
Tocopherol (Vitamin E)	0.9993	0.0121
Retinol palmitate (Vitamin A)	0.9933	0.0077

From the calibration curve, the r^2 of ergocalciferol was found to be less than 0.900, indicating less linearity of Vitamin D and therefore less precision, hence the decision not to analyse Vitamin D content. Furthermore, when analysing a sample of the known standard and comparing with similar concentration of the test sample, the peak of the standard was much lower than the test sample.

The chromatogram after forced degradation showed that decomposition products did not interfere with the vitamin peaks of interest. An example of the chromatogram after exposure to sunlight is shown in Figure 2.7.

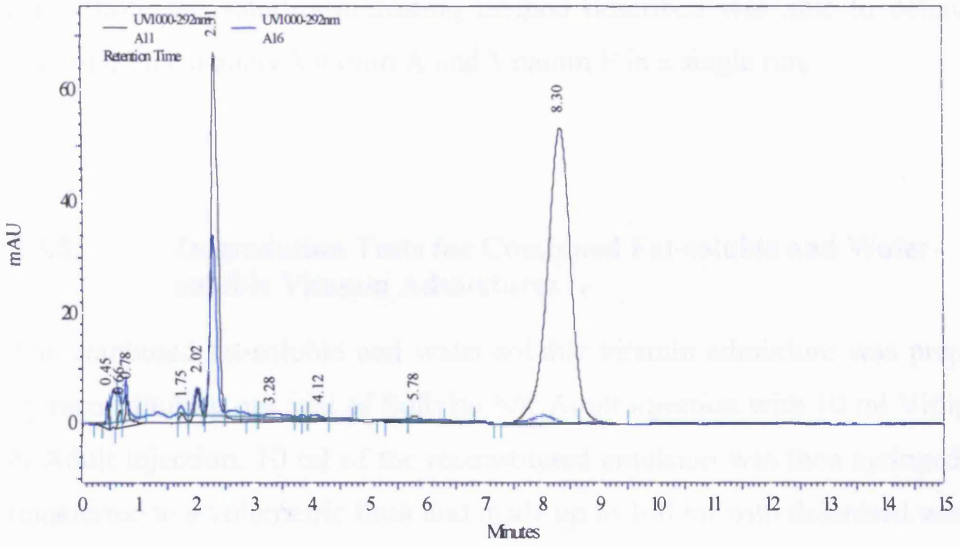


Figure 2.7: Chromatogram of Vitlipid® N Adult sample before (black) and after (blue) exposure to sunlight

In Figure 2.7, very large losses of Vitamins A and E were observed which were as expected as the sample was exposed to sunlight, but the degradation products did not interfere with the vitamin peaks.

Frozen samples were tested for up to 43 nights. Results of tests of frozen samples were found to be inconsistent as at times vitamins were shown to be stable but at other times were also found to have degraded to 72%, as shown in Table 2.7.

Table 2.7: Percentage of fat-soluble vitamins remaining after 43 nights from freezing

Vitamin	*Vitamin D*	*Vitamin E*	*Vitamin A*
% Vitamin remaining	75.3	74.7	72.0

Samples refrigerated for up to 3 nights and samples left in the autosampler for up to 22 hours showed losses of less than 2%. With these data gathered, it was

decided that test samples could be kept refrigerated for up to 3 days before HPLC analysis without deterioration and it was decided that analysis should be checked against a fresh sample.

For the fat-soluble Vitlipid® N Adult (Fresenius Kabi) vitamins assay, in conclusion, the stability-indicating method described was able to determine two vitamins, namely Vitamin A and Vitamin E in a single run.

2.3.5. Degradation Tests for Combined Fat-soluble and Water-soluble Vitamin Admixtures

The combined fat-soluble and water-soluble vitamin admixture was prepared by reconstituting one vial of Solivito N® Adult injection with 10 ml Vitlipid® N Adult injection. 10 ml of the reconstituted emulsion was then syringed out, transferred to a volumetric flask and made up to 100 ml with deionised water.

HPLC assays were carried out according to the water-soluble vitamins assay for the water-soluble vitamin components, and according to the fat-soluble vitamins assay for the fat-soluble vitamin components. Forced degradation of this emulsion sample was carried out at conditions similar to those performed with the water-soluble vitamins as described in 2.4.6.

Examples of chromatograms of the water-soluble and the fat-soluble vitamins in the combined admixture after forced degradation are shown in Figure 2.8 and Figure 2.9 respectively. The chromatograms after forced degradation did not show any decomposition products that would interfere with the peaks of interest.

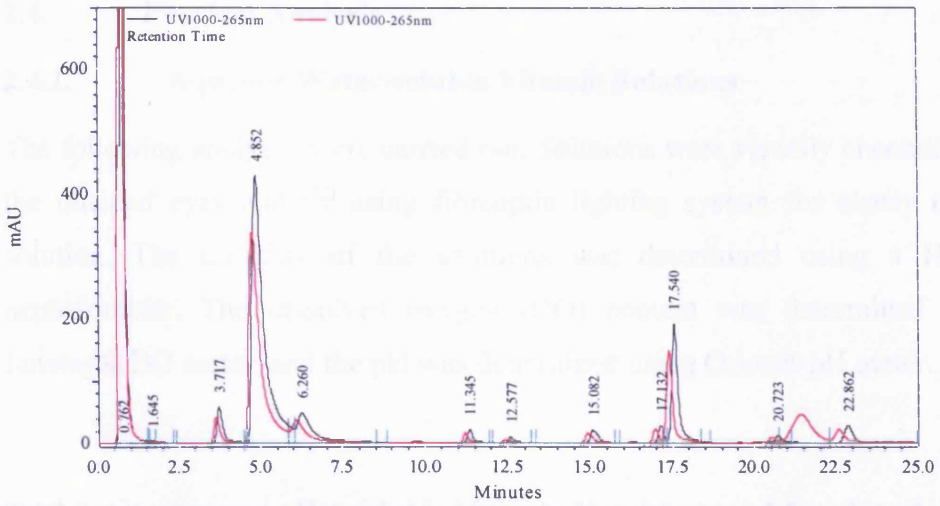


Figure 2.8: Chromatogram of a water-soluble vitamin analysis of combined Solivito® N Adult with Vitlipid® N Adult sample before (black) and after (red) exposure to sunlight

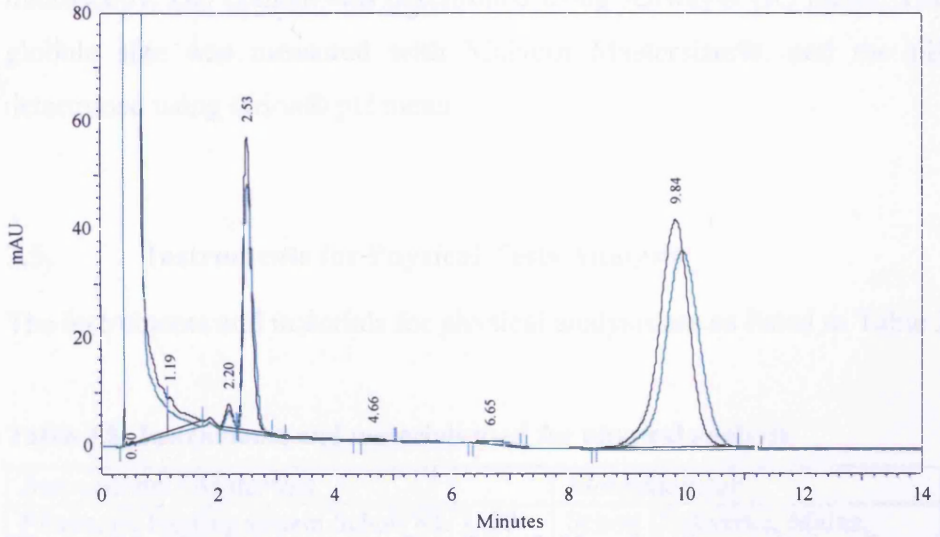


Figure 2.9: Chromatogram of a fat-soluble vitamin analysis of combined Solivito® N Adult and Vitlipid® N Adult sample (black) and sample degraded with sodium hydroxide (green).

2.4. Physical Analysis

2.4.1. Aqueous Water-soluble Vitamin Solutions

The following analyses were carried out. Solutions were visually checked with the unaided eyes and by using fibreoptic lighting system for clarity of the solution. The turbidity of the solutions was determined using a Hach® nephelometer. The dissolved oxygen (DO) content was determined using Jenway® DO meter, and the pH was determined using Orion® pH meter.

2.4.2. Aqueous Fat-soluble Vitamin Emulsions and Combined Water-soluble and Fat-soluble Vitamin Emulsions

The following analyses were carried out. Emulsions were visually checked with the unaided eye, by using fibreoptic lighting system and by light microscopy. DO content was determined using Jenway® DO meter. The lipid globule size was measured with Malvern Mastersizer®, and the pH was determined using Orion® pH meter.

2.5. Instruments for Physical Tests Analysis

The instruments and materials for physical analysis are as listed in Table 2.8.

Table 2.8: Instruments and materials used for physical analysis

Instruments / Materials	*Manufacturer*
Fibreoptic lighting system Schott KL 1500	Schott Glaswerke, Mainz, Germany
Hach Turbidimeter Model 43900 Ratio/XR	Hach Company, Ames, Iowa
Orion pH Meter Model 420A	Orion Research, Inc., Beverly, Massachusetts
Jenway Dissolved Oxygen meter model 9500	Jenway, Felsted, Dunmow, Essex
Microflow Laminar Flow cabinet, horizontal	Bioquell UK Limited, Andover, Hants
Microscope Olympus BH-2	Olympus Optical Co. Limited, Shinjuku-ku, Tokyo
Thoma welled slides and cover slips	Weber Scientific International Limited, Teddington, Middlesex

Instruments / Materials	*Manufacturer*
Weighing balance Sartorius Model A02	Sartorius AG, Goettingen, Germany
Malvern Mastersizer® *X*	Malvern Instruments Limited, Malvern, Worcestershire
Pharmaceutical Stability Chamber PSC 062	Sanyo Weiss Gallenkamp, Loughborough, Leicestershire
Pharmaceutical Refrigerator	Lec Refrigeration, Prescot, Merseyside
Magnetic stirrers	Fisher Scientific UK, Loughborough, Leicestershire
pH Buffers	Fisher Scientific UK, Loughborough, Leicestershire
Sodium sulphite anhydrous	Fisher Scientific UK, Loughborough, Leicestershire
Glassware	Fisher Scientific UK, Loughborough, Leicestershire

2.5.1. Nephelometer – Hach Turbidimeter

A nephelometer measures the intensity of light scattered at right angles to the incident light beam, this scattered light relates to the samples actual turbidity. Turbidity is the interaction between light and suspended particles in water, producing the Tyndall effect (European Pharmacopoeia Commission 2005). The higher the intensity of scattered light, the higher is the turbidity. A 90°-detection angle offers a system that is relatively free from stray light (Hach Company Manual 1991)

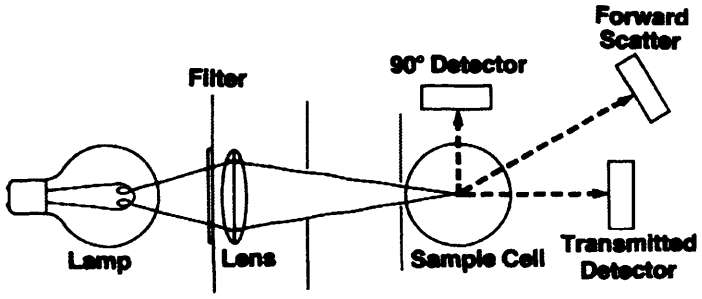


Figure 2.10: Schematic diagram of Hach® Ratio/XR nephelometer (Hach Manual 1991)

The nephelometer used is the Hach Ratio™/XR Turbidimeter (Figure 2.10) which can measure nephelometric turbidity unit (NTU) as low as 0.001 NTU to as high as 1999 NTU. The signal output measures the ratio between the scattered light at right angles and the weighted sum of forward-scattered light and transmitted light. It also compensates for absorption due to colour in the sample solution, changes in lamp output variation, or any film build-up on the Hach® tubes.

To measure turbidity with this meter, the glass Hach® tube was filled with 25 ml solution and coated with a thin film of silicone oil to mask any imperfections in the glass. The tube was then placed into the sample holder, with its index mark aligned with the mark on the spill ring around the holder opening, and covered with the light shield cap. The turbidity of the sample was measured from the digital display.

2.5.2. Dissolved Oxygen Meter

The DO meter measures the amount of oxygen dissolved in water. Jenway® DO meter uses the principle of the Clark oxygen electrode or polarographic oxygen sensor as illustrated in Figure 2.11.

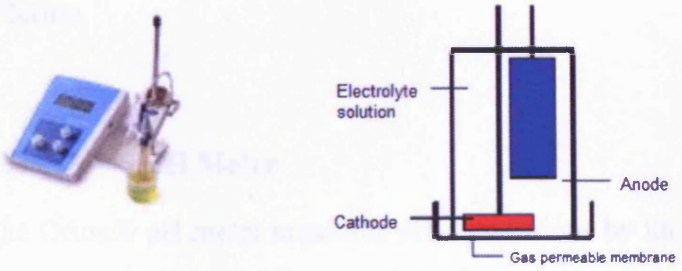


Figure 2.11: Jenway® DO meter and its schematic representation.

The meter consists of a probe with gold and silver electrodes supplied with a protective cap, a gas-permeable membrane and a 5% potassium chloride (KCl) electrolyte solution. The probe is connected to the membrane filled with KCl solution and is polarised at a constant voltage. Dissolved oxygen in the sample

diffuses through the membrane into the KCl solution and is reduced at the cathode. The resulting current flow is directly proportional to the amount of oxygen present, and the sensor converts the current measurement into concentration units. The oxidation-reduction reactions are as follows:

Reaction at cathode (oxygen is reduced):

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

Reaction at anode (silver is oxidised):

$$4Ag + 4Cl^- \rightarrow 4AgCl + 4e^-$$

Because diffusion gradients are set up across the membrane, a magnetic stirrer is needed to replenish the fluid across the membrane surface. This probe comes with a temperature compensating element and the DO meter can be adjusted for salinity.

To measure DO, the probe was immersed in the solution such that it covered the silver ring of the probe. The solution was stirred with a magnetic stirrer to avoid oxygen starvation at the membrane. Sufficient time was allowed when taking measurement to allow the reading to become stable. After each test, the probe was rinsed with ultrafiltered water. The probe was calibrated regularly using freshly prepared sodium sulphite 2% solution as the zero standard solution.

2.5.3. pH Meter

The Orion® pH meter measures pH of solutions by the glass electrode method, that is by measuring the potential difference between the glass and reference electrode. The pH meter was calibrated using either two of the three standard buffer solutions, namely phthalic acid buffer (pH 4.01), phosphate buffer (pH 7.00), and borate buffer (pH 10.01).

2.5.4. Fibre-optic Lighting System

The fibre-optic light uses halogen cold light source where the infrared component is removed by a filter, therefore at the point of light output, heat is not generated but yet it produces intensive illumination (Schott KL 1500 Instruction Manual).

For examination of the water-soluble vitamin samples using this light, the samples were placed above the light source and viewed against a dark background. This enhanced visualisation of haze and small particles (Trissel and Bready 1992). For examination of the fat-soluble vitamins, the samples were viewed against dark and white backgrounds with the light placed at approximately 45^{o} above the sample to enhance visualisation of creaming and oil globules.

2.5.5. Mastersizer® *X* Laser Diffraction Particle Size Analyser

The Malvern MasterSizer® *X* particle size analyser uses the laser diffraction technique which is based on the principle of laser light scattering. Laser diffraction technique relies on the fact that the diffraction angle is directly proportional to particle size. The instrument consists of a laser light, a detector, and a small volume sampler as a means of passing the sample through the laser light beam (Malvern Application Note).

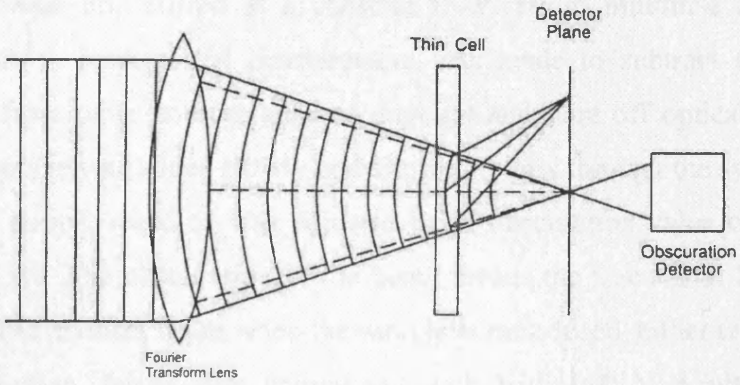


Figure 2.12: Reverse Fourier Optical Configuration (adapted from Malvern Reference Manual)

The Malvern MasterSizer® *X* employs reverse Fourier optics (Figure 2.12). For conventional Fourier optics configuration, particles from the sample cell scatter the collimated beam of laser light and through use of a Fourier transform lens, focus it on a detector. In the reverse Fourier optics configuration, the laser output beam, instead of forming a collimated laser beam, is focused to a detector plane. The sample cell is placed at a distance from the detector plane in the converging analyser beam. The advantage of the reverse Fourier optics configuration is that it allows measurement size range of samples dispersed in liquids to be extended down to 0.1 μm (Malvern Instruments Ltd 1993).

For analysis of the Vitlipid® N Adult in 0.9% sodium chloride sample, the Mastersizer® was set to polydisperse mode of analysis and "SOYA" presentation, which is the optical presentation optimised for soybean oil lipids, based on their refractive index and light absorption properties. The number of sweeps of the detector readings was set to 30,000.

For the measurement of lipid globules, they are measured by passing the laser beam through the emulsion samples dispersed in ultrapure deionised water. To

analyse the sample, the small volume sampler was filled with ultrapure deionised water and stirred at a constant flow rate to minimise air bubble introduction. A background measurement was made to subtract stray light picked up from other sources such as daylight and flare off optical surfaces. Then the sample was added slowly and allowed to mix through the system. The amount of sample required was adjusted to an obscuration value of between 19% and 21%. The obscuration of the beam means the fraction of light 'lost' from the incident laser beam when the sample is introduced, either to scattering or to absorption. Initial tests carried out with Vitlipid® N Adult emulsion resulted in about 3 ml sample being required to obtain this obscuration concentration. The particle size data of the measured sample was analysed using the Malvern Mastersizer® software version 2.19.

2.5.6. Light Microscope

A pre-calibrated light microscope (Olympus® BH-2) with a phase contrast attachment was employed to view the appearance and size of the lipid globules of the Vitlipid® N Adult vitamin samples. A phase-contrast optical system was selected to enable better visibility of the samples.

When counting under the microscope, slight refocusing was required occasionally to keep the globules in sharp focus. Air bubbles, which may pose a problem as they appear similar in shape and appearance to lipid globules, were distinguished from lipid globules by their halo rings surrounding them.

Each test sample was placed on a Thoma welled counting chamber slide having a height of 0.2 mm, and with gridlines having a total of 256 squares. The slide was covered with a disposable cover slip, care being taken not to introduce any air bubbles. Samples within the calibrated grid were viewed at a magnification of 200 times, one unit gridline of the scale being equivalent to 5 μm in size, and any globules size greater than 5 μm diameter were then recorded.

2.5.7. Pharmaceutical Stability Chamber

The pharmaceutical stability chamber Pharma-Safe PSC 062 (Sanyo Gallenkamp) meets the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) standards for stability and photostability (ICH Steering Committee 1996; 2003a). The cabinet provides uniform lighting conditions as well as uniform temperature and humidity, thus ensuring that samples are exposed in identical manner. The PSC 062 cabinet has two shelves with visible light sources and another two shelves with near ultraviolet (UV) light sources, placed alternately and spaced evenly, 24 cm apart. Each shelf has light sensor for measurement of the respective light exposure. The lux sensor measures visible light in Lux unit, while the UVA sensor measures UVA radiation between 320 – 380 nm from the UV lamp, with units in Watts per square metre (W/m^2) (PSC 062 Instruction Manual).

In all the studies conducted, unless otherwise specified, this stability chamber was used to subject all samples to test conditions. The stability chamber was set at 60% ± 5% relative humidity, while the temperature settings deviation was set at ± 2°C.

2.5.8. Laminar Flow Cabinet

This horizontal laminar-flow cabinet, Microflow® (Bioquell), is fitted with high efficiency particulate air (HEPA) filter with 99.97% efficiency, thus providing ultrafiltered air and allowing a clean air work environment.

All compounding works for the minibag vitamin formulations were performed in the laminar-flow cabinet throughout the entire project thesis. Aseptic technique was used during preparation and during sample taking to avoid bacterial contamination.

2.6. Conclusions

Validated stability-indicating assay methods were carried out to identify the vitamins that could be analysed in single runs for the water-soluble vitamins in Solivito® N Adult and the fat-soluble vitamins in Vitlipid® N Adult injections. These were found to be the vitamins pyridoxine, nicotinamide, thiamine, folic acid and riboflavin sodium phosphate for the water-soluble vitamins, and retinol palmitate (vitamin A) and tocopherol (vitamin E) for the fat-soluble vitamins. These vitamins to be analysed were based on the convenience of assay methods as they could be determined simultaneously in single runs. The other vitamins in the formulation such as ascorbic acid, Vitamin D and Vitamin K were not looked at in the following chapters.

The five water-soluble vitamins analysed were assayed simultaneously by reversed phase HPLC and gradient run method. The fat-soluble Vitamin A and Vitamin E were assayed by reversed phase HPLC assay method, and using two different wavelengths in a single run.

Both methods produced satisfactory results and had good separation of the peaks of interest. Forced degradation of the samples (Solivito® N Adult solution, Vitlipid® N Adult emulsion and the combined Solivito® N Adult and Vitlipid® N Adult emulsion) showed no interference of the degradation products with the peaks of interest. The methods developed for these vitamins were found to be repeatable and stability indicating.

CHAPTER THREE

DISSOLVED OXYGEN CONTENT ASSESSMENT

3.1. Introduction

The use of plastic delivery containers in parenteral formulations is widely increasing, they are lighter, unbreakable and more economical than glass bottles. However, a number of studies have shown that the phthalate plasticizers in the polyvinyl chloride (PVC) plastics pose problems of interactions such as sorption of drugs or leaching of chemicals from the PVC containers (Allwood 1986; Moorhatch and Chiou 1974; Pearson and Trissel 1993). Recent innovations have seen the development of plastic containers free from such plasticizers and able to withstand heat sterilisation (Lund 1994). One such innovation is the Freeflex® (Fresenius Kabi) plastic container.

For this project, Freeflex® plastic minibags were employed. The Freeflex® plastic bag is made from polyolefine, a polymer that is free from phthalate plastizers and PVC material, as well as free from adhesives or latex. It can also be heat sterilised (Freeflex® Technical Manual). A study by Henton and Merritt (1990) found increased recovery of vitamin A from polyolefin tubing than from PVC, indicating greater interaction of vitamin A with PVC than with polyolefin. Therefore, problems of interactions are greatly minimised with this Freeflex® bag.

One of the factors that can affect vitamins stability in PN formulations is the presence of air or dissolved oxygen (DO) in the solution. This study therefore aimed to assess the permeability of Freeflex® 100 ml minibags to oxygen, by assessing the DO content of the base system, in this case 0.9% sodium chloride. 100 ml capacity glass bottles were used as comparison. This study

also assessed the DO content when the containers were subjected to various storage conditions, to be used as baseline information.

3.2. Materials and Methods

The instruments and general methods for measurement of DO content are as described in Chapter Two.

Other materials used are as outlined in Table 3.1.

Table 3.1: Materials used for DO assessment

Materials	*Supplier/ Manufacturer*
Specimen glass tubes with the screw caps	Fisher Scientific UK, Loughborough, Leicestershire
0.9% Sodium Chloride (NaCl) Intravenous infusion BP, 100 ml, Freeflex®	Fresenius Kabi Limited, Birchwood, Warrington
Magnetic stirrers	Fisher Scientific UK, Loughborough, Leicestershire
Type 1 glass bottles, 100 ml, clear Flurotec injection stopper, 20 mm Aluminium caps, 20 mm	Adelphi (Tubes) Limited, Haywards Heath, West sussex
0.9% Sodium Chloride (NaCl) Intravenous infusion BP, 500 ml	Fresenius Kabi Limited, Birchwood, Warrington
Vial capping hand crimping tongs Vial decapping hand crimping tongs	Adelphi Manufacturing, Haywards Heath, West sussex
Needles 21G, sterile Syringe 10 ml, sterile	Becton Dickinson, Drogheda, Ireland

3.2.1. Experimental Design

3.2.1.1. Experiment 1

The containers were stored in the pharmaceutical stability chamber (PSC) for one week at 4, 25, 30 and 40°C, at a relative humidity of 60%. In addition, with the exception of the samples stored at 4°C, experimental solutions were also tested by exposing them to artificial fluorescent white light having an illuminance of 0.7 klux, this light intensity is equivalent to office and general hospital ward lighting conditions that have specifications of around 0.5 klux (Baker et al. 1993).

The DO content of the solutions was measured at 0, 48 and 168 hours.

3.2.1.2. Experiment 2

The containers were stored in the pharmaceutical refrigerator at 4°C for either 7 days or 30 days. This was followed by exposing the containers to either 25°C or 40°C in the PSC for the next 24 hours. The fluorescent white light was set to 1.2 klux for containers exposed to light, this light intensity is equivalent to hospital operating theatre lighting condition (Baker et al. 1993).

The DO content of the solutions was measured at 0 hour, on day 7 or day 30, and at 24 hours after subjection to exposure condition.

3.2.2. Sample Preparation

Using a sterile syringe and needle, 10 ml of air was added to 100 ml 0.9% sodium chloride in either 100 ml Freeflex® plastic bags or 100 ml glass bottles. 0.9% sodium chloride in 100 ml glass bottles were prepared by aseptically transferring 100 ml of 0.9% Sodium Chloride Intravenous Infusion from 500 ml Freeflex® bags into sterile 100 ml type 1 glass bottles and capped with sterile Flurotec® stoppers and aluminium seals. The solutions were then stored according to the experimental design as described above. Normal saline solutions without addition of air acted as controls. For Freeflex® bags, the outer overwraps were also removed.

3.2.3. Sampling

For DO analysis of normal saline from glass bottle, the bottle was decapped and about 25 ml sample solution was run down the side of a marked glass specimen tube, taking care to minimise air introduction. In the case of the Freeflex® bag, the addition port was snipped off and the solution was run down the side of the marked tube, care was taken not to introduce any air

bubbles. The glass specimen tube was immediately capped and DO measurement was carried out as early as possible. This handling technique was adapted from the work of Ball (1997).

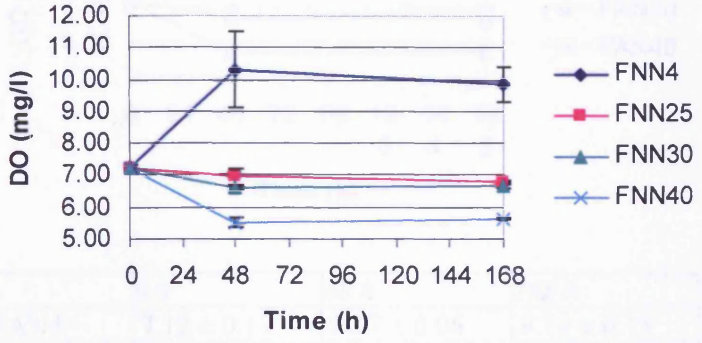
Containers wrapped in aluminium foil serve as the light protected containers. Separate bags were used for measurement at each time point. In each case, triplicate readings were taken from each sample solution and triplicate samples were prepared for each set of conditions.

3.3. Results

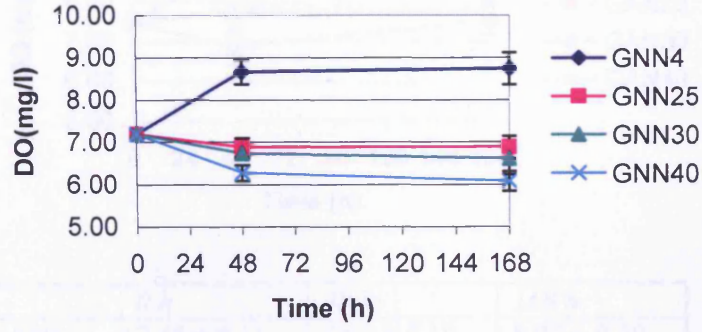
The following abbreviations are used to denote the type of containers and conditions for which the solutions are stored:

FNN4 (25/30/40)	Freeflex® bags, no air added, light protected, stored at 4°C (or at 25°C /30°C /40°C)
FAN4 (25/30/40)	Freeflex® bags, air added, light protected, stored at 4°C (or at 25°C /30°C /40°C)
FNL25 (30/40)	Freeflex® bags, no air added, light exposed, stored at 25°C (or at 30°C /40°C)
FAL25 (30/40)	Freeflex® bags, air added, light exposed, stored at 25°C (or at 30°C /40°C)
GNN4 (25/30/40)	glass bottles, no air added, light protected, stored at 4°C (or at 25°C /30°C /40°C)
GAN4 (25/30/40)	glass bottles, air added, light protected, stored at 4°C (or at 25°C /30°C /40°C)
GNL25 (30/40)	glass bottles, no air added, light exposed, stored at 25°C (or at 30°C /40°C)
GAL25 (30/40)	glass bottles, air added, light exposed, stored at 25°C (or at 30°C /40°C)

The results of the DO content for experiment 1 are shown from Figure 3.1, to Figure 3.4, while the results for experiment 2 are shown from Figure 3.5 to Figure 3.8.

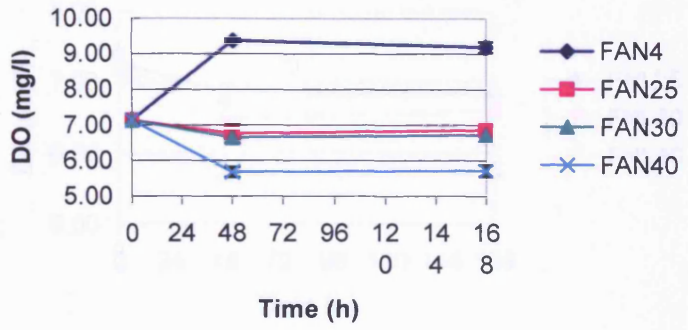


	0 h	48 h	168 h
FNN4	7.21 ± 0.12	10.29 ± 1.21	9.83 ± 0.56
FNN25	7.21 ± 0.12	6.99 ± 0.25	6.81 ± 0.05
FNN30	7.21 ± 0.12	6.57 ± 0.06	6.64 ± 0.06
FNN40	7.21 ± 0.12	5.51 ± 0.15	5.62 ± 0.03

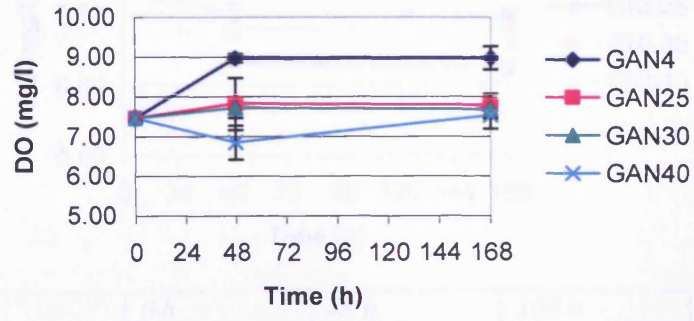


	0 h	48 h	168 h
GNN4	7.18 ± 0.10	8.65 ± 0.29	8.74 ± 0.38
GNN25	7.18 ± 0.10	6.88 ± 0.22	6.87 ± 0.26
GNN30	7.18 ± 0.10	6.73 ± 0.11	6.60 ± 0.37
GNN40	7.18 ± 0.10	6.27 ± 0.18	6.07 ± 0.23

Figure 3.1: Dissolved oxygen content of Freeflex® bags/ glass bottles, no air added, light protected, stored at 4°C/25°C/30°C/40°C (control)

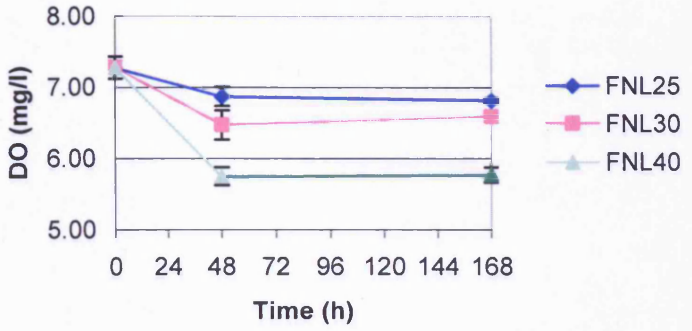


	0 h	*48 h*	*168 h*
FAN4	7.12 ± 0.17	9.37 ± 0.06	9.19 ± 0.15
FAN25	7.12 ± 0.17	6.75 ± 0.26	6.85 ± 0.18
FAN30	7.12 ± 0.17	6.63 ± 0.04	6.71 ± 0.18
FAN40	7.12 ± 0.17	5.68 ± 0.14	5.71 ± 0.16

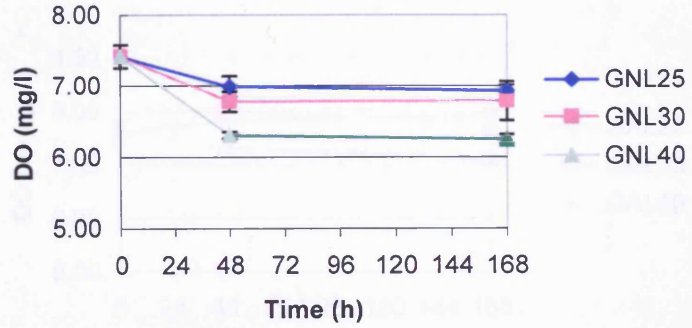


	0 h	*48 h*	*168 h*
GAN4	7.45 ± 0.11	8.97 ± 0.10	8.97 ± 0.29
GAN25	7.45 ± 0.11	7.82 ± 0.66	7.79 ± 0.28
GAN30	7.45 ± 0.11	7.71 ± 0.24	7.68 ± 0.23
GAN40	7.45 ± 0.11	6.84 ± 0.43	7.52 ± 0.33

Figure 3.2: Dissolved oxygen content of Freeflex® bags/ glass bottles, air added, light protected, stored at 4°C/25°C/30°C/40°C

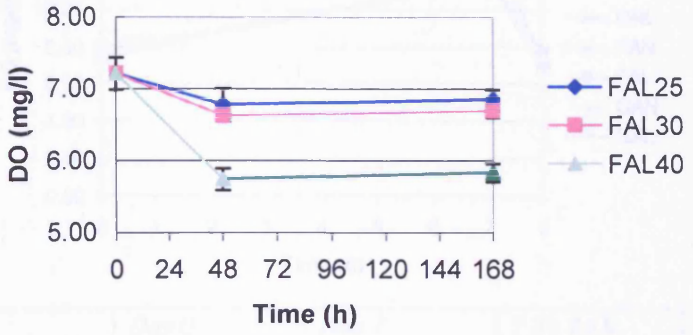


	0 h	*48 h*	*168 h*
FNL25	7.28 ± 0.16	6.87 ± 0.14	6.81 ± 0.04
FNL30	7.28 ± 0.16	6.47 ± 0.21	6.58 ± 0.02
FNL40	7.28 ± 0.16	5.75 ± 0.13	5.78 ± 0.10

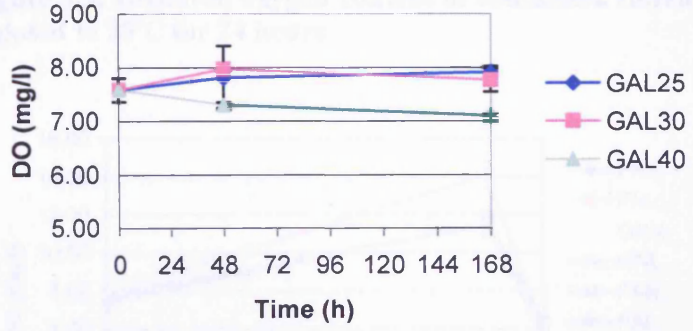


	0 h	*48 h*	*168 h*
GNL25	7.42 ± 0.16	7.00 ± 0.15	6.93 ± 0.08
GNL30	7.42 ± 0.16	6.78 ± 0.14	6.78 ± 0.28
GNL40	7.42 ± 0.16	6.31 ± 0.05	6.25 ± 0.07

Figure 3.3: Dissolved oxygen content of Freeflex® bags / glass bottles, no air added, light exposed, stored at 25°C/30°C/40°C

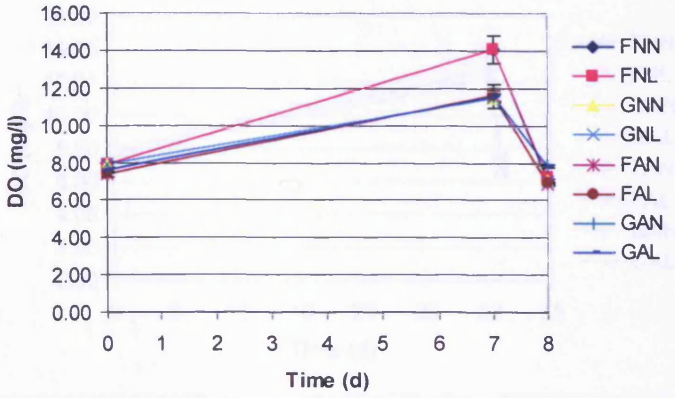


	0 h	*48 h*	*168 h*
FAL25	7.21 ± 0.22	6.78 ± 0.24	6.85 ± 0.13
FAL30	7.21 ± 0.22	6.62 ± 0.03	6.69 ± 0.09
FAL40	7.21 ± 0.22	5.74 ± 0.15	5.83 ± 0.12



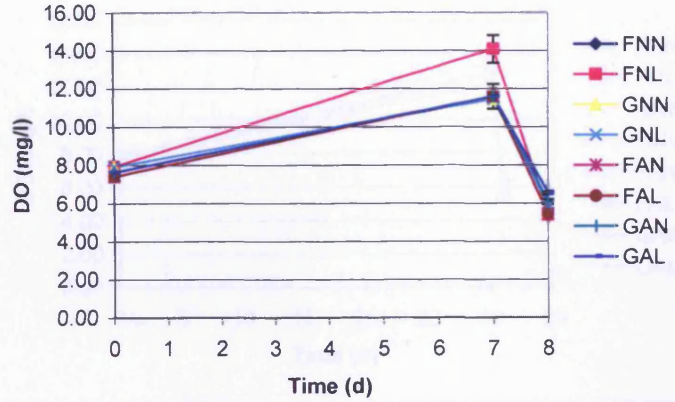
	0 h	*48 h*	*168 h*
GAL25	7.58 ± 0.22	7.80 ± 0.60	7.91 ± 0.12
GAL30	7.58 ± 0.22	7.96 ± 0.03	7.76 ± 0.22
GAL40	7.58 ± 0.22	7.30 ± 0.05	7.09 ± 0.05

Figure 3.4: Dissolved oxygen content of Freeflex® bags, air added, light exposed, stored at 25°C/30°C/40°C



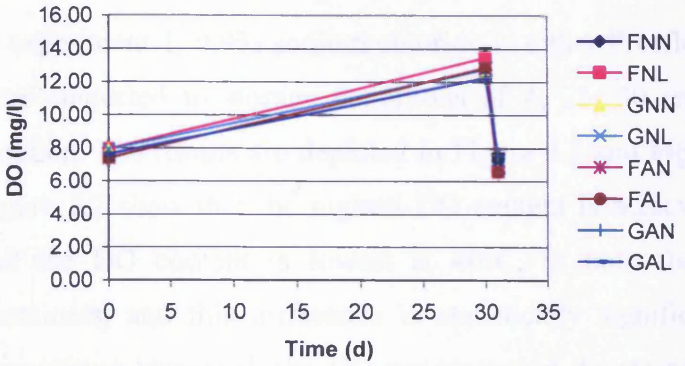
	Day 0	*Day 7*	*7 d+ 24 h*
FNN	7.90 ± 0.13	14.07 ± 0.74	7.04 ± 0.07
FNL	7.90 ± 0.13	14.07 ± 0.74	7.18 ± 0.08
GNN	7.87 ± 0.16	11.45 ± 0.20	7.06 ± 0.09
GNL	7.87 ± 0.16	11.45 ± 0.20	6.98 ± 0.17
FAN	7.38 ± 0.17	11.58 ± 0.66	6.89 ± 0.08
FAL	7.38 ± 0.17	11.58 ± 0.66	6.89 ± 0.08
GAN	7.60 ± 0.13	11.57 ± 0.27	7.82 ± 0.01
GAL	7.60 ± 0.13	11.57 ± 0.27	7.83 ± 0.19

Figure 3.5: Dissolved oxygen content of containers stored for 7 days at 4°C, then exposed to 25°C for 24 hours



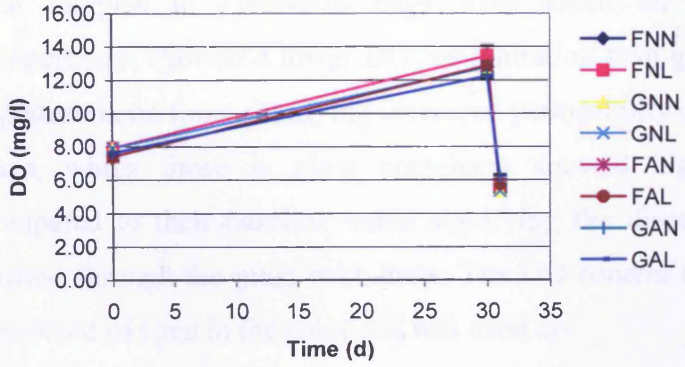
	Day 0	*Day 7*	*7 d+ 24 h*
FNN	7.90 ± 0.13	14.07 ± 0.74	5.45 ± 0.04
FNL	7.90 ± 0.13	14.07 ± 0.74	5.37 ± 0.10
GNN	7.87 ± 0.16	11.45 ± 0.20	5.50 ± 0.08
GNL	7.87 ± 0.16	11.45 ± 0.20	5.86 ± 0.09
FAN	7.38 ± 0.17	11.58 ± 0.66	5.41 ± 0.09
FAL	7.38 ± 0.17	11.58 ± 0.66	5.39 ± 0.10
GAN	7.60 ± 0.13	11.57 ± 0.27	6.08 ± 0.12
GAL	7.60 ± 0.13	11.57 ± 0.27	6.52 ± 0.12

Figure 3.6: Dissolved oxygen content of containers stored for 7 days at 4°C, then exposed to 40°C for 24 hours



	Day 0	*Day 30*	*30 d + 24 h*
FNN	7.90 ± 0.13	13.43 ± 0.69	7.24 ± 0.29
FNL	7.90 ± 0.13	13.43 ± 0.69	6.57 ± 0.14
GNN	7.87 ± 0.16	12.75 ± 0.48	6.65 ± 0.19
GNL	7.87 ± 0.16	12.75 ± 0.48	6.69 ± 0.03
FAN	7.38 ± 0.17	12.85 ± 0.38	6.59 ± 0.03
FAL	7.38 ± 0.17	12.85 ± 0.38	6.57 ± 0.05
GAN	7.60 ± 0.13	12.23 ± 0.19	7.59 ± 0.06
GAL	7.60 ± 0.13	12.23 ± 0.19	7.56 ± 0.15

Figure 3.7: Dissolved oxygen content of containers stored for 30 days at 4°C, then exposed to 25°C for 24 hours



	Day 0	*Day 30*	*30 d + 24 h*
FNN	7.90 ± 0.13	13.43 ± 0.69	5.71 ± 0.04
FNL	7.90 ± 0.13	13.43 ± 0.69	6.57 ± 0.04
GNN	7.87 ± 0.16	12.75 ± 0.48	6.65 ± 0.14
GNL	7.87 ± 0.16	12.75 ± 0.48	6.69 ± 0.04
FAN	7.38 ± 0.17	12.85 ± 0.38	6.59 ± 0.06
FAL	7.38 ± 0.17	12.85 ± 0.38	6.57 ± 0.15
GAN	7.60 ± 0.13	12.23 ± 0.19	7.59 ± 0.15
GAL	7.60 ± 0.13	12.23 ± 0.19	7.56 ± 0.13

Figure 3.8: Dissolved oxygen content of containers stored for 30 days at 4°C, then exposed to 40°C for 24 hours

3.4. Discussion

In experiment 1, 0.9% sodium chloride in either Freeflex® or glass containers were subjected to storage conditions of 4, 25, 30 and 40°C for 168 hours duration. The results are depicted in Figure 3.1 and Figure 3.2. Figure 3.1 and Figure 3.2 show that the highest DO content is achieved when stored at 4°C and the DO content is lowest at 40°C, in both the glass and Freeflex® containers, and this difference is statistically significant ($p<0.001$). As the temperature increased, the DO concentration decreased, this is comparable to the dissolved oxygen content of the base solution being dependent on temperature, with the oxygen solubility decreasing with higher temperatures. It is a known fact that the solubility of oxygen in a particular solvent depends on the temperature, pressure, and the nature of the solvent (Florence and Attwood 1998).

Comparison between Figure 3.1 and Figure 3.2 shows that with the addition of air, the graph patterns of the solutions upon subjecting to different temperatures are similar to those without air added. In addition, in Figure 3.2, it was found that samples in Freeflex® bags with added air and stored at higher temperatures showed a lower DO concentration than glass bottles and reached a plateau upon time, signifying increased permeability of air through the plastic bags, whilst those in glass containers showed higher DO concentration compared to their baseline value signifying the dissolved oxygen could not diffuse through the glass containers. The DO content reached a plateau as the dissolved oxygen in the solutions was used up.

In addition, for storage conditions of 25, 30 and 40°C, the containers were also exposed to fluorescent white light, and the results of the DO content upon the effects of light are depicted in Figure 3.3 and Figure 3.4. Figure 3.3 and Figure 3.4 show that exposing the samples to light did not have a significant effect on the dissolved oxygen content of the base solution.

It would be noted that only three sampling times were taken for the measurement of the dissolved oxygen, when marked changes of the DO readings were already noticeable at 48 hours. These sampling times were not changed to an earlier time in order to have better graphical illustrations of the DO content within 48 hours storage in the containers, as the aim was more of looking at the total DO content over the one week storage as baseline information for clinical practice.

In experiment 2, the samples were stored for a longer duration at 4°C, and all the samples showed a high DO content compared to 0 hour. Upon exposure to 25°C or 40°C, the DO content decreased, again signifying that the solubility of oxygen being dependent on temperature. Exposure to light did not have significant effect on the DO content. It is interesting to note that the DO content after storage for 7 days in the refrigerator from experiment 2 (Figure 3.5 and Figure 3.6) was much higher than from experiment 1 (Figure 3.1 and Figure 3.2). Possibilities are that in experiment 1, the containers were stored in the PSC whilst in experiment 2, they were stored in the pharmaceutical refrigerator which may result in the difference in DO content. Also, for experiment 2, there was less opening of the refrigerator door during experimental duration compared to that of experiment 1 when samples had to be taken to be tested.

In both these experiments, there was minimal air headspace in the containers which would be of a consistent size as the capacities of the containers are the same throughout the experiments. Saline solutions in the Freeflex® bags are prepared by an automated process, whereby the bags are dosed with sterile filtered air before in-line filling of the bags with sterile filtered saline solutions (Freeflex® Technical Manual). Preparation of the saline solutions in glass containers have been discussed under 3.2.2.

In hospital practice, variable amounts of air can be introduced into PN formulations during compounding (Ball and Barnett 1996). The problem with

oxidation is that it requires only a small amount of oxygen to initiate a chain reaction. Therefore, although it is helpful to keep the oxygen content of PN solutions at a minimum, it is not sufficient to prevent degradation from occurring. One method to obtain the minimum amount of free oxygen is to purge with inert gas such as nitrogen or helium. For plain water, Lachman et al (1986) recommended that it be first boiled and then purged with carbon dioxide or nitrogen gas.

Compounded PN bags may be wrapped with a gas-impermeable material, such as aluminium foil (Allwood et al. 1996). Another possible way to minimise oxygen transmission is by using low permeability multilayered bags (Balet et al. 2004), which are manufactured commercially. Parenteral preparations nowadays also have plastic overwraps to protect from moisture loss and some come with oxygen absorber sachets within the overwraps to minimise the amount of oxygen available to permeate the plastic bags. These commercial modalities improve the stability of the parenteral preparations and are increasingly used in PN formulations for hospital inpatients and for some home PN patients.

3.5. Conclusions

This study provides baseline information of DO content of normal saline in Freeflex® bags and glass bottles. The results confirm that the level of dissolved oxygen is dependent on temperature and that different types of packaging have different levels of permeability to air or oxygen. Therefore, when carrying out PN stability studies, monitoring the DO content of the formulations is essential to check on the effects of DO upon the stability of the vitamins.

CHAPTER FOUR

STABILITY OF WATER-SOLUBLE VITAMINS UPON DIFFERENT STORAGE CONDITIONS

4.1. Introduction

It has been shown that standardised PN formulation made from ready mixed formulae can have considerable advantages for PN patients (Mansell et al. 1989). These include a reduction in time and cost of preparation as well as ease of use by less experienced medical and pharmacy staff, thus reducing any potential errors. Thus, many pharmaceutical companies nowadays have introduced ready-made compartmentalized multichamber PN bags of longer shelf life, consisting of the three macronutrient solutions, which are glucose, amino acids and fats, with or without the electrolytes (Muhlebach 2005). These solutions can easily be mixed by opening the seals within the bag system. However, this PN bag is still incomplete because there are no vitamins and trace elements in the bag as these micronutrients limit the shelf life of the PN admixture.

In a hospital setting, these micronutrients are added to the bag shortly before use by trained staff, however for home patients, who may be on PN for life, this may pose some difficulties. Some vitamins need reconstitution before they can be added to the big bag, hence requiring a lot of manipulations by the home patients or their care-providers, besides having to add the vitamins to the bag themselves. Because of the potential market to home patients, a compounded micronutrient-based, vitamin minibag preparation is being looked into for possible development, where it can be infused together with the PN. In addition, this small volume minibag formulation would be an ideal ready-to-use product which does not require reconstitution or for the solution being drawn into a syringe, thus releasing home patients from making their own

additions. Throughout this thesis, 100 ml 0.9% sodium chloride (normal saline) and Freeflex® plastic containers were used as the base system.

In this chapter, a water-soluble multivitamin infusion in Freeflex® plastic minibag was formulated and assessed for physico-chemical stability parameters. The formulation consists of Solivito® N Adult multivitamin injection, prepared in 0.9% sodium chloride in 100 ml Freeflex® plastic bags. The abbreviation SV is given to this test solution throughout this thesis.

The aims of the study were:

a) To examine the physical stability of the multivitamin solutions stored for one week at different temperature conditions over varying time intervals as well as to examine the effects when exposed to artificial lighting conditions that are equivalent to general ward light exposure.

b) To determine the chemical stability of pyridoxine (P), nicotinamide (N), thiamine (T), folic acid (FA) and riboflavin sodium phosphate (RSP) in the multivitamin solutions when stored as outlined in (a) above. These vitamins were selected for analysis based on their identification by HPLC in a single gradient run as described in Chapter Two.

4.2. Methods

4.2.1. Experimental Design

The bags were stored and subjected to storage conditions as outlined under Experiment 1 of Chapter Three.

4.2.2. Preparation of Test Solutions SV

Each vial of Solivito® N Adult injection was reconstituted with 10 ml of Water for Injections, an additional vial was reconstituted as overage to make

allowance during transfer. These reconstituted solutions were then pooled to a 250 ml EVA Freka® IV-Bag (Fresenius Kabi) in order to minimise dilution error. 10 ml reconstituted Solivito® N Adult solution was then syringed out and added to the 100 ml sterile 0.9% sodium chloride in Freeflex® minibag. This test solution is given the abbreviation SV throughout this thesis.

Two bags were prepared for each set of conditions, one bag was for HPLC analysis and another bag for physical analysis. Sample bags wrapped in aluminium foil acted as dark Freeflex® bags. Bags wrapped in aluminium foil unexposed to light acted as controls. In each case, triplicate samples were prepared for the set of conditions. Therefore, 12 bags of SV were prepared for storage condition at 4°C while 24 bags of SV were each prepared for the other storage conditions.

4.2.3. Sampling

For physical analysis, 25 ml samples were withdrawn from the minibags using sterile 21G needles and 20 ml syringes, and placed in the Hach® glass tubes and capped. With the needle detached from the syringe, care was taken to run the sample from the syringe down the side of the tube to minimise air introduction during handling. The glass tubes were only uncapped when performing the DO measurement where the probe was required to be immersed in the solution. Samples were tested at times 0, 24, 48 and 168 hours. The tests were performed in the following order: firstly, the physical appearance by visual inspections followed by nephelometry test, DO measurement and lastly pH measurement. Visual inspections were performed with the unaided eye and by using the fibreoptic lighting system. The test instruments and their principles and methods of operation have been described in Chapter Two.

For chemical analysis, 3 ml samples were withdrawn via the additive port using sterile 21G needles and 5 ml syringes at 0 hour immediately after preparation, then at 3, 6, 24, 48, 72 and 168 hours and placed into two separate

microcentrifuge tubes. Samples were stored in the pharmaceutical freezer at -80°C before analysis and analysed using a validated HPLC stability-indicating method as described in Chapter Two. The peak areas of all the samples at 0 hour were averaged and the content analysis of these samples was regarded as the baseline content of 100%.

4.3. Results

The following abbreviations are used to denote the type of containers and conditions for which the multivitamin solutions are stored. The numerals in parentheses denote the different temperature settings used for each group of experiment.

FF4 (25/30/40)	in clear Freeflex® minibags, stored at 4°C (or at 25°C/30°C/40°C)
FPL4 (25/30/40)	in dark Freeflex® minibags, stored at 4°C (or at 25°C/30°C/40°C)
FFW25 (30/40)	in clear Freeflex® minibags, stored at 25°C (or at 30°C/40°C), exposed to light
FPLW25 (30/40)	in dark Freeflex® minibags, stored at 25°C (or at 30°C/40°C), exposed to light

4.3.1. Physical Analysis

4.3.1.1. Colour and Appearance

All solutions wrapped in aluminium foil and solutions in clear bags protected from light had no change in colour from the initial light yellow colour. When viewed with the fibreoptic light, the solutions had a light green haze appearance.

Exposure of the clear bags to fluorescent white light turned the solutions to pale yellow, and upon increasing temperature, the haze became less intense at 30°C and disappeared at 40°C.

4.3.1.2. Turbidity

The results of the turbidity measurement of SV solutions at different storage conditions are tabulated in Table 4.1.

Table 4.1: Mean turbidity measurement in NTU of SV at different storage conditions

Storage condition	*0 h (n=12)*	*24 h*	*48 h*	*168 h*
FF4	5.10 ± 0.26#	5.17 ± 0.40	5.09 ± 0.15	5.14 ± 0.18
FPL4	5.10 ± 0.26#	5.21 ± 0.37	5.15 ± 0.31	5.16 ± 0.13
FF25	4.58 ± 0.45	4.51 ± 0.47	4.56 ± 0.43	4.68 ± 0.14
FFW25	4.58 ± 0.45	4.47 ± 0.37	4.19 ± 0.26	0.22 ± 0.01 *
FPLW25	4.58 ± 0.45	4.50 ± 0.54	4.46 ± 0.57	4.65 ± 0.03
FPL25	4.58 ± 0.45	4.54 ± 0.43	4.54 ± 0.37	4.72 ± 0.11
FF30	4.89 ± 0.37	4.85 ± 0.04	4.89 ± 0.02	4.63 ± 0.03
FFW30	4.89 ± 0.37	4.90 ± 0.14	3.76 ± 0.16	0.17 ± 0.05 *
FPLW30	4.89 ± 0.37	4.47 ± 0.64	4.38 ± 0.60	4.05 ± 0.66 *
FPL30	4.89 ± 0.37	4.98 ± 0.13	4.89 ± 0.04	4.68 ± 0.04
FF40	5.01 ± 0.07	4.81 ± 0.11	4.65 ± 0.15	4.08 ± 0.12 *
FFW40	5.01 ± 0.07	4.33 ± 0.02	2.30 ± 0.97	0.17 ± 0.01 *
FPLW40	5.01 ± 0.07	4.88 ± 0.08	4.69 ± 0.11	4.21 ± 0.09 *
FPL40	5.01 ± 0.07	4.76 ± 0.02	4.62 ± 0.06	4.03 ± 0.05 *

#: n=6

*: change in NTU of more than 0.5 units

The results showed that test solutions exposed to light and those stored at 40°C had NTU change of more than 0.5 units (marked with asterisks *).

4.3.1.3. Dissolved Oxygen

The mean DO measurements of SV solutions are tabulated in Table 4.2.

Table 4. 2: Mean DO measurement (mg/l) of SV solutions at different storage conditions

Storage condition	*0 h (n=12)*	*24 h*	*48 h*	*168 h*
FF4	2.52 ± 1.56#	5.58 ± 0.71	5.26 ± 1.27	5.37 ± 0.58
FPL4	2.52 ± 1.56#	6.42 ± 0.14	5.56 ± 1.02	5.55 ± 0.47
FF25	2.58 ± 1.05	3.19 ± 0.19	2.88 ± 0.60	3.59 ± 0.49
FFW25	2.58 ± 1.05	1.10 ± 0.12	2.06 ± 0.81	6.12 ± 0.23
FPLW25	2.58 ± 1.05	2.08 ± 0.75	1.98 ± 0.99	3.85 ± 0.32
FPL25	2.58 ± 1.05	3.3 ± 0.30	3.22 ± 0.53	3.99 ± 0.59
FF30	2.25 ± 1.34	1.90 ± 1.11	2.36 ± 1.24	1.40 ± 1.70
FFW30	2.25 ± 1.34	1.10 ± 0.34	3.30 ± 0.11	6.37 ± 0.07
FPLW30	2.25 ± 1.34	1.04 ± 0.99	1.62 ± 1.68	1.56 ± 2.08
FPL30	2.25 ± 1.34	2.30 ± 0.80	3.07 ± 0.33	1.61 ± 2.14
FF40	2.87 ± 0.70	2.59 ± 0.10	2.53 ± 1.07	3.84 ± 0.55
FFW40	2.87 ± 0.70	1.14 ± 0.10	4.56 ± 0.44	5.65 ± 0.14
FPLW40	2.87 ± 0.70	1.89 ± 0.57	2.34 ± 1.09	4.30 ± 0.16
FPL40	2.87 ± 0.70	2.44 ± 0.36	2.73 ± 1.07	4.30 ± 0.12

#: n=6

The above results are illustrated by the following graphs from Figure 4.1 to Figure 4.4:

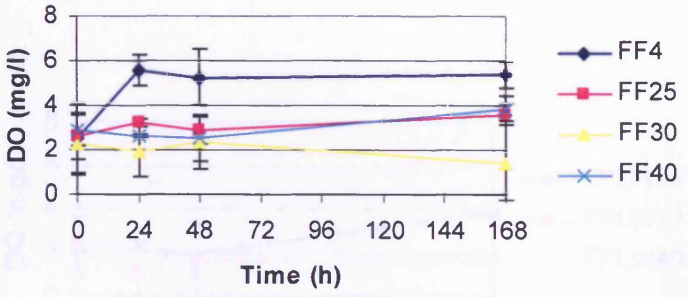


Figure 4.1: DO content of SV in clear minibags stored at different temperatures

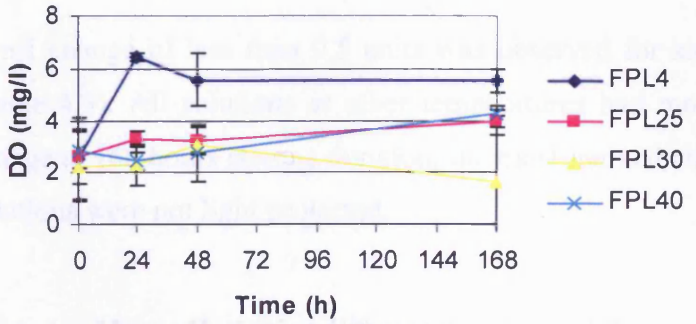


Figure 4.2: DO content of SV in dark minibags stored at different temperatures

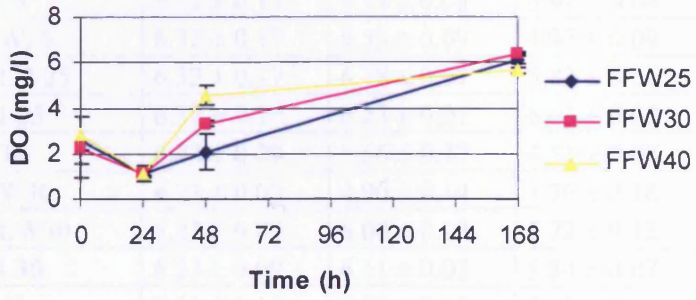


Figure 4.3: DO content of SV in clear minibags exposed to artificial light and stored at different temperatures

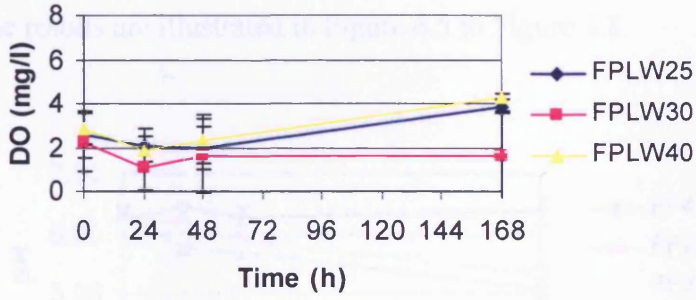


Figure 4.4: DO content of SV in dark minibags exposed to artificial light and stored at different temperatures

4.3.1.4. pH

A pH change of less than 0.5 units was observed for solutions stored at 4°C (Table 4.3). All solutions at other temperatures had more than 0.5 units pH change at 168 hours storage duration, the most marked changes were when the solutions were not light protected.

Table 4.3: Mean pH of SV at different storage conditions.

Storage condition	*0 h (n=12)*	*24 h*	*48 h*	*168 h*
FF4	6.23 ± 0.05#	6.44 ± 0.06	6.30 ± 0.14	6.37 ± 0.20 *
FPL4	6.23 ± 0.05#	6.32 ± 0.06	6.41 ± 0.07	6.39 ± 0.09 *
FF25	6.32 ± 0.17	6.14 ± 0.08	5.91 ± 0.04	5.34 ± 0.08
FFW25	6.32 ± 0.17	5.53 ± 0.09	4.95 ± 0.09	4.10 ± 0.04
FPLW25	6.32 ± 0.17	6.18 ± 0.07	5.82 ± 0.06	5.08 ± 0.09
FPL25	6.32 ± 0.17	6.23 ± 0.01	6.03 ± 0.08	5.44 ± 0.04
FF30	6.23 ± 0.09	5.56 ± 0.47	4.51 ± 0.04	4.17 ± 0.03
FFW30	6.23 ± 0.09	4.90 ± 0.14	3.76 ± 0.16	0.17 ± 0.05
FPLW30	6.23 ± 0.09	6.00 ± 0.13	5.72 ± 0.12	5.19 ± 0.09
FPL30	6.23 ± 0.09	6.11 ± 0.02	5.84 ± 0.07	5.45 ± 0.04
FF40	6.31 ± 0.14	5.75 ± 0.08	5.69 ± 0.08	5.06 ± 0.11
FFW40	6.31 ± 0.14	5.02 ± 0.06	4.37 ± 0.04	4.27 ± 0.06
FPLW40	6.31 ± 0.14	5.75 ± 0.11	5.55 ± 0.12	4.90 ± 0.10
FPL40	6.31 ± 0.14	5.79 ± 0.14	5.65 ± 0.06	5.11 ± 0.10

#:n= 6

*: change in pH of *less than* 0.5 units

The results are illustrated in Figure 4.5 to Figure 4.8.

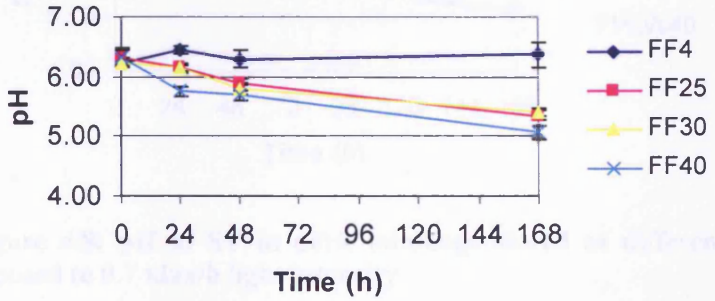


Figure 4.5: pH of SV in clear minibags at different storage temperatures

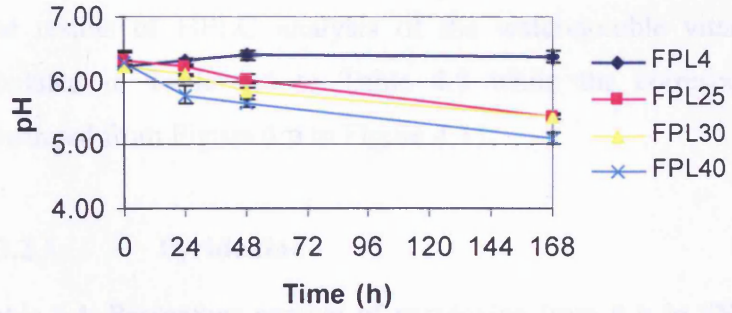


Figure 4.6: pH of SV in dark minibags at different storage temperatures

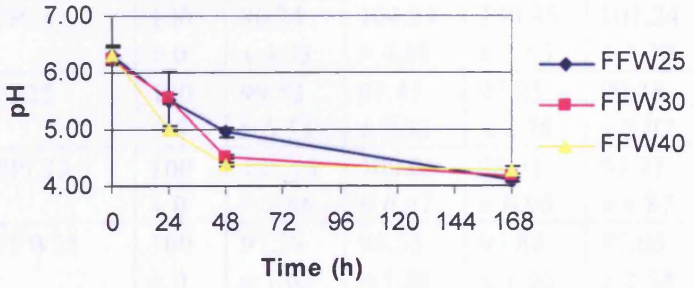


Figure 4.7: pH of SV in clear minibags stored at different temperatures and exposed to 0.7 klux/h light intensity

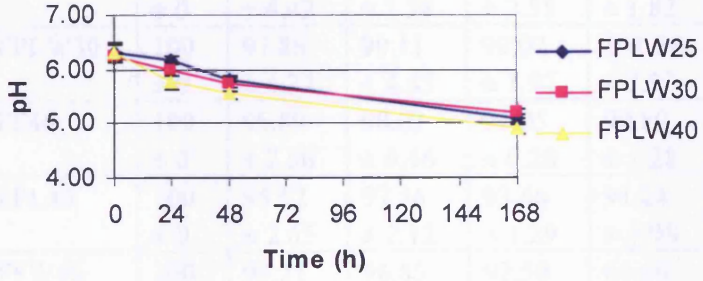


Figure 4.8: pH of SV in dark minibags stored at different temperatures and exposed to 0.7 klux/h light intensity

4.3.2. HPLC Analysis

The results of HPLC analysis of the water-soluble vitamins analysed are tabulated in Table 4.4 to Table 4.8 while the corresponding graphs are illustrated from Figure 4.9 to Figure 4.13.

4.3.2.1. Pyridoxine

Table 4.4: Percentage content of pyridoxine from 0 h in SV stored at different storage conditions

Storage condition	*0 h*	*3 h*	*6 h*	*24 h*	*48 h*	*72 h*	*168 h*
PFF4	100 ± 0	101.65 ± 2.57	103.84 ± 4.28	101.69 ± 2.98	99.43 ± 2.88	99.59 ± 1.59	99.11 ± 2.72
PFPL4	100 ± 0	96.24 ± 4.33	104.23 ± 4.88	100.95 ± 4.55	101.24 ± 3.32	99.16 ± 4.74	98.54 ± 4.76
PFF25	100 ± 0	99.53 ± 5.53	97.45 ± 2.32	97.25 ± 2.78	99.38 ± 3.07	95.47 ± 3.99	92.33 ± 3.83
PFPL25	100 ± 0	101.15 ± 1.86	100.00 ± 6.47	98.41 ± 6.96	97.77 ± 4.87	97.04 ± 6.25	97.31 ± 5.63
PFFW25	100 ± 0	97.29 ± 1.62	95.55 ± 1.88	95.80 ± 1.96	85.85 ± 2.58	82.27 ± 3.41	nd nd
PFPLW25	100 ± 0	98.60 ± 2.15	97.02 ± 1.27	95.28 ± 2.02	94.67 ± 1.56	92.73 ± 3.13	90.88 ± 1.11
PFF30	100 ± 0	104.29 ± 4.32	100.62 ± 2.60	101.24 ± 4.68	100.32 ± 3.31	100.54 ± 4.29	96.91 ± 5.00
PFPL30	100 ± 0	100.34 ± 1.55	103.67 ± 0.31	97.27 ± 3.13	98.61 ± 3.12	100.51 ± 2.63	99.35 ± 2.05
PFFW30	100 ± 0	96.24 ± 4.42	100.43 ± 2.38	96.21 ± 2.55	87.81 ± 1.82	77.90 ± 4.93	nd nd
PFPLW30	100 ± 0	97.86 ± 4.27	99.11 ± 4.45	99.02 ± 1.97	101.34 ± 3.93	95.76 ± 5.33	96.01 ± 2.25
PFF40	100 ± 0	95.89 ± 2.56	98.03 ± 0.46	94.05 ± 0.20	90.80 ± 1.28	88.55 ± 1.25	86.27 ± 2.29
PFPL40	100 ± 0	95.52 ± 2.65	97.56 ± 2.12	93.66 ± 1.29	91.24 ± 1.98	89.44 ± 1.11	88.90 ± 1.63
PFFW40	100 ± 0	99.21 ± 2.70	96.85 ± 0.43	92.50 ± 2.24	64.66 ± 2.78	nd nd	nd nd
PFPLW40	100 ± 0	97.69 ± 0.92	96.44 ± 2.33	93.05 ± 2.37	91.16 ± 1.51	89.60 ± 3.70	88.94 ± 1.25

nd: not detected

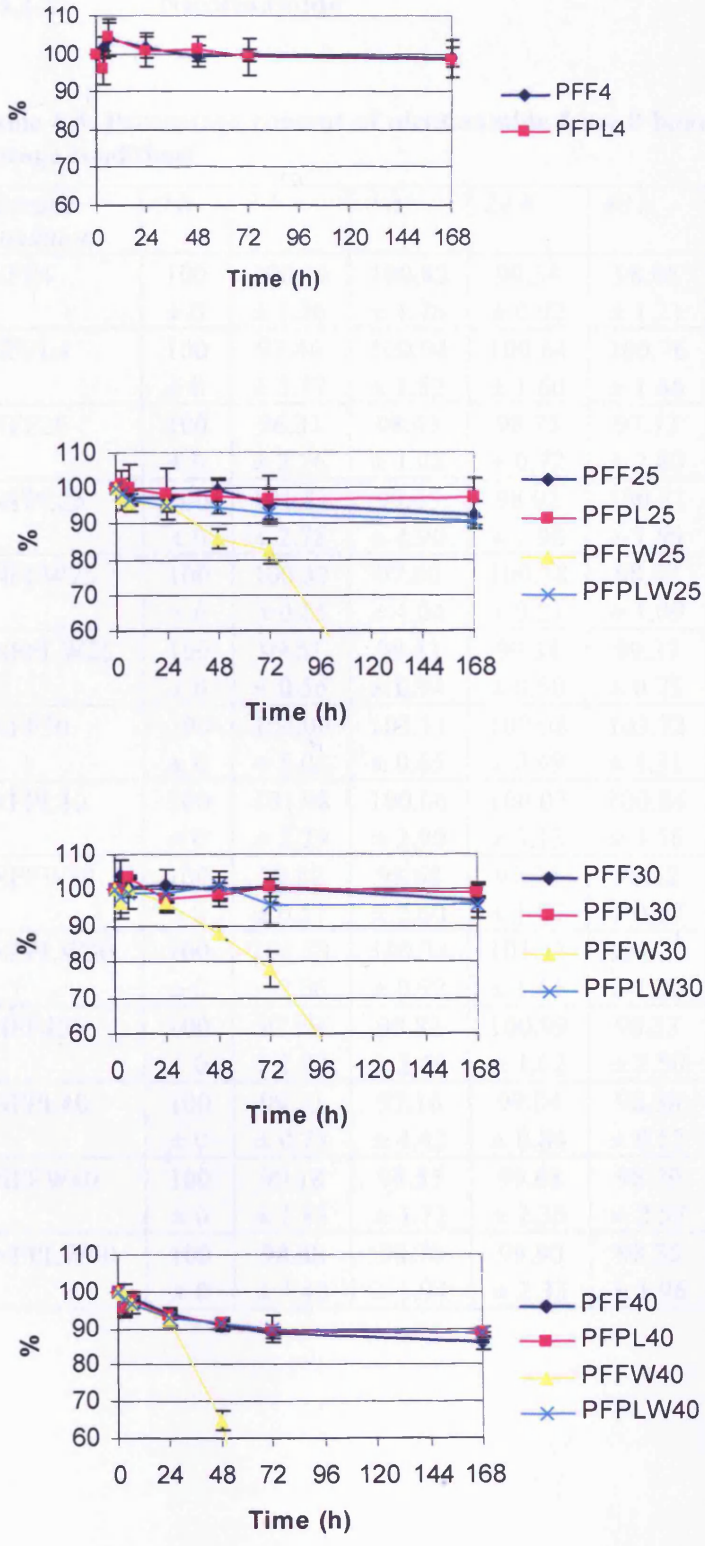


Figure 4.9: Percentage content of pyridoxine in SV solutions upon different storage conditions

4.3.2.2. Nicotinamide

Table 4.5: Percentage content of nicotinamide from 0 hour in SV upon different storage conditions

Storage condition	*0 h*	*3 h*	*6 h*	*24 h*	*48 h*	*72 h*	*168 h*
NFF4	100 ± 0	100.16 ± 1.36	100.82 ± 1.76	99.34 ± 0.92	98.06 ± 1.21	99.73 ± 0.72	99.94 ± 1.38
NFPL4	100 ± 0	97.46 ± 3.77	100.94 ± 1.52	100.64 ± 1.60	100.76 ± 1.46	99.48 ± 0.30	97.28 ± 3.34
NFF25	100 ± 0	96.33 ± 2.76	98.43 ± 1.08	98.75 ± 0.72	97.13 ± 2.80	98.71 ± 1.09	98.27 ± 0.68
NFPL25	100 ± 0	101.83 ± 2.78	99.45 ± 4.90	98.92 ± 1.98	100.31 ± 1.99	101.08 ± 1.85	101.46 ± 2.03
NFFW25	100 ± 0	100.37 ± 0.85	97.60 ± 4.04	100.38 ± 0.21	98.58 ± 1.09	99.29 ± 0.17	97.64 ± 2.51
NFPLW25	100 ± 0	99.51 ± 0.56	99.43 ± 0.94	99.34 ± 0.50	99.37 ± 0.75	98.35 ± 0.47	98.74 ± 1.10
NFF30	100 ± 0	104.06 ± 5.05	103.11 ± 0.65	107.08 ± 0.49	103.72 ± 4.31	103.74 ± 5.24	100.76 ± 2.93
NFPL30	100 ± 0	101.08 ± 2.29	100.06 ± 2.90	100.07 ± 3.13	100.84 ± 3.56	100.13 ± 3.92	102.27 ±0.61
NFFW30	100 ± 0	99.80 ± 0.51	98.68 ± 2.60	97.94 ± 1.07	98.52 ± 0.67	99.20 ± 0.76	97.69 ± 1.20
NFPLW30	100 ± 0	101.53 ± 2.06	100.33 ± 0.92	101.11 ± 1.61	101.26 ± 1.30	98.03 ± 3.21	99.81 ± 0.81
NFF40	100 ± 0	97.39 ± 1.92	99.82 ± 2.49	100.99 ± 1.02	98.33 ± 2.50	97.41 ± 2.19	98.77 ± 1.67
NFPL40	100 ± 0	99.22 ± 0.73	97.16 ± 4.42	99.04 ± 0.84	98.58 ± 0.57	98.42 ± 0.88	98.37 ± 0.40
NFFW40	100 ± 0	99.18 ± 1.85	98.55 ± 1.72	99.68 ± 2.35	98.79 ± 2.57	99.84 ± 2.76	99.54 ± 2.57
NFPLW40	100 ± 0	98.88 ± 1.42	98.70 ± 1.94	99.90 ± 2.33	98.35 ± 1.96	99.76 ± 2.74	99.56 ± 2.58

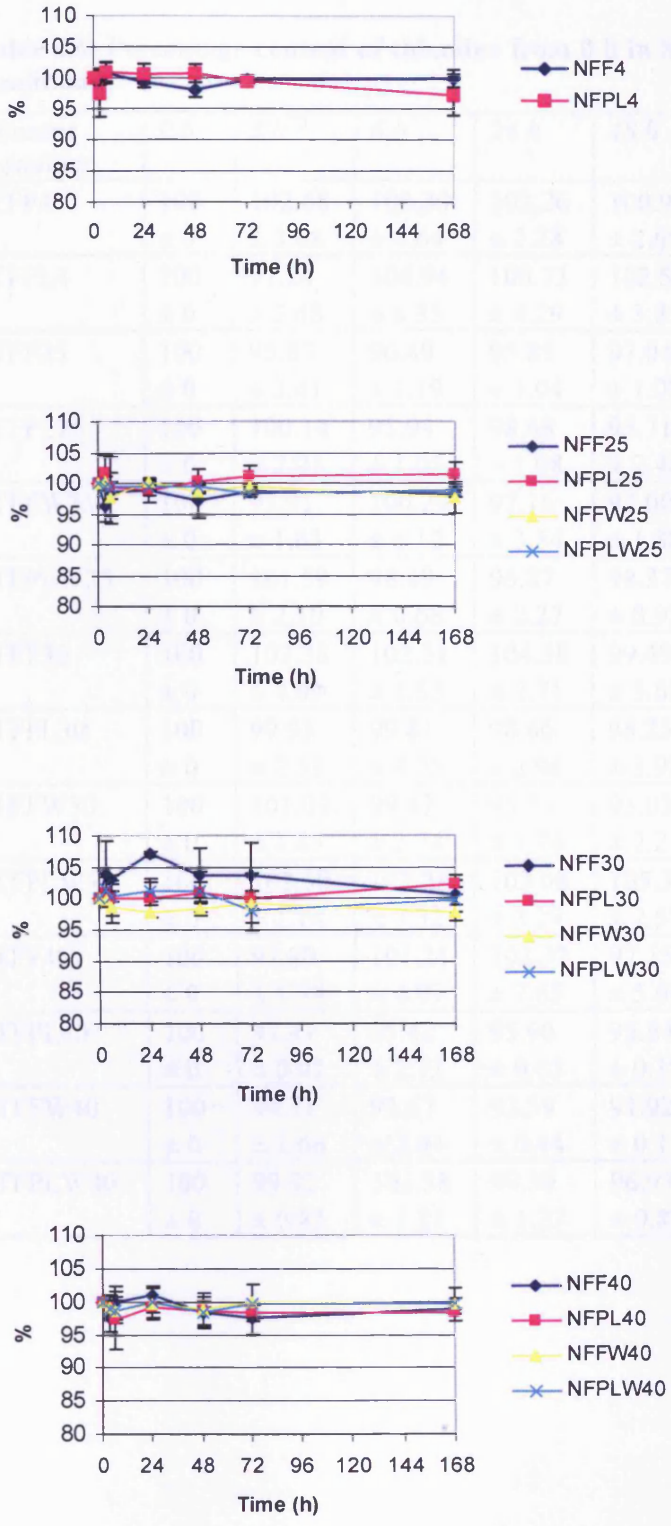


Figure 4.10: Percentage content of nicotinamide in SV solutions upon different storage conditions

4.3.2.3. Thiamine

Table 4.6: Percentage content of thiamine from 0 h in SV upon different storage conditions

Storage condition	*0 h*	*3 h*	*6 h*	*24 h*	*48 h*	*72 h*	*168 h*
TFF4	100 ± 0	102.68 ± 3.08	105.30 ± 4.64	102.26 ± 2.28	100.91 ± 2.65	99.89 ± 2.18	100.11 ± 2.76
TFPL4	100 ± 0	97.24 ± 2.48	104.94 ± 6.35	100.73 ± 4.29	102.59 ± 3.81	99.97 ± 8.05	99.35 ± 6.93
TFF25	100 ± 0	95.83 ± 3.41	96.49 ± 1.19	95.85 ± 1.04	97.04 ± 1.08	94.73 ± 2.24	93.14 ± 2.43
TFPL25	100 ± 0	100.14 ± 2.91	95.94 ± 1.05	98.68 ± 1.08	95.71 ± 2.48	95.54 ± 1.54	95.79 ± 2.08
TFFW25	100 ± 0	97.91 ± 1.63	100.29 ± 1.12	97.16 ± 3.54	92.00 ± 1.88	89.77 ± 2.60	86.82 ± 2.49
TFPLW25	100 ± 0	101.59 ± 2.10	98.49 ± 0.68	96.87 ± 2.27	98.37 ± 0.92	95.24 ± 3.50	94.59 ± 1.07
TFF30	100 ± 0	102.38 ± 4.09	102.31 ± 1.53	104.58 ± 2.71	99.49 ± 3.60	99.05 ± 4.93	98.17 ± 4.93
TFPL30	100 ± 0	99.93 ± 2.53	99.81 ± 4.25	98.66 ± 2.94	98.23 ± 3.98	100.09 ± 7.08	100.73 ± 2.59
TFFW30	100 ± 0	101.07 ± 1.45	99.47 ± 2.74	95.73 ± 1.74	93.03 ± 2.21	95.05 ± 5.13	90.78 ± 1.89
TFPLW30	100 ± 0	102.50 ± 1.14	102.25 ± 3.32	103.08 ± 3.29	105.31 ± 2.57	95.50 ± 2.29	98.38 ± 2.67
TFF40	100 ± 0	97.80 ± 1.38	101.24 ± 4.99	102.37 ± 7.65	97.15 ± 5.68	95.59 ± 5.48	91.53 ± 0.83
TFPL40	100 ± 0	97.41 ± 0.92	95.42 ± 2.73	95.90 ± 0.65	95.84 ± 0.19	94.48 ± 3.16	93.89 ± 2.94
TFFW40	100 ± 0	94.71 ± 1.66	93.67 ± 2.04	92.39 ± 0.44	91.92 ± 0.15	78.24 ± 2.80	80.80 ± 1.13
TFPLW40	100 ± 0	99.92 ± 0.83	101.38 ± 1.21	99.39 ± 1.27	96.97 ± 0.82	96.17 ± 1.56	95.38 ± 1.04

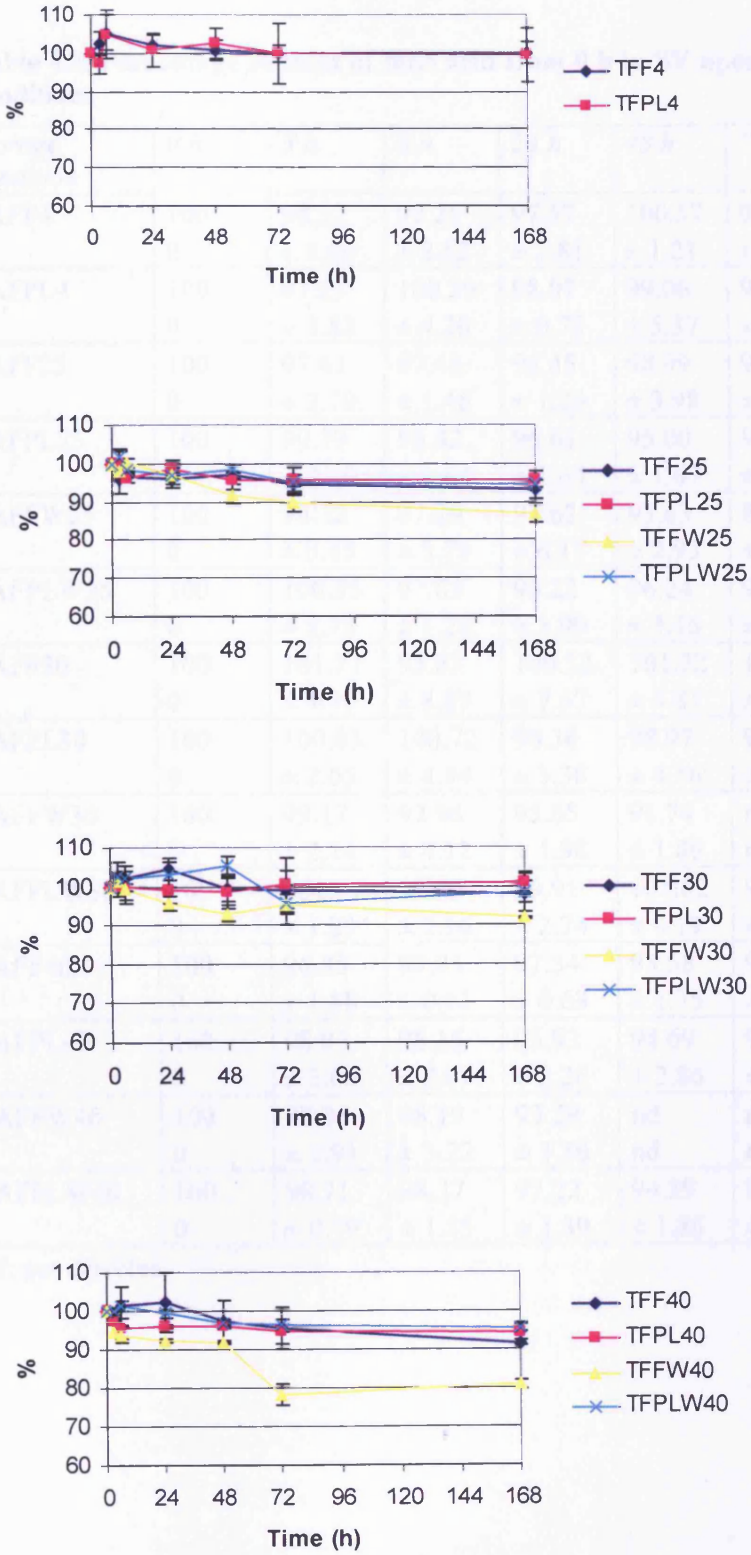


Figure 4.11: Percentage content of thiamine in SV solutions upon different storage conditions

4.3.2.4. Folic Acid

Table 4.7. Percentage content of folic acid from 0 h in SV upon different storage conditions

Storage condition	*0 h*	*3 h*	*6 h*	*24 h*	*48 h*	*72 h*	*168 h*
FAFF4	100 0	98.52 ± 4.00	97.25 ± 2.62	97.57 ± 1.81	100.57 ± 1.21	97.98 ± 3.89	100.17 ± 2.37
FAFPL4	100 0	97.21 ± 3.82	100.59 ± 4.20	98.07 ± 0.75	99.06 ± 5.37	99.55 ± 2.27	96.87 ± 3.08
FAFF25	100 0	97.61 ± 2.79	97.48 ± 1.46	98.45 ± 1.26	98.99 ± 3.98	96.38 ± 4.34	95.03 ± 2.16
FAFPL25	100 0	99.79 ± 2.46	96.42 ± 1.64	96.61 ± 1.64	95.00 ± 1.67	93.66 ± 0.89	93.37 ± 1.71
FAFFW25	100 0	98.72 ± 3.45	97.29 ± 3.79	97.62 ± 6.17	93.63 ± 2.95	89.58 ± 3.23	nd nd
FAFPLW25	100 0	100.55 ± 3.78	97.63 ± 1.28	96.22 ± 3.99	96.24 ± 3.16	93.89 ± 2.25	92.34 ± 0.39
FAFF30	100 0	101.77 ± 4.40	95.83 ± 8.89	100.12 ± 7.57	101.72 ± 4.81	101.60 ± 6.40	92.70 ± 2.27
FAFPL30	100 0	100.83 ± 2.05	100.72 ± 4.94	98.36 ± 3.38	98.97 ± 4.16	97.56 ± 4.22	95.87 ± 4.79
FAFFW30	100 0	99.17 ± 2.14	97.96 ± 4.12	95.85 ± 1.98	91.74 ± 1.80	nd nd	nd nd
FAFPLW30	100 0	101.34 ± 1.23	99.90 ± 2.16	99.91 ± 2.74	101.62 ± 0.19	96.80 ± 2.48	92.29 ± 1.70
FAFF40	100 0	96.85 ± 1.88	99.93 ± 0.11	97.54 ± 0.63	95.58 ± 1.75	93.50 ± 0.81	90.31 ± 1.11
FAFPL40	100 0	98.04 ± 3.05	96.16 ± 2.07	95.93 ± 2.26	94.69 ± 2.86	94.54 ± 5.19	91.61 ± 4.64
FAFFW40	100 0	98.26 ± 2.91	98.19 ± 3.22	93.29 ± 3.56	nd nd	nd nd	nd nd
FAFPLW40	100 0	98.71 ± 0.79	98.37 ± 1.55	97.22 ± 1.39	94.39 ± 1.86	93.44 ± 1.66	87.78 ± 2.36

nd: not detected

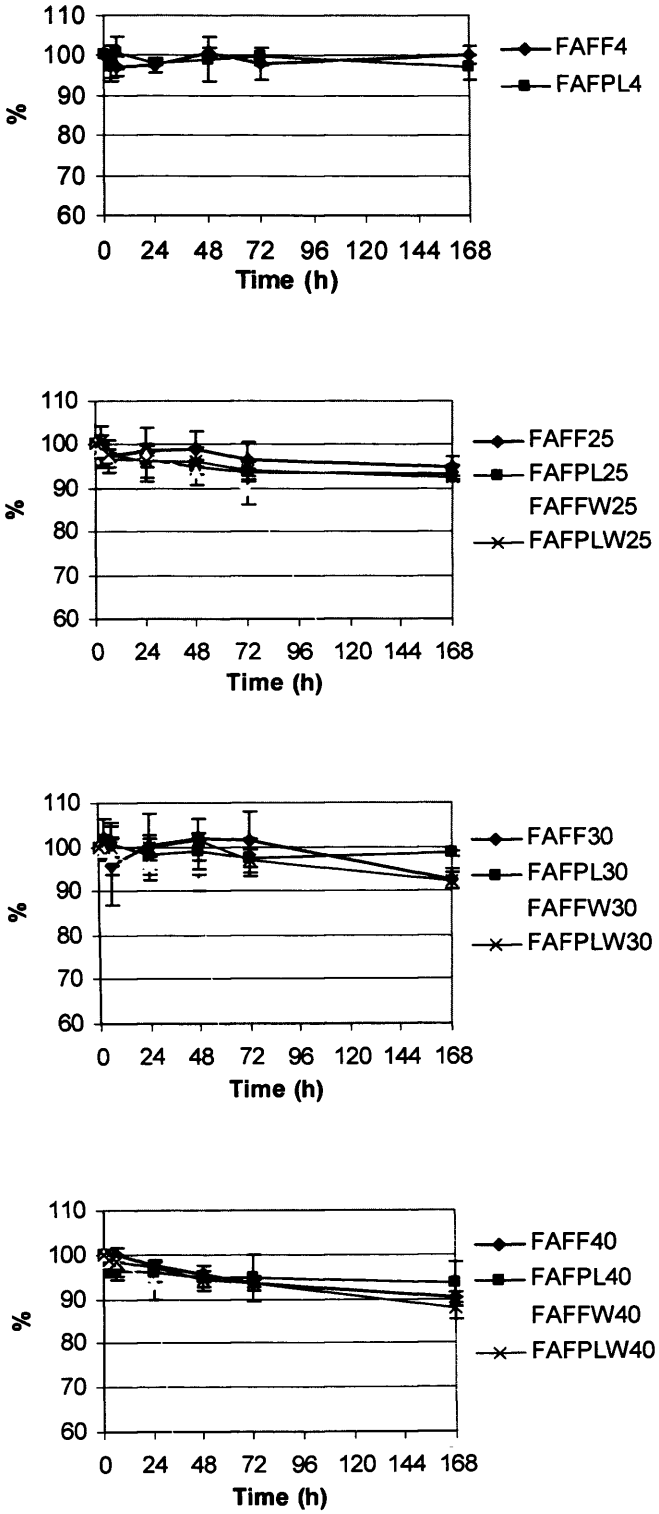


Figure 4.12: Percentage content of folic acid in SV solutions upon different storage conditions

4.3.2.5. Riboflavin Sodium Phosphate

Table 4.8: Percentage content of riboflavin sodium phosphate from 0 h in SV upon different storage conditions

Storage condition	*0 h*	*3 h*	*6 h*	*24 h*	*48 h*	*72 h*	*168 h*
RSPFF4	100 0	102.24 ± 3.95	102.71 ± 5.21	100.44 ± 4.69	99.56 ± 3.68	100.32 ± 4.07	98.35 ± 2.72
RSPFPL4	100 0	101.43 ± 3.17	102.34 ± 3.94	99.63 ± 3.77	99.39 ± 2.76	97.05 ± 1.36	96.31 ± 1.41
RSPFF25	100 0	97.89 ± 3.67	97.23 ± 3.27	98.10 ± 0.88	97.19 ± 0.89	96.98 ± 1.24	94.30 ± 1.68
RSPFPL25	100 0	97.65 ± 4.62	99.28 ± 5.32	99.63 ± 4.13	98.63 ± 3.25	99.85 ± 3.57	100.25 ± 2.72
RSPFFW25	100 0	96.48 ± 3.40	92.48 ± 4.66	87.29 ± 1.89	65.33 ± 3.11	37.37 ± 1.08	nd nd
RSPFPL25	100 0	97.65 ± 4.62	99.28 ± 5.32	99.63 ± 4.13	98.63 ± 3.25	99.85 ± 3.57	100.25 ± 2.72
RSPFPLW25	100 0	99.48 ± 0.42	97.61 ± 0.55	95.88 ± 2.41	94.70 ± 1.33	93.09 ± 2.97	88.99 ± 2.51
RSPFF30	100 0	101.18 ± 5.23	99.53 ± 4.75	100.73 ± 6.88	97.51 ± 5.80	95.74 ± 6.34	92.03 ± 3.61
RSPFPL30	100 0	100.53 ± 2.64	98.98 ± 3.46	97.46 ± 4.41	96.54 ± 4.58	94.89 ± 4.28	93.67 ± 2.15
RSPFFW30	100 0	95.13 ± 0.90	91.22 ± 1.22	71.66 ± 0.79	49.32 ± 1.60	12.85 ± 1.54	nd nd
RSPFPLW30	100 0	100.96 ± 2.02	100.27 ± 1.16	98.40 ± 1.07	97.70 ± 1.62	93.68 ± 4.21	90.34 ± 0.15
RSPFF40	100 0	96.63 ± 2.47	98.53 ± 0.45	94.57 ± 0.08	90.83 ± 1.38	88.50 ± 1.13	84.84 ± 1.83
RSPFPL40	100 0	96.83 ± 1.40	98.40 ± 2.25	93.80 ± 0.50	91.26 ± 1.09	87.78 ± 1.86	85.38 ± 1.37
RSPFFW40	100 0	92.52 ± 3.15	86.80 ± 3.00	62.29 ± 4.83	15.73 ± 0.31	nd nd	nd nd
RSPFPLW40	100 0	97.14 ± 0.68	95.98 ± 0.46	94.78 ± 3.75	91.79 ± 2.74	87.26 ± 2.37	85.62 ± 3.15

nd: not detected

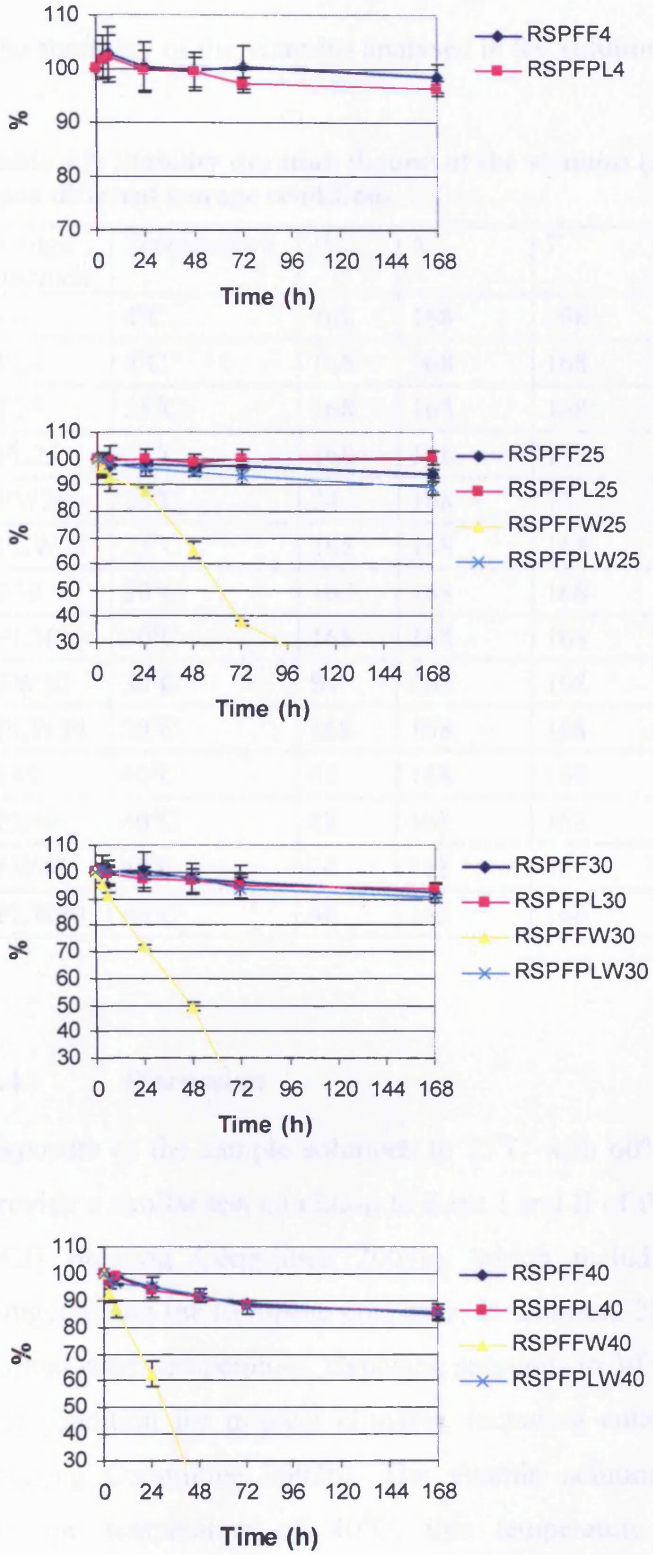


Figure 4.13: Percentage content of riboflavin sodium phosphate in SV solutions upon different storage conditions

4.3.3. Summary of Results of HPLC Analysis

The summary of the vitamins analysed in SV solutions is shown in Table 4.9.

Table 4.9: Stability duration (hours) of the vitamins (more than 90% remaining) upon different storage conditions

Storage condition	*Temperature*	*P*	*N*	*T*	*FA*	*RSP*
FF4	4°C	168	168	168	168	168
FPL4	4°C	168	168	168	168	168
FF25	25°C	168	168	168	168	168
FPL25	25°C	168	168	168	168	168
FFW25	25°C	24	168	72	72	6
FPLW25	25°C	168	168	168	168	168
FF30	30°C	168	168	168	168	168
FPL30	30°C	168	168	168	168	168
FFW30	30°C	24	168	168	48	6
FPLW30	30°C	168	168	168	168	168
FF40	40°C	48	168	168	168	48
FPL40	40°C	48	168	168	168	48
FFW40	40°C	24	168	48	24	3
FPLW40	40°C	48	168	168	72	48

4.4. Discussion

Exposure of the sample solutions to 25°C with 60% relative humidity would provide a similar test condition to Zone I and II of the ICH Stability Guideline (ICH Steering Committee 2003a), which includes countries like United Kingdom and the European countries. In addition, 25°C is also regarded as the normal room temperature. Exposing solutions to 30°C would provide a similar test condition for tropical climates, including countries like Malaysia (ICH Steering Committee 2003b). The vitamin solution was also tested at the extreme temperature of 40°C, this temperature being chosen to make allowances for preparations that are to be administered to infants and preterm infants who are placed in incubators. For solutions exposed to light, light

intensity was set at 0.7 klux. This is equivalent to office and hospital ward lightings that have specifications of around 0.5 klux (Baker et al. 1993).

The multivitamin solution SV showed maximum stability when stored at 4°C, where physical tests showed no obvious colour change and minimum change in pH and turbidity, and chemical analysis of all vitamins tested showed negligible loss of the vitamins.

Turbidity measurement showed that there was no obvious NTU change over time when stored at 4°C, but the NTU readings decreased over time at higher temperatures. In the presence of light, the difference in NTU from 0 hour was more than 0.5 units. Generally, NTU measurement should not be more than 0.5 units in range from its initial value as this could indicate solution incompatibility (Trissel and Bready 1992), however, the 0.5 units in range is for an increase in NTU. On the contrary, in this case, the turbidity of SV solutions showed decreased NTU instead of an increase, therefore the NTU readings of the SV solutions are acceptable. SV solution is from lyophilised powder of Solivito® N Adult injection. The fact that NTU decreased over time and that the decrease is greater at higher temperatures in the presence of light may indicate that the lyophilised powder solubilises over time, and temperature and light aid with the solubilisation.

The DO measurement showed similar trends as has been discussed in Chapter Three with increased DO content at lower temperature. The DO contents of this study and that of Chapter Three however, were different as the solvent systems for DO content measurements were different. In Chapter Three, measurement was in the normal saline base vehicle while in this Chapter, DO measurement was in water-soluble SV vitamin solution. As pointed out in Chapter Three, the solubility of oxygen is also dependent on the type of solvent used.

As for pH, with the exception of storage at 4°C which showed negligible pH change, there was more than 0.5 units of pH difference from 0 hour at other conditions and was towards a decrease. The pH difference of 0.5 units is the generally accepted marker of physical instability by the present laboratory research group. Tests of the pH of the individual vitamin components were not carried out, but the possible decrease in pH value could be due to the degradation of the vitamin components, such as ascorbic acid and folic acid, to more acidic degradation products. It is interesting to note that with multiple vitamins present in the test solution, pH test alone is not a good marker of instability as can be seen from the results of the chemical stability of the individual vitamins still remaining stable.

From the HPLC analysis, as the solution was exposed to higher temperature, different stability profiles of the vitamins were noted. At 25°C and 30°C (see Table 4.9, FF25/30/40), the vitamins remained stable, with less than 10% potency loss, however at 40°C, thiamine, nicotinamide and folic acid were stable but the stability duration of pyridoxine and riboflavin sodium phosphate was reduced to 48 hours.

From the results (see 4.3.3), in general it can be seen that nicotinamide is the most stable of all the five vitamins tested. Nicotinamide showed stability over the range of conditions tested, which is to be expected as nicotinamide is not heat labile nor light sensitive. Overall, these results are comparable to referenced data on the individual vitamins (DeRitter 1982) and to an earlier study by Dahl et.al (1986) who studied the stability of Soluvit® N vitamins up to 96 h storage at 2-8°C but in a different container.

Pyridoxine was stable for 168 hours at 25°C and 30°C when light protected but the stability was reduced to 24 hours when exposed to the artificial fluorescent light. Riboflavin sodium phosphate too was stable at 25°C and 30°C when light protected but the stability was reduced to 6 hours when exposed to artificial fluorescent light. Chen et al. (1983) reported pyridoxine and riboflavin sodium

phosphate as stable under fluorescent light, as their study was conducted over 8 hours duration. So far, no vitamin studies have been carried out over 168 hours for conditions exposed to artificial fluorescent light.

Thiamine stability was also comparable to the study conducted by Baumgartner and colleagues (Baumgartner et al. 1997), where they found no significant degradation of thiamine in PN salt solution containing neither carbohydrates nor amino acids, stored at either room temperature (25°C) or refrigerated (4°C). A study by Allwood (1982b) also reported thiamine to be stable under refrigeration for up to 28 days. It must be stressed that if the solution contained sulphites, then thiamine stability may be compromised as shown by studies performed by many investigators (Bowman and Nguyen 1983; Kearney et al. 1995; Scheiner et al. 1981). However, amino acid solutions in the European market contain no sulphites.

According to Baumgartner and colleagues (Baumgartner et al. 1997) too, their study showed that thiamine, as a component of multivitamin, degraded faster in aqueous PN solution with less than 90% thiamine available when stored at 25°C. They attributed the thiamine loss to either direct or indirect interaction with either the amino acids and/or carbohydrates used, as well as with one or more of the other vitamins. In their study, a different multivitamin product and a different plastic bag container were used when compared to this study.

For folic acid, studies conducted by Barker et al. (1984) revealed that the stability of folic acid depended on pH. If the pH is greater than 5.0, then folic acid remains in solution. Thus, they found folic acid to be stable for at least 2 weeks in amino acid solutions but not in glucose solutions which have a low pH. In this study, the pH of SV vitamin solution is greater than 5.0, hence folic acid is found to be stable in the normal saline solution.

When SV solutions are, in addition, exposed to artificial fluorescent white light, the stability of the solutions is greatly reduced. Notable changes in colour

of the solutions from light yellow to pale yellow were observed, and the light green haze appearance became less intense when light exposed at 30°C and 40°C. Chemical analysis confirmed the decrease in stability of some vitamins. At 25°C and exposed to light, the stability of pyridoxine, folic acid and riboflavin sodium phosphate was reduced from 168 hours to 24 hours, 72 hours and 6 hours, respectively. At 30°C, the stability of folic acid was reduced to 48 hours and riboflavin sodium phosphate was reduced to 6 hours, and at 40°C, the stability of riboflavin sodium phosphate was further reduced to 3 hours.

It has been pointed out that vitamins are less sensitive to artificial light as compared to ultraviolet light. However, this study has shown that loss of vitamins does occur even when exposed to continuous artificial light. Most stability studies from the effects of artificial light exposure carried out were only for a duration of between 8 to 24 hours (Chen et al. 1983), but in this case, the solutions were subjected to continuous exposure to fluorescent white light for up to 168 hours. Therefore when exposed for a longer duration to fluorescent white light, there is a potential for vitamins to degrade. Besides, there is also the possibility that difference in light intensity, and wavelengths absorbed, may affect the degradation of the vitamins differently, as studied by Ahmad et al. (2006) on the effects of riboflavin.

4.5. Conclusions

All the water-soluble vitamins analysed were stable at 4°C throughout the 168 hours study duration. Nicotinamide was found to be the most stable of all the vitamins tested. If the water-soluble SV vitamin solution is to be kept at 25°C, it is best to protect the solution from light where stability is maintained for up to 168 hours. Vitamin loss is least when the solutions are stored in the refrigerator and light protected.

CHAPTER FIVE

STABILITY STUDY OF FAT-SOLUBLE VITAMINS SUBJECTED TO VARIOUS STORAGE CONDITIONS

5.1. Introduction

Similar to Chapter Four, the stability of a fat-soluble multivitamin infusion upon different storage conditions was tested. This study evaluates the stability of Vitlipid® N Adult vitamin injection, prepared in 0.9% sodium chloride in 100ml Freeflex® minibags. The abbreviation VL is given to this test emulsion throughout this thesis.

The physical stability of the emulsion was assessed and the chemical stability of Vitamin A (retinol palmitate) and Vitamin E (tocopherol) was examined, the HPLC method has been described in Chapter Two.

5.2. Methods

The experimental set-up was similar to the study as described under Experiment 1 of Chapter Three.

5.2.1. Preparation of Test Solutions VL

Each ampoule of Vitlipid® N Adult injection was pooled into a 250 ml EVA Freka® IV-Bag (Fresenius Kabi), with an extra ampoule added as excess allowance. 10 ml of this Vitlipid® N Adult injection was then added to 100 ml sterile 0.9% sodium chloride in Freeflex® minibags.

Different bags were prepared for physical analysis and for HPLC analysis respectively, as outlined in Chapter Four.

5.2.2. Sampling

For physical analysis, samples were tested at times 0, 24, 48 and 168 hours. Using sterile 20 ml syringes and 21G needles, 20 ml samples were withdrawn from the minibags via the additive port. The needles were then detached from the syringes and the samples were collected into Hach® glass tubes, running the samples down the side of the tubes, and then capped immediately. These samples were for visual inspection, and to test the DO content and pH. Another set of 5 ml samples were withdrawn for particle size analysis and for examination by light microscopy. This amount was determined by conducting preliminary trials which showed that about 3 ml of sample emulsion was required for the particle size analysis by laser diffraction. These methods for determining physical stability have been described under Chapter Two.

For chemical analysis, 3 ml samples were withdrawn using sterile 21G needles and 5 ml syringes at 0 hour immediately after preparation and split into two separate 2 ml amber autosampler vials, then at 3, 6, 24, 48, 72 and 168 hours. Samples were then stored in the pharmaceutical refrigerator at 4°C before analysis and analysed within 3 days using a validated HPLC stability-indicating method as described in Chapter Two. A fresh sample prepared in a 100 ml glass bottle was also prepared and analysed as reference. Samples taken at 168 hours were also analysed against the freshly prepared reference sample. Adjustments were made in relation to the fresh reference peaks. Content analysis of these samples at 0 hour was regarded as the baseline content of 100%.

5.3. Results

The abbreviations used to denote the type of containers and conditions for storage of the multivitamin emulsions are the same as those used in Chapter Four.

5.3.1. Physical Analysis

5.3.1.1. Colour and Appearance

All emulsions showed no change in colour from the initial white colour for all storage conditions. There seemed to be no separation of the oily phase in all emulsions, except for the samples stored at 40°C for 168 h, which seemed to have an oily streak along the side of the Hach® tube. No other changes were observed even when viewed with the fibreoptic light.

5.3.1.2. Dissolved Oxygen

The results of DO content of the sample emulsions are tabulated in Table 5.1 with the corresponding graphs illustrated in Figure 5.1 to Figure 5.4.

Table 5.1: Mean DO measurement in mg/l of VL emulsions at different storage conditions

VL	*0 h (n=12)*	*24 h*	*48 h*	*168 h*
FF4	5.46 ± 0.16 #	6.91 ± 0.19	6.94 ± 0.15	7.01 ± 0.22
FPL4	5.46 ± 0.16 #	7.02 ± 0.17	7.06 ± 0.07	6.94 ± 0.08
FF25	5.26 ± 0.33	5.01 ± 0.11	4.90 ± 0.13	4.78 ± 0.38
FFW25	5.26 ± 0.33	5.06 ± 0.09	4.98 ± 0.23	4.76 ± 0.13
FPLW25	5.26 ± 0.33	4.99 ± 0.40	4.46 ± 0.09	4.80 ± 0.24
FPL25	5.26 ± 0.33	4.95 ± 0.13	5.16 ± 0.23	5.12 ± 0.15
FF30	4.73 ± 0.32	4.88 ± 0.11	4.58 ± 0.13	4.42 ± 0.38
FFW30	4.73 ± 0.32	4.65 ± 0.23	4.38 ± 0.19	4.39 ± 0.36
FPLW30	4.73 ± 0.32	4.68 ± 0.24	4.70 ± 0.18	4.34 ± 0.30
FPL30	4.73 ± 0.32	4.08 ± 0.04	4.03 ± 0.19	3.09 ± 0.30
FF40	4.68 ± 0.18	3.94 ± 0.21	3.57 ± 0.04	3.87 ± 0.23
FFW40	4.68 ± 0.18	3.83 ± 0.05	3.65 ± 0.06	4.06 ± 0.28
FPLW40	4.68 ± 0.18	3.87 ± 0.08	3.74 ± 0.26	3.99 ± 0.02
FPL40	4.68 ± 0.18	2.99 ± 0.11	2.52 ± 0.27	2.74 ± 0.03

: n=6

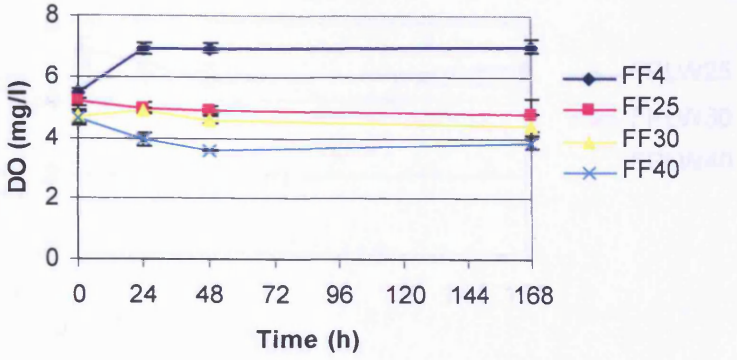


Figure 5.1: Mean DO content of VL in clear minibags at different storage conditions

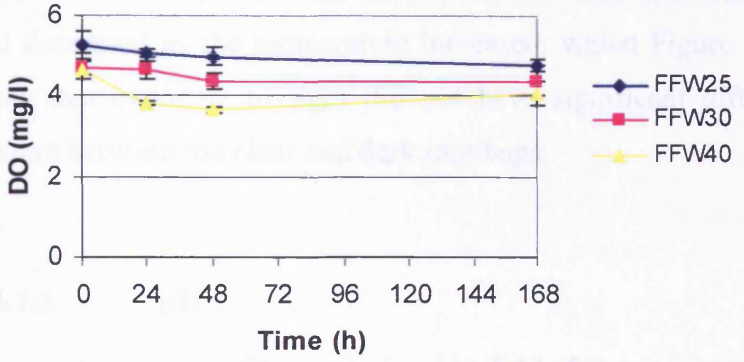


Figure 5.2: Mean DO content of VL in clear minibags at different storage conditions and exposed to light

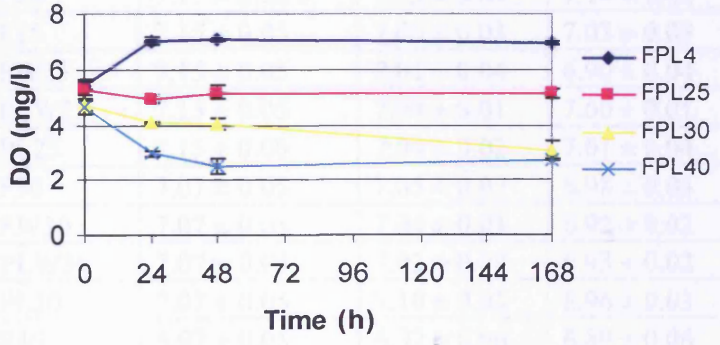


Figure 5.3: Mean DO content of VL in dark minibags at different storage conditions

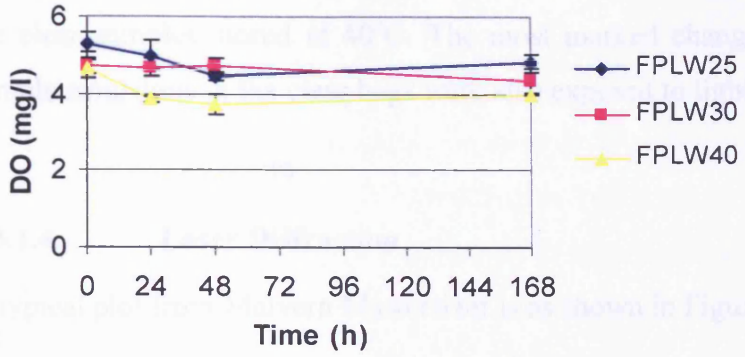


Figure 5.4: Mean DO content of VL in dark minibags at different storage conditions and exposed to light

Figures 5.1 and 5.3 show that the DO content increased with lower temperature and decreased as the temperature increased, whilst Figure 5.2 and Figure 5.4 show that exposure to light did not have significant difference in the DO content between the clear and dark minibags.

5.3.1.3. pH

The results of mean pH are tabulated in Table 5.2.

Table 5.2: Mean pH of VL at different storage conditions

VL	*0 h (n=12)*	*24 h*	*48 h*	*168 h*
FF4	7.17 ± 0.08 #	7.08 ± 0.06	7.11 ± 0.06	6.83 ± 0.05
FPL4	7.17 ± 0.08 #	7.13 ± 0.04	7.14 ± 0.02	6.84 ± 0.03
FF25	7.15 ± 0.05	7.05 ± 0.03	7.02 ± 0.03	6.74 ± 0.04
FFW25	7.15 ± 0.05	7.01 ± 0.04	6.90 ± 0.04	6.71 ± 0.05
FPLW25	7.15 ± 0.05	7.04 ± 0.01	7.00 ± 0.01	6.72 ± 0.04
FPL25	7.15 ± 0.05	7.04 ± 0.02	7.01 ± 0.04	6.75 ± 0.07
FF30	7.07 ± 0.05	7.05 ± 0.07	6.98 ± 0.03	6.70 ± 0.07
FFW30	7.07 ± 0.05	7.04 ± 0.04	6.92 ± 0.02	6.64 ± 0.05
FPLW30	7.07 ± 0.05	7.03 ± 0.03	6.93 ± 0.02	6.74 ± 0.02
FPL30	7.07 ± 0.05	7.10 ± 0.03	6.96 ± 0.03	6.75 ± 0.03
FF40	6.97 ± 0.05	6.92 ± 0.06	6.89 ± 0.06	6.41 ± 0.16 *
FFW40	6.97 ± 0.05	6.93 ± 0.04	6.84 ± 0.03	6.28 ± 0.08 *
FPLW40	6.97 ± 0.05	6.96 ± 0.01	6.91 ± 0.03	6.54 ± 0.06
FPL40	6.97 ± 0.05	6.98 ± 0.00	6.91 ± 0.01	6.54 ± 0.05

#: n= 6

*: change in pH of more than 0.5 units

Changes in pH of more than 0.5 units (marked with asterisks) were noted with the clear samples stored at 40°C. The most marked changes were when the sample emulsions in the clear bags were also exposed to light.

5.3.1.4. Laser Diffraction

A typical plot from Malvern Mastersizer is as shown in Figure 5.5.

Result: Analysis Table

ID: fpl4-0c	Run No: 12	Measured: 21/4/2005 14:30PM
File: SITIVIT	Rec. No: 110	Analysed: 21/4/2005 14:30PM
Path: H:\SIZERX\DATA\		Source: Analysed
Range: 45 mm	Beam: 2.40 mm	Sampler: MS1	Obs': 20.0 %
Presentation: 2-SOYA		Analysis: Polydisperse	Residual: 0.694 %
Modifications: None			
Conc. = 0.0218 %Vol	Density = 1.000 g/cm^3	S.S.A.= 21.1871 m^2/g
Distribution: Volume	D[4, 3] = 0.36 um	D[3, 2] = 0.28 um
D(v, 0.1) = 0.17 um	D(v, 0.5) = 0.31 um	D(v, 0.9) = 0.59 um
Span = 1.339E+00	Uniformity = 4.542E-01	

Size (um)	Volume In %	Size (um)	Volume In %	Size (um)	Volume In %	Size (um)	Volume In %
0.05	0.32	0.53	7.80	2.83	0.09	15.04	0.00
0.12	4.83	0.65	4.02	3.49	0.02	18.54	0.00
0.15	9.37	0.81	1.39	4.30	0.00	22.84	0.00
0.19	13.11	1.00	0.10	5.29	0.00	28.15	0.00
0.23	15.51	1.23	0.00	6.52	0.00	34.69	0.00
0.28	16.12	1.51	0.15	8.04	0.00	42.75	0.00
0.35	14.81	1.86	0.36	9.91	0.00	52.68	0.00
0.43	11.78	2.30	0.24	12.21	0.00	64.92	0.00
0.53		2.83		15.04		80.00	

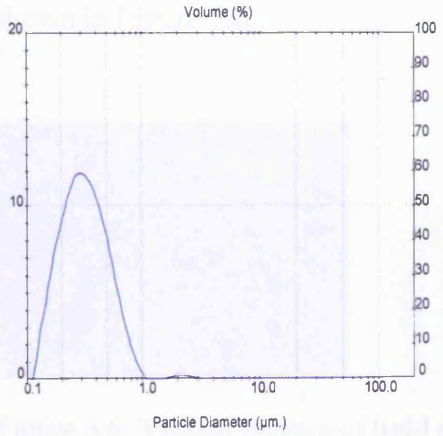


Figure 5.5: Typical laser diffraction graph of VL by Malvern® Mastersizer

In the Malvern® analysis table, D[4,3] is the volume mean, D[3,2] is the surface area mean while D(υ,0.5) is the volume median diameter or the mean diameter at 50% volume distribution. D[4,3] is the mean diameter chosen in the analysis of the sample emulsions as this volume mean has good reproducibility

and a narrow margin of error for determining particle size (Malvern Application Note).

At 0 hour, all samples had maximum diameter of less than 4.30 μm with D[4,3] of between 0.35 to 0.37 μm. The results remained the same at 168 hours with the exception of samples that were exposed to temperature at 40°C.

At 168 hours, samples stored at 40°C had maximum diameter of more than 12.21 μm with the percentages of lipid globules with a diameter of greater than 4.30 μm ranging from 0.14 to 10.12%. The D[4,3] of these samples also increased with results ranging from 0.52 μm to 1.60 μm.

5.3.1.5. Light Microscopy

At 0 hour, occasional samples contained lipid globules size of 5 μm, but always with less than 5 globules of 5 μm size within the calibrated grid. A typical picture of lipid globules at 0 hour as seen under the microscope is shown in Figure 5.6.

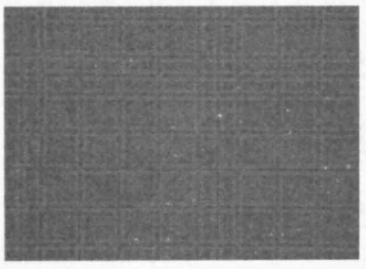


Figure 5.6: Typical picture of lipid globules in VL. Magnification 200 times

When exposed to higher temperatures at 30°C and 40°C, a greater number of 5 μm size lipid globules (range 7-25) was noted at 168 hours. In addition, larger lipid globules from 7.5 μm to 12.5 μm in size were identified, with some smaller globules tending to aggregate around the larger lipid globules.

5.3.2. HPLC Analysis

The results of Vitamin A and Vitamin E analysed by HPLC are tabulated in Table 5.3 and Table 5.4 respectively, and the corresponding graphs at different temperature conditions are shown in Figure 5.7 and Figure 5.8.

5.3.2.1. Vitamin A

Table 5.3: Percentage content of Vitamin A in VL from 0 hour upon different storage conditions

Storage condition	*0 h (n=12)*	*3 h*	*6 h*	*24 h*	*48 h*	*72 h*	*168 h*
FF4	100 # ± 0	103.94 ± 0.44	104.67 ± 0.81	106.80 ± 0.71	107.31 ± 1.02	109.97 ± 0.72	114.54 ± 4.02
FPL4	100 # ± 0	104.45 ± 1.39	104.81 ± 2.03	106.49 ± 1.92	107.21 ± 1.69	109.37 ± 1.60	115.36 ± 3.94
FF25	100 ± 0	99.92 ± 3.23	98.52 ± 2.69	98.99 ± 1.58	99.99 ± 0.42	101.55 ± 1.51	95.03 ± 2.22
FPL25	100 ± 0	100.19 ± 0.55	98.62 ± 1.22	97.06 ± 0.52	98.94 ± 1.11	100.69 ± 1.68	93.14 ± 0.54
FFW25	100 ± 0	99.50 ± 2.06	99.48 ± 1.10	93.23 ± 0.63	81.42 ± 1.47	80.28 ± 0.93	55.84 ± 1.63
FPLW25	100 ± 0	101.19 ± 1.57	100.72 ± 3.81	98.91 ± 3.26	97.45 ± 2.99	101.51 ± 2.24	92.70 ± 1.40
FF30	100 ± 0	98.45 ± 1.50	98.67 ± 1.21	97.73 ± 0.42	101.11 ± 1.05	106.35 ± 1.15	72.63 ± 3.40
FPL30	100 ± 0	96.33 ± 0.94	98.14 ± 1.35	96.57 ± 1.32	103.73 ± 0.68	106.61 ± 0.91	73.19 ± 1.07
FFW30	100 ± 0	94.71 ± 0.68	95.58 ± 1.03	88.41 ± 0.42	84.74 ± 0.63	80.58 ± 0.36	40.06 ± 0.87
FPLW30	100 ± 0	97.50 ± 1.19	99.09 ± 1.26	97.82 ± 0.40	103.48 ± 0.26	106.68 ± 2.72	74.51 ± 1.46
FF40	100 ± 0	98.86 ± 1.25	96.72 ± 1.21	95.52 ± 0.68	94.63 ± 0.86	92.57 ± 0.80	77.83 ± 0.53
FPL40	100 0	98.43 ± 0.63	96.65 ± 1.16	96.09 ± 0.53	94.25 ± 0.90	92.02 ± 0.88	77.70 ± 0.48
FFW40	100 0	96.48 ± 0.56	92.98 ± 0.77	84.86 ± 2.55	74.90 ± 3.47	66.04 ± 5.23	34.28 ± 7.86
FPLW40	100 0	99.04 ± 0.95	96.11 ± 0.55	95.88 ± 0.53	94.25 ± 0.78	92.53 ± 0.78	75.90 ± 1.91

#: n=6

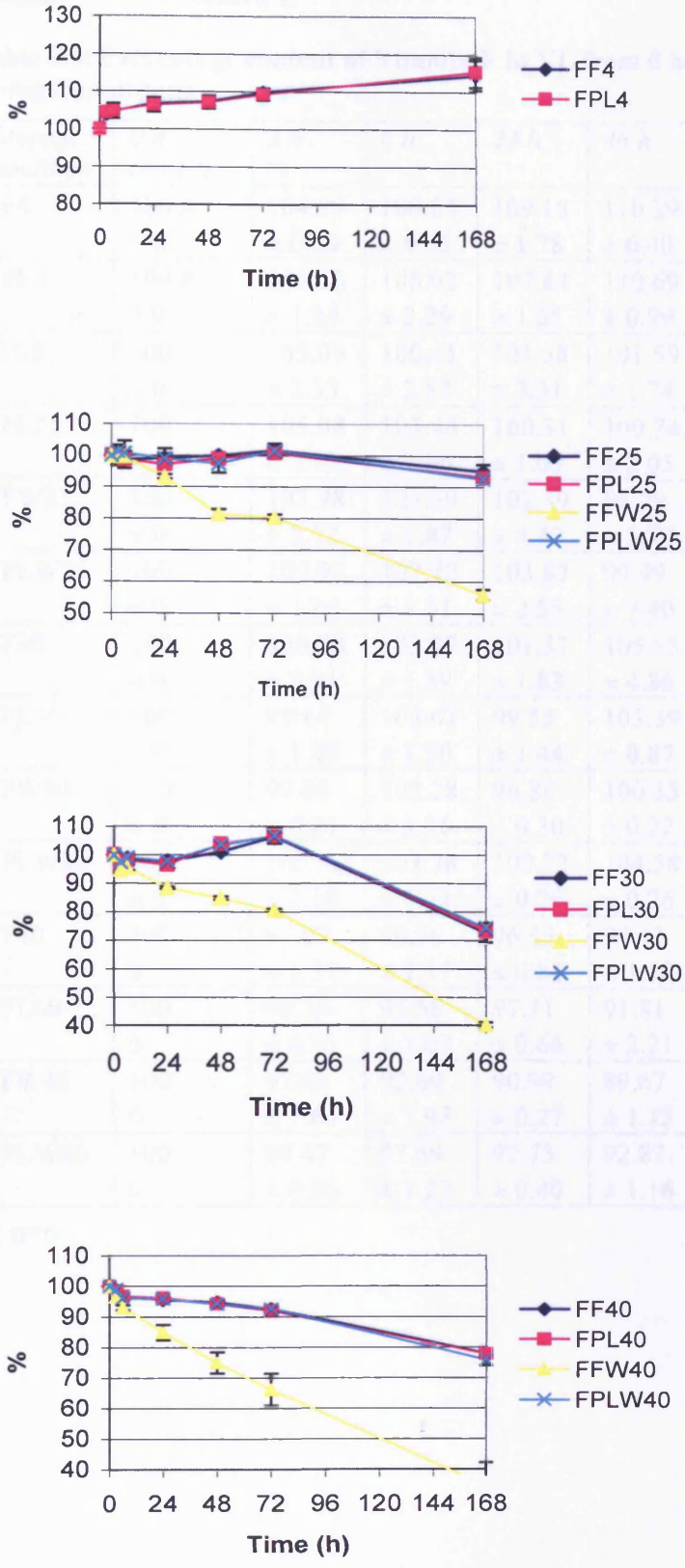


Figure 5.7: Percentage content of Vitamin A upon different storage conditions

5.3.2.2. Vitamin E

Table 5.4: Percentage content of Vitamin E in VL from 0 hour upon different storage conditions

Storage condition	*0 h (n=12)*	*3 h*	*6 h*	*24 h*	*48 h*	*72 h*	*168 h*
FF4	100 # ± 0	104.99 ± 0.69	106.05 ± 0.65	109.18 ± 1.78	110.29 ± 0.40	112.70 ± 1.12	116.72 ± 4.34
FPL4	100 # ± 0	106.16 ± 1.24	105.92 ± 2.29	107.84 ± 1.35	110.69 ± 0.99	110.76 ± 1.85	117.22 ± 3.27
FF25	100 ± 0	105.09 ± 2.53	100.41 ± 2.54	101.58 ± 3.31	101.59 ± 1.74	100.38 ± 5.59	96.25 ± 0.98
FPL25	100 ± 0	105.08 ± 2.42	103.48 ± 2.06	100.31 ± 1.03	100.74 ± 2.05	97.44 ± 5.27	98.35 ± 0.82
FFW25	100 ± 0	103.98 ± 2.72	105.69 ± 1.87	102.39 ± 4.42	96.29 ± 5.72	97.84 ± 4.56	87.00 ± 1.01
FPLW25	100 ± 0	105.98 ± 1.84	107.72 ± 1.61	103.87 ± 2.55	99.99 ± 7.40	93.47 ± 0.31	93.23 ± 4.66
FF30	100 ± 0	100.78 ± 2.41	103.37 ± 1.39	101.37 ± 1.83	105.55 ± 4.86	102.13 ± 4.20	73.08 ± 5.32
FPL30	100 ± 0	98.14 ± 1.29	105.07 ± 1.50	99.55 ± 1.44	103.39 ± 0.87	100.34 ± 1.16	73.00 ± 2.79
FFW30	100 ± 0	97.05 ± 0.21	102.28 ± 1.36	96.80 ± 0.30	100.33 ± 0.22	98.75 ± 1.70	58.14 ± 2.08
FPLW30	100 ± 0	102.52 ± 2.14	103.78 ± 1.14	100.22 ± 0.29	104.58 ± 0.76	105.42 ± 2.09	74.26 ± 1.52
FF40	100 0	97.07 ± 1.27	96.36 ± 1.17	96.65 ± 0.88	94.13 ± 1.58	89.00 ± 0.57	74.03 ± 1.14
FPL40	100 0	99.39 ± 0.91	95.58 ± 1.07	97.31 ± 0.66	91.81 ± 2.21	88.16 ± 0.70	73.72 ± 0.28
FFW40	100 0	97.45 ± 1.64	92.69 ± 1.98	90.99 ± 0.27	89.67 ± 1.12	83.02 ± 0.02	55.79 ± 0.55
FPLW40	100 0	99.47 ± 0.26	97.69 ± 1.22	92.73 ± 0.40	92.87 ± 1.16	87.73 ± 0.66	71.09 ± 2.68

#: n=6

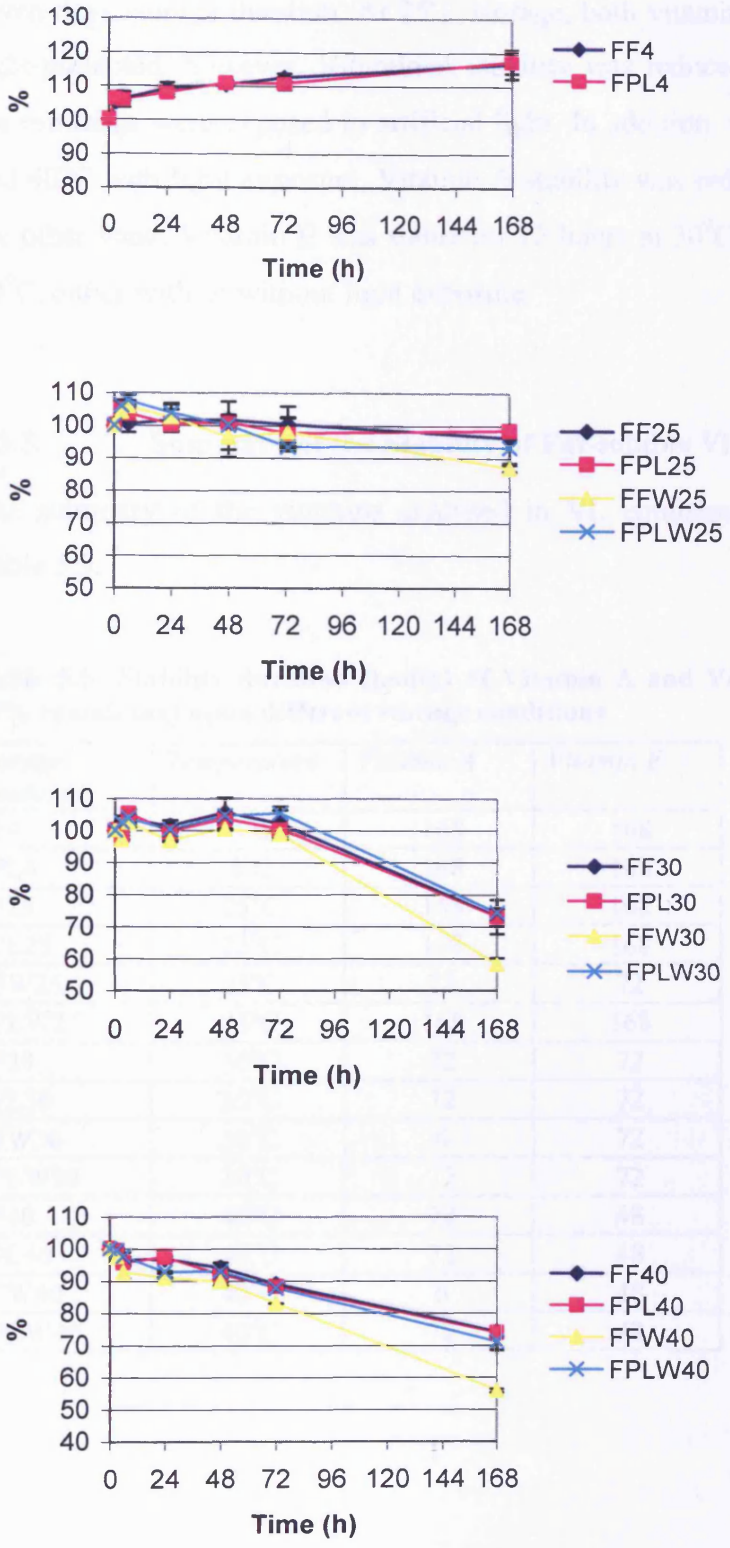


Figure 5.8: Percentage content of Vitamin E upon different storage conditions

Vitamin A and Vitamin E remained stable when stored at 4°C throughout the seven days storage duration. At 25°C storage, both vitamins were stable when light-protected, however, Vitamin A stability was reduced to 24 hours when the minibags were exposed to artificial light. In addition, when stored at 30°C and 40°C with light exposure, Vitamin A stability was reduced to 6 hours. On the other hand, Vitamin E was stable for 72 hours at 30°C and for 48 hours at 40°C, either with or without light exposure.

5.3.3. Summary of the Stability of Fat-soluble Vitamins Analysed

The summary of the vitamins analysed in VL emulsions is summarised in Table 5.5.

Table 5.5: Stability duration (hours) of Vitamin A and Vitamin E (more than 90% remaining) upon different storage conditions

Storage condition	*Temperature*	*Vitamin A*	*Vitamin E*
FF4	4°C	168	168
FPL4	4°C	168	168
FF25	25°C	168	168
FPL25	25°C	168	168
FFW25	25°C	24	72
FPLW25	25°C	168	168
FF30	30°C	72	72
FPL30	30°C	72	72
FFW30	30°C	6	72
FPLW30	30°C	72	72
FF40	40°C	72	48
FPL40	40°C	72	48
FFW40	40°C	6	48
FPLW40	40°C	72	48

5.4. Discussion

Vitlipid® N injection is recommended by the supplier to be added to a fat emulsion before infusion. The stability of these vitamins in lipid based emulsions or in 'all-in-one' PN admixture has been previously studied. In this case however, the stability of the fat-soluble vitamins in an aqueous based formulation using a normal saline diluent was studied.

The formulation was found to be homogenous upon mixing. The emulsifying agent present in Vitlipid® N injection formulation is egg phospholipids, which has been proven to be a good emulsifying agent for intravenous emulsions since it possesses both hydrophilic and hydrophobic molecules which orient at the oil-water interface. As a result, phospholipids also confer stability by creating electrostatic repulsive forces in the hydrophilic portion. Lipid emulsions have a surface potential of about -35 mV (Brown et al. 1986), the greater the surface potential or zeta potential of the oil globule, the more effective is the emulsion system in preventing coalescence. Mixing of lipid emulsions with electrolytes may reduce the zeta potential due to ion interactions and thereby causing emulsion instability. The dilution with normal saline in these experiments showed a homogenous emulsion and did not result in phase separation.

A number of methods can be used to determine the physical stability of fat emulsions (Driscoll et al. 2001; Washington 1990). These include particle sizing techniques such as laser diffraction, optical microscopy and visual inspection.

Particle size measurement of oil globules is one of the requirements of the BP 1980, whereby it states that the diameter should not exceed 5 μm. 5 μm is taken as the upper limit since larger particles may pose a risk of lung embolism. In the later editions of the BP, this requirement has been removed, when new perception of the behaviour of lipid globules in vivo was presented, such as the reversible nature of the blockade since the lipid globules are biodegradable,

and that a globule with a diameter of even greater than 7.5 μm can deform and pass through the pulmonary capillaries without difficulty (Koster et al. 1996). Nevertheless, particle size testing of lipid globules continues as a physical marker of emulsion stability. Driscoll et al (1995) showed that emulsions in which greater than 0.4% of the lipid globules are above 5 μm are likely to become unstable. Recently, the USP has proposed a standard for testing lipid emulsion where the volume-weighted percentage of the lipid globule size greater than 5 μm ($PFAT_5$) is to be limited to less than 0.05% (Driscoll 2005). Studies on animals have shown that the infusion of unstable lipid emulsions having $PFAT_5$ greater than 5 μm may have pathological consequences such as injury to the liver and lungs as a result of abnormal deposition of lipid globules causing oxidative stress and reduced blood flow from embolised lipid globules (Driscoll et al. 2006; Driscoll et al. 2005).

The results of particle size measurement by laser diffraction indicated that there was destability of the emulsions at 40°C after 168 hours storage, with the emulsions having lipid globules of diameter greater than 4.30 μm to be more than 0.14%. In addition, when viewed under the light microscope, larger lipid globules of more than 7.5 μm diameter were noted, with smaller globules tending to aggregate around the larger lipid globules.

When the emulsions were exposed to higher temperature of 40°C, with visual inspection, it was difficult to detect any sign of creaming or phase separation such as an oil layer in the minibags as they were handled when taking them out from the stability chamber, thus they had been subjected to agitation, which could have redispersed the phases. This was despite the fact that they were handled with care. A trace of oil on the Hach® tubes for 40°C storage at the end of the study was noted whilst washing the tubes. There is a possibility too that while withdrawing the sample with a syringe and needle, shearing of the sample could occur (Washington 1990), thus causing coalescence although this was not detected by laser diffraction.

The DO content again showed similar trend to Chapter Three and Chapter Four with respect to temperature, and the DO content at 0 hour was different from Chapter Three or Chapter Four since DO measurement was carried out in a different solvent system. These data serve as baseline information for future work.

With respect to pH, the emulsions showed negligible pH change from time 0 hour when exposed to the different storage conditions, the only exception was when the emulsions were stored in clear bags at 40°C where changes in pH of more than 0.5 units were noted.

Chemical analysis by HPLC showed that both the fat-soluble vitamins were stable when stored at 4°C throughout the study duration. These are in agreement with other studies (Dahl et al. 1986; Dupertuis et al. 2002). Dahl et al (1986) observed insignificant loss of the vitamins during the six days study duration in darkness. Another study carried out by Allwood and Plane (1984) on Vitamin A found no degradation of the vitamin when stored for 28 days in the refrigerator.

During storage at 4°C, there was an apparent higher content of the vitamins at 168 hours. In a study on Vitamin E, Balet et al (2004) attributed the higher concentration of the vitamin after 7 days storage at 4°C as being due to the natural variability of the vitamin in vegetable oils. In this study, for Vitamin E for example, the higher concentration of the vitamin may be attributed to the variability of the synthetic dl-alpha-tocopherol which consists of a mixture of the tocopherol stereoisomers, as discussed in Chapter One. Another possibility may be due to moisture loss from the minibag when stored in the pharmaceutical stability chamber, although this requires further investigation.

Table 5.5 summarises the stability duration of Vitamin A and Vitamin E upon various storage conditions. Vitamins A and E were also stable for 168 hours at 25°C, besides storage at 4°C, and for 72 hours at 30°C when light protected. At

40°C and protected from light, Vitamin A stability was reduced to 72 hours while Vitamin E stability was reduced to 48 hours. With artificial light exposure, Vitamin A stability was reduced to 24 hours at 25°C and to 6 hours at 30°C and 40°C, while Vitamin E was stable for 72 hours at 25°C and 30°C, and for 48 hours at 40°C.

The results of this study show that degradation is least when samples are protected from light and this is in agreement with results from other studies (Dahl et al. 1986; Drott et al. 1991). In a study by Allwood (1982a) on the effects of light on Vitamin A palmitate over a 24 hour period, it was pointed out that fluorescent light did not significantly degrade the vitamin, and that photodegradation of the vitamin is caused by the ultraviolet light below the wavelength of 400 nm. The result of this study after storage at 25°C for 24 hours was comparable to the study by Allwood (1982a). However, when exposed for a longer duration, artificial fluorescent light was seen to affect the stability of Vitamin A. Therefore, the same precautions need to be taken even when exposing the vitamin in emulsion formulation to fluorescent light.

Upon exposure to artificial fluorescent light, both vitamins showed greater loss of concentrations when compared to the results when subjected to temperature effects only, and the extent of degradation was higher for vitamin A.

5.5. Conclusions

The fat-soluble Vitamin A and Vitamin E in the final aqueous test formulations were stable for the storage duration of the study period at 4°C and at 25°C when protected from light. The results indicate that Vitamin A was more light-sensitive whilst Vitamin E stability was more affected by temperature.

CHAPTER SIX

SIMULATION STUDY OF VITAMINS MIMICKING REAL CLINICAL SET-UP

6.1. Introduction

Besides considering the stability of vitamins during storage immediately after preparation or compounding, their stability must also be considered during administration, as some vitamins can undergo degradation during administration (Allwood and Kearney 1998).

This chapter investigates the stability of the water-soluble and fat-soluble vitamins in situations simulating hospital clinical practice relating to 7 days storage before use and 24 hours infusion. In addition, the increasing demand for PN for home patients and the fact that many studies have shown that patients receiving long term PN have inadequate vitamin status (Forbes and Forbes 1997; Steephen et al. 1991; Zak III et al. 1991), have created a demand for PN formulations with longer shelf lives. Thus, a clinical set-up with an extended 30 days storage period for the vitamins in minibag formulations mimicking use by home patients was also examined.

During the 24 hours infusion, multivitamin bags may be exposed to either artificial fluorescent light or to sunlight (ultraviolet light). This study therefore looked into the stability of the vitamins under these exposure conditions after their designated storage durations at 4°C.

It is also most convenient to add the water-soluble and the fat-soluble vitamins together to a minibag and infuse as one infusion fluid. In view of this, the stability of the vitamins as combined admixtures of Solivito® N Adult and Vitlipid® N Adult in normal saline minibags was investigated.

6.2. Methods

There were 3 different test solutions evaluated, which were Solivito® N Adult in 0.9% sodium chloride minibags (SV), Vitlipid® N Adult in 0.9% sodium chloride minibags (VL) and the combination of these fat and water-soluble vitamins in 0.9% sodium chloride minibags (CB).

6.2.1. Experimental Design

The minibags were stored in the pharmaceutical refrigerator for either one week or for 30 days at 4°C. This was followed by subjecting the minibags to either 25°C or 40°C in the pharmaceutical stability chamber (PSC) for the next 24 hours. One set of minibags wrapped with aluminium foil was placed on the PSC shelf without the lights turned on, another set was exposed to artificial fluorescent light having an illuminance of 1.2 klux/hour intensity, this light intensity being similar to exposure under extreme hospital unit lighting, such as in the operating theatre (Baker et al. 1993). The third set of minibags was placed on a shelf with ultraviolet A (UVA) lamps turned on, whereby the minibags were irradiated with UVA light of 3.5 Watts per square metre per hour ($W/m^2/h$). The total UVA irradiation was about 84 W/m^2, and this exposure simulates about 10 – 20 hours of exposure on a sunny window sill (Anderson and Byard 2004).

A summary of the experimental set-up is shown in Figure 6.1. Hence, there were four simulation conditions, two conditions at 7 days and two conditions at 30 days, i.e: 7 days storage in the refrigerator followed by 25°C or 40°C for 24 hours (7d25C and 7d40C), and 30 days storage in the refrigerator followed by 25°C or 40°C for 24 hours (30d25C and 30d40C). These simulation studies mimicked real clinical settings for best case (7d25C, light protect) and worst case (30d40C, exposed to UVA) storage and administration conditions for use by ward inpatients and home patients.

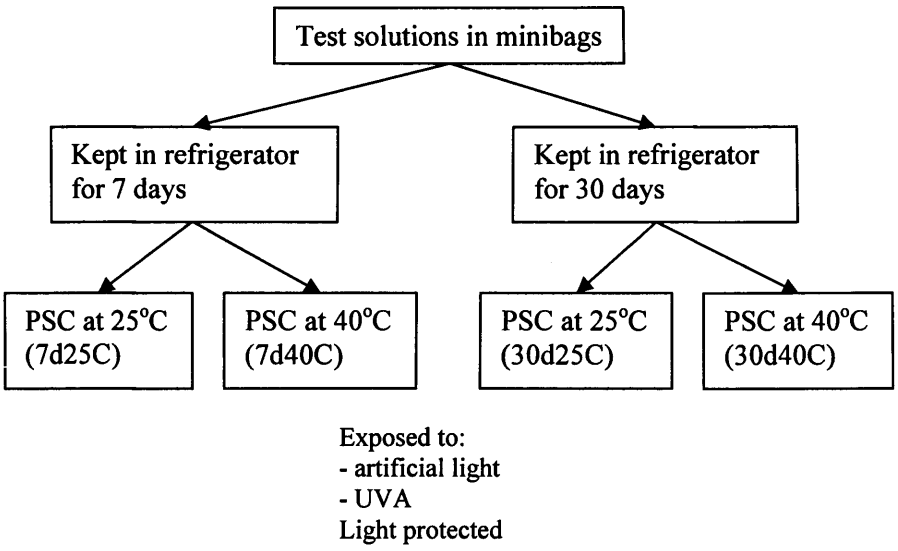


Figure 6.1: Experimental set-up of simulation study

6.2.2. Preparation of Test Solutions

A total of 18 minibags were compounded for each set of simulation conditions, with 2 bags required for chemical and physical analyses, for each of the three exposure conditions, carried out in triplicate.

Test solutions SV were prepared as in Chapter Four (see 4.2.2) while test emulsions VL were prepared as in Chapter Five (see 5.2.1).

Test emulsions CB were prepared in the following way:

The Solivito® N Adult injection vial was reconstituted with the 10 ml vial of Vitlipid® N Adult Injection. These reconstituted emulsions were then pooled to a 250 ml EVA Freka® IV-Bag (Fresenius Kabi). 10 ml of the reconstituted emulsion formulation was then syringed out and added to the 100 ml sterile 0.9% sodium chloride in Freeflex® minibag.

6.2.3. Sampling

For both physical and chemical analyses, samples were taken on day 0 before storage in the refrigerator, on day 7 or day 30, and then 24 hours after the temperature with/without light exposure. The physical and HPLC analyses carried out are described in Chapter Two.

For SV and VL samples, the sampling methods are described in Chapter Four for SV (see 4.2.3) and in Chapter Five for VL (see 5.2.2).

For CB, the method of sampling for its physical analysis is the same as for VL (see 5.2.2). For its chemical analysis, 3 ml samples were withdrawn from the minibags via the additive port using 21G needles and 5 ml syringes. 1.5 ml of the sample taken was placed into a microcentrifuge tube and another 1.5 ml sample into a 2 ml amber autosampler vial. Samples placed into the microcentifuge tubes were stored at -80°C in the pharmaceutical freezer, and analysed for the water-soluble vitamin components at the end of the study period for each simulation condition, as described in Chapter Two (see 2.3.3.2) and Chapter Four.

CB samples placed in the amber autosampler vials were stored in the pharmaceutical refrigerator and analysed within 3 days for analysis of the fat-soluble vitamin components, as described in Chapter Two (see 2.3.4.2). A fresh CB sample prepared in a 100 ml glass bottle was analysed together as a reference for fresh peaks, and adjustments were made in relation to the fresh reference peaks. Content analysis of samples at 0 hour was regarded as the baseline content of 100%.

For HPLC analysis, the peak areas of all the 9 samples on day 0 were averaged and the content analysis of day 0 was regarded as 100%.

6.3. Results

The following abbreviations are used to denote the exposure conditions after the stipulated storage durations:

FFlgt - minibags exposed to artificial fluorescent light, intensity 1.2 klux/h

FFuv - minibags irradiated with UVA, intensity 3.5 W/m^2/h

FPL - minibags protected from light

The abbreviations for the simulation conditions have been described under experimental design of this chapter.

6.3.1. Physical Analysis

6.3.1.1. Water-soluble Vitamin Solutions SV

6.3.1.1.1. Appearance

There was no change of colour when the SV samples were stored for either 7 days or for 30 days. No apparent colour change was observed when these samples were then exposed to the simulation conditions at 25°C. However, at 40°C, the following observations were noted, as in Table 6.1.

Table 6.1: Appearance of water-soluble vitamins SV for 7d40C and 30d40C simulation conditions

Exposure condition	*Day 0*	*Day 7/ 30*	*7d40C*	*30d40C*
FFlgt	LY; LGH	LY; LGH	PY; less intense LGH	PY; less intense LGH
FFuv	LY; LGH	LY; LGH	PY; less intense LGH	PY; less intense LGH
FPL	LY; LGH	LY; LGH	LY; LGH	LY; LGH

LY: light yellow, PY: pale yellow, LGH: light green haze

6.3.1.1.2. Turbidity

Turbidity measurement results are tabulated in Table 6.2.

Table 6.2: Results of turbidity measurement in NTU of SV

Simulation condition	*Exposure condition*	*Day 0 (n=9)*	*Day 7/30*	*7d/30d + 24 h*
7d25C	FFlgt	4.99 ± 0.07	4.99 ± 0.05	4.55 ± 0.18
	FFuv	4.99 ± 0.07	4.81 ± 0.10	3.54 ± 0.06 *
	FPL	4.99 ± 0.07	5.04 ± 0.14	4.91 ± 0.19
7d40C	FFlgt	4.85 ± 0.23	4.90 ± 0.18	2.24 ± 0.76 *
	FFuv	4.85 ± 0.23	4.90 ± 0.06	0.74 ± 0.08 *
	FPL	4.85 ± 0.23	4.94 ± 0.29	4.73 ± 0.29
30d25C	FFlgt	5.00 ± 0.12	4.90 ± 0.13	4.40 ± 0.23 *
	FFuv	5.00 ± 0.12	4.81 ± 0.10	3.54 ± 0.06 *
	FPL	5.00 ± 0.12	4.98 ± 0.10	4.90 ± 0.09
30d40C	FFlgt	4.94 ± 0.15	4.78 ± 0.37	1.36 ± 0.63 *
	FFuv	4.94 ± 0.15	4.88 ± 0.09	0.44 ± 0.01 *
	FPL	4.94 ± 0.15	4.88 ± 0.32	4.75 ± 0.34

*: values with change in NTU of more than 0.5 units

The NTU results above clearly show that there was a decrease in NTU units from the time of preparation at 0 hour. The change in NTU of more than 0.5 units (marked with asterisk *) were observed for 7d40C, 30d25C and 30d40C samples exposed to either artificial light or UVA light.

6.3.1.1.3. Dissolved Oxygen

The DO results of SV minibags are shown in Figure 6.2 and 6.3. They show increasing trend in DO when minibags were kept in the refrigerator, and then decreased upon exposure to higher temperatures.

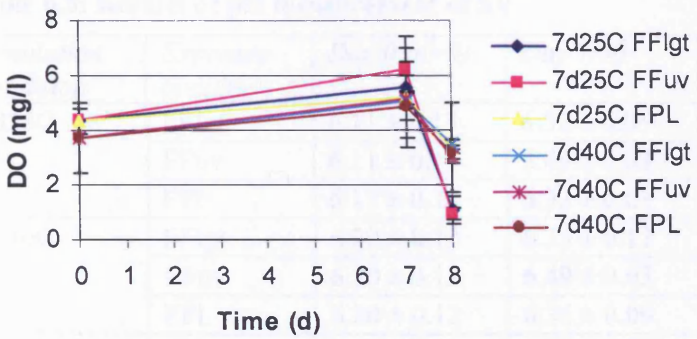


Figure 6.2: DO content of SV for 7 days + 24 hours simulation study

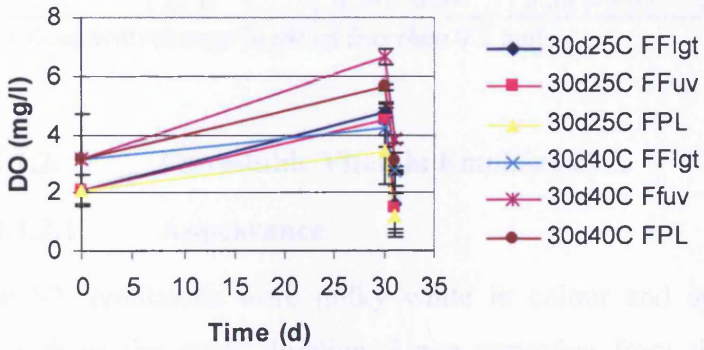


Figure 6.3: DO content of SV for 30 days + 24 hours simulation study

6.3.1.1.4. pH

The results of pH measurements of SV samples are tabulated in Table 6.3. Only the light protected SV sample exposed to 7d25C simulation condition had a pH change of less than 0.5 units.

Table 6.3: Results of pH measurement of SV

Simulation condition	*Exposure condition*	*Day 0 (n=9)*	*Day 7/30*	*7d/30d + 24 h*
7d25C	FFlgt	6.11 ± 0.13	6.32 ± 0.05	5.08 ± 0.18
	FFuv	6.11 ± 0.13	6.49 ± 0.03	5.13 ± 0.03
	FPL	6.11 ± 0.13	6.33 ± 0.03	5.84 ± 0.02 *
7d40C	FFlgt	6.20 ± 0.12	6.35 ± 0.12	4.43 ± 0.03
	FFuv	6.20 ± 0.12	6.49 ± 0.03	4.38 ± 0.02
	FPL	6.20 ± 0.12	6.36 ± 0.09	5.47 ± 0.02
30d25C	FFlgt	6.16 ± 0.11	5.61 ± 0.00	5.00 ± 0.02
	FFuv	6.16 ± 0.11	5.53 ± 0.01	4.80 ± 0.04
	FPL	6.16 ± 0.11	5.64 ± 0.04	5.44 ± 0.05
30d40C	FFlgt	6.24 ± 0.09	5.55 ± 0.02	4.34 ± 0.04
	FFuv	6.24 ± 0.09	5.48 ± 0.01	4.22 ± 0.01
	FPL	6.24 ± 0.09	5.58 ± 0.03	5.38 ± 0.08

*: values with change in pH of *less than* 0.5 units

6.3.1.2. Fat-soluble Vitamin Emulsions VL

6.3.1.2.1. Appearance

The VL emulsions were milky white in colour and appeared homogenous throughout the study duration. Upon removing from the refrigerator, some white emulsion was noted to stick on to the sides of the plastic bags but redispersed upon agitation and upon exposing to simulation temperatures.

6.3.1.2.2. Dissolved Oxygen

The results of DO content in VL minibags are shown in Figure 6.4 and Figure 6.5. The trend of DO contents of the VL minibags is similar to those of SV bags.

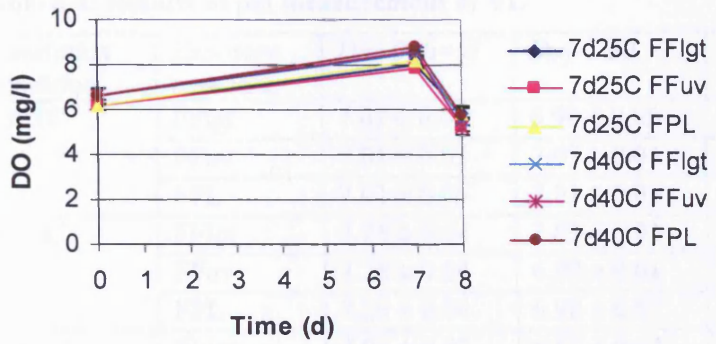


Figure 6.4: DO content of VL for 7 days + 24 hours simulation study

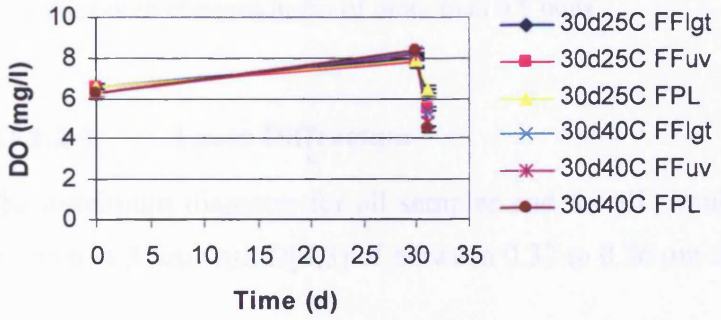


Figure 6.5: DO content of VL for 30 days + 24 hours simulation study

6.3.1.2.3. pH

The results of pH of VL minibags are shown in Table 6.4. From the table, VL minibags exposed to UVA during 40°C administration condition produced pH changes of more than 0.5 units.

Table 6.4: Results of pH measurement of VL

Simulation condition	*Exposure condition*	*Day 0 (n=9)*	*Day 7/30*	*7d/30d + 24 h*
7d25C	FFlgt	7.01 ± 0.03	6.99 ± 0.05	6.95 ± 0.06
	FFuv	7.01 ± 0.03	7.03 ± 0.04	6.82 ± 0.03
	FPL	7.01 ± 0.03	7.01 ± 0.03	6.99 ± 0.02
7d40C	FFlgt	7.26 ± 0.04	7.03 ± 0.01	6.92 ± 0.02
	FFuv	7.26 ± 0.04	6.99 ± 0.01	6.55 ± 0.07 *
	FPL	7.26 ± 0.04	6.98 ± 0.03	6.94 ± 0.01
30d25C	FFlgt	7.04 ± 0.02	6.63 ± 0.02	6.81 ± 0.01
	FFuv	7.04 ± 0.02	6.68 ± 0.01	6.71 ± 0.04
	FPL	7.04 ± 0.02	6.65 ± 0.01	6.82 ± 0.01
30d40C	FFlgt	7.08 ± 0.15	6.64 ± 0.07	6.81 ± 0.01
	FFuv	7.08 ± 0.15	6.54 ± 0.04	6.54 ± 0.01 *
	FPL	7.08 ± 0.15	6.54 ± 0.05	6.78 ± 0.05

*: values with changes in pH of more than 0.5 units

6.3.1.2.4. Laser Diffraction

The maximum diameter for all samples and for all simulation conditions was less than 4.3 μm with D[4,3] of between 0.32 to 0.36 μm throughout the study.

6.3.1.2.5. Light Microscopy

All samples had lipid globules sizes of less than 7.5 μm diameter and were seen as individual globules with occasional 5 μm size globules seen.

6.3.1.3. Fat-soluble and Water-soluble Vitamin Admixtures CB

6.3.1.3.1. Appearance

For the CB samples, the presented admixtures were a homogenous milky yellow and had a milky white meniscus. There was no change in appearance when kept in the refrigerator although some milky yellow emulsion was noted to stick to the side of the plastic bags. The admixture returned to a homogenous emulsion upon gentle agitation. No change in appearance was noted upon exposure at 25°C after the storage durations in the refrigerator, but a decrease

in colour intensity was noted upon exposure to 40°C and light, as shown in Table 6.5.

Table 6.5: Appearance of Combined Fat and Water-soluble Vitamin Admixtures CB

Exposure condition	*Day 0*	*Day 7/ 30*	*7 days, then 40°C for 24 h*	*30 days, then 40°C for 24 h*
FFlgt	MY	MY	PMY	PMY
FFuv	MY	MY	PMY	PMY
FPL	MY	MY	MY	MY

MY: milky yellow; PMY: pale milky yellow

6.3.1.3.2. Dissolved Oxygen

The results of DO mesurement of CB minibags are tabulated in Table 6.6 while the graphs are depicted in Figure 6.6 and Figure 6.7.

Table 6.6: Results of DO measurement in mg/l for CB

Simulation condition	*Exposure condition*	*Day 0 (n=9)*	*Day 7/30*	*7d/30d + 24 h*
7d25C	FFlgt	4.93 ± 0.56	6.32 ± 0.06	1.35 ± 0.33
	Ffuv	4.93 ± 0.56	5.55 ± 0.56	1.13 ± 0.18
	FPL	4.93 ± 0.56	5.71 ± 1.65	4.46 ± 0.56
7d40C	FFlgt	3.89 ± 0.55	5.94 ± 0.22	2.45 ± 0.30
	Ffuv	3.89 ± 0.55	5.66 ± 0.51	2.73 ± 0.27
	FPL	3.89 ± 0.55	6.18 ± 0.27	3.38 ± 0.21
30d25C	FFlgt	3.38 ± 0.33	5.51 ± 0.55	1.10 ± 0.53
	Ffuv	3.38 ± 0.33	5.26 ± 0.22	1.74 ± 0.71
	FPL	3.38 ± 0.33	5.37 ± 0.57	2.22 ± 0.34
30d40C	FFlgt	4.97 ± 0.71	1.26 ± 0.43	2.76 ± 0.11
	Ffuv	4.97 ± 0.71	2.81 ± 0.65	3.83 ± 0.40
	FPL	4.97 ± 0.71	4.66 ± 0.67	3.07 ± 0.32

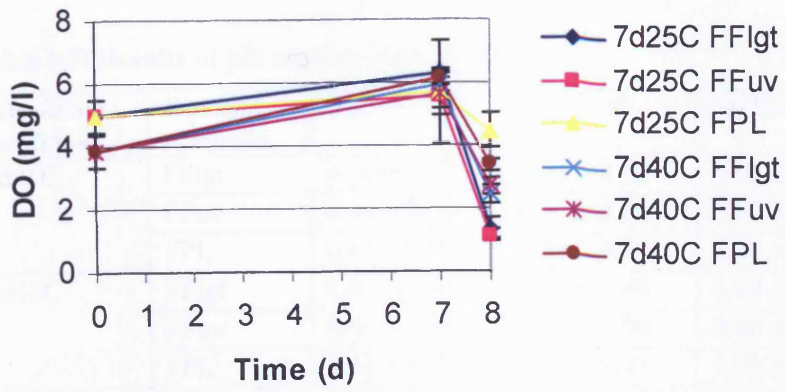


Figure 6.6: DO for CB minibags 7 days + 24 hours simulation study

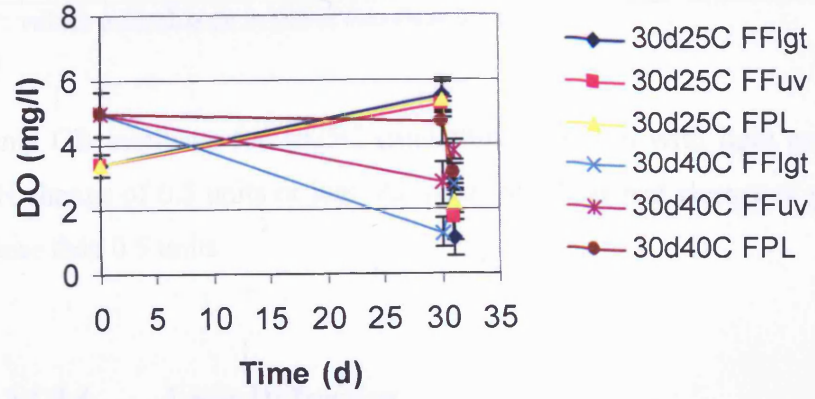


Figure 6.7: DO for CB minibags 30 days + 24 hours simulation study

6.3.1.3.3. pH

The results of pH measurements of CB minibags are tabulated in Table 6.7.

Table 6.7: Results of pH measurement of CB

Simulation condition	*Exposure condition*	*Day 0 (n=9)*	*Day 7/30*	*7d/30d + 24 h*
7d25C	FFlgt	6.44 ± 0.03	6.35 ± 0.03	5.10 ± 0.04
	FFuv	6.44 ± 0.03	6.33 ± 0.05	5.13 ± 0.08
	FPL	6.44 ± 0.03	6.41 ± 0.10	5.94 ± 0.10 *
7d40C	FFlgt	6.41 ± 0.06	6.34 ± 0.04	4.69 ± 0.02
	FFuv	6.41 ± 0.06	6.35 ± 0.00	4.66 ± 0.00
	FPL	6.41 ± 0.06	6.34 ± 0.01	5.75 ± 0.04
30d25C	FFlgt	6.30 ± 0.03	5.73 ± 0.03	4.82 ± 0.16
	FFuv	6.30 ± 0.03	5.68 ± 0.01	4.71 ± 0.01
	FPL	6.30 ± 0.03	5.70 ± 0.03	5.58 ± 0.01
30d40C	FFlgt	6.27 ± 0.06	5.93 ± 0.01	4.56 ± 0.02
	FFuv	6.27 ± 0.06	5.90 ± 0.04	4.54 ± 0.03
	FPL	6.27 ± 0.06	5.89 ± 0.04	5.66 ± 0.01

*: values with change in pH of *less than* 0.5 units

Only CB minibags for 7d25C simulation condition with light protection had pH change of 0.5 units or less. All other minibags had change of pH values of more than 0.5 units.

6.3.1.3.4. Laser Diffraction

For the simulation temperature of 25°C after the stipulated storage durations in the refrigerator, all samples had a maximum diameter of less than 4.3 μm with a D[4,3] of 0.35 to 0.37 μm.

However, for the simulation temperature of 40°C after the storage durations in the refrigerator, the samples had a maximum diameter of more than 15.04 μm, with the percentage having diameter more than 4.3 μm ranging from 0.36% to 2.0%. The D[4,3] values had increments ranging from 0.36/0.37 μm at time 0 hour to 0.36 μm – 0.49 μm at the end of the simulation conditions.

6.3.1.3.5. Light Microscopy

There was no change observed in the number and size of the lipid globules for the 7d25C simulation condition. All the lipid globules remained at less than 7.5 μm with an occasional 5 μm globule seen. For 30d25C simulation condition, the number of 5 μm lipid globules had increased, with one or two 7.5 μm globules seen and one sample that was exposed to UVA had one 10 μm globule observed.

For 7d40C and 30d40C simulation conditions, there was no obvious change of the size of the lipid globules during storage in the refrigerator observed. However upon exposure at 40°C, 7.5 μm size lipid globules were observed with a range from 10 – 12 in numbers. Larger globule sizes were also observed; a 25 μm lipid globule size was observed for the 7d40C simulation condition while a 40 μm lipid globule size was observed for the 30d40C simulation condition.

6.3.2. Chemical Analysis

6.3.2.1. Water-soluble Vitamins in SV

The results of pyridoxine, nicotinamide, thiamine, folic acid and riboflavin sodium phosphate in SV solutions are tabulated from Table 6.8 to Table 6.12, while the graphs are displayed from Figure 6.8 to Figure 6.17.

6.3.2.1.1. Pyridoxine

Table 6.8: Percentage content of pyridoxine in SV upon simulation conditions

Simulation condition	*Exposure condition*	*Day 0 %*	*Day 7/30 %*	*7d/30d + 24 h %*
7d25C	FFlgt	100 ± 0	97.21 ± 0.39	88.74 ± 3.80
	FFuv	100 ± 0	96.17 ± 3.99	56.60 ± 3.30
	FPL	100 ± 0	95.01 ± 0.78	96.00 ± 3.06
7d40C	FFlgt	100 ± 0	98.09 ± 2.73	87.47 ± 2.01
	FFuv	100 ± 0	98.58 ± 1.33	46.12 ± 1.23
	FPL	100 ± 0	95.98 ± 3.80	95.21 ± 2.29
30d25C	FFlgt	100 ± 0	95.16 ± 2.24	86.34 ± 3.33
	FFuv	100 ± 0	95.68 ± 5.86	58.57 ± 1.62
	FPL	100 ± 0	97.23 ± 1.52	92.82 ± 2.07
30d40C	FFlgt	100 ± 0	93.51 ± 1.14	92.23 ± 3.79
	FFuv	100 ± 0	92.84 ± 5.55	45.58 ± 1.90
	FPL	100 ± 0	96.54 ± 2.52	94.21 ± 4.99

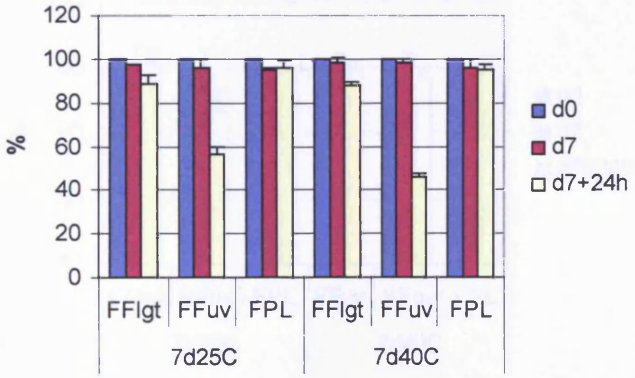


Figure 6.8: Content of pyridoxine in SV for 7d25C and 7d40C simulation conditions

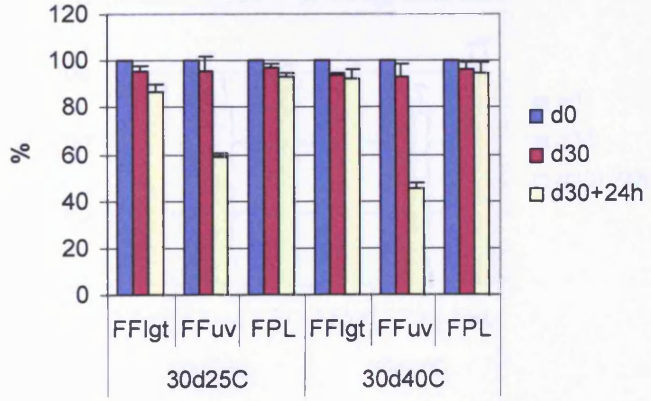


Figure 6.9: Content of pyridoxine in SV for 30d25C and 30d40C simulation conditions

6.3.2.1.2. Nicotinamide

Table 6.9: Percentage content of nicotinamide in SV upon simulation conditions

Simulation condition	*Exposure condition*	*Day 0 %*	*Day 7/30 %*	*7d/30d + 24 h %*
7d25C	FFlgt	100 ± 0	100.90 ± 0.21	100.34 ± 2.38
	FFuv	100 ± 0	99.18 ± 4.48	93.28 ± 3.50
	FPL	100 ± 0	98.84 ± 1.33	99.41 ± 0.58
7d40C	FFlgt	100 ± 0	100.66 ± 1.09	99.55 ± 1.31
	FFuv	100 ± 0	100.86 ± 1.60	98.65 ± 1.04
	FPL	100 ± 0	99.70 ± 2.52	99.93 ± 1.68
30d25C	FFlgt	100 ± 0	99.12 ± 0.68	97.83 ± 0.64
	FFuv	100 ± 0	97.13 ± 5.55	95.34 ± 3.52
	FPL	100 ± 0	100.41 ± 1.06	98.88 ± 2.29
30d40C	FFlgt	100 ± 0	99.39 ± 0.87	99.60 ± 1.46
	FFuv	100 ± 0	93.33 ± 1.27	94.20 ± 3.64
	FPL	100 ± 0	100.80 ± 3.26	98.53 ± 5.90

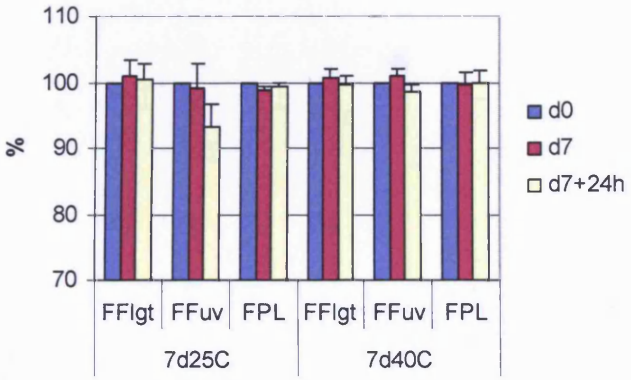


Figure 6.10: Content of nicotinamide in SV for 7d25C and 7d40C simulation conditions

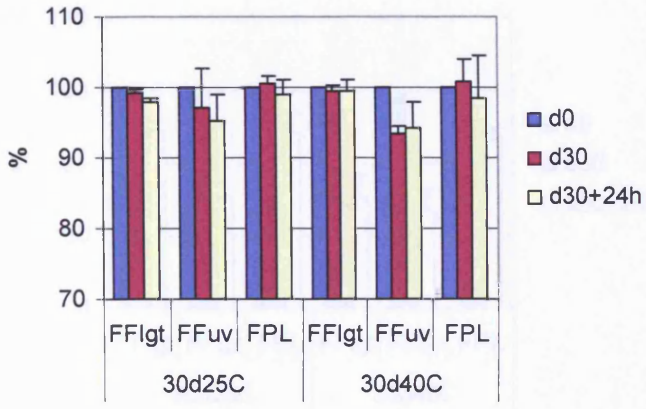


Figure 6.11: Content of nicotinamide in SV for 30d25C and 30d40C simulation conditions

6.3.2.1.3. Thiamine

Table 6.10: Percentage content of thiamine in SV upon simulation conditions

Simulation condition	*Exposure condition*	*Day 0 %*	*Day 7/30 %*	*7d/30d + 24 h %*
7d25C	FFlgt	100 ± 0	97.99 ± 0.91	96.14 ± 2.99
	FFuv	100 ± 0	97.33 ± 4.00	88.18 ± 2.87
	FPL	100 ± 0	95.95 ± 2.24	96.15 ± 3.29
7d40C	FFlgt	100 ± 0	99.47 ± 2.45	93.87 ± 1.75
	FFuv	100 ± 0	104.00 ± 0.62	87.50 ± 2.24
	FPL	100 ± 0	96.75 ± 2.12	97.28 ± 0.40
30d25C	FFlgt	100 ± 0	97.50 ± 1.65	94.69 ± 1.29
	FFuv	100 ± 0	95.14 ± 5.51	85.48 ± 2.19
	FPL	100 ± 0	99.29 ± 1.98	94.27 ± 2.55
30d40C	FFlgt	100 ± 0	95.37 ± 1.97	85.06 ± 3.63
	Ffuv	100 ± 0	93.80 ± 1.14	83.40 ± 2.76
	FPL	100 ± 0	95.88 ± 1.93	93.27 ± 1.48

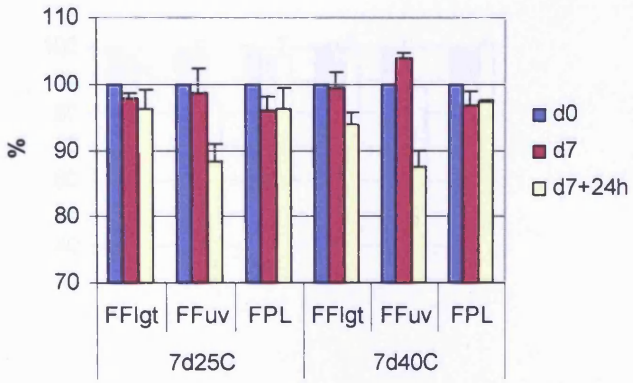


Figure 6.12: Content of thiamine in SV for 7d25C and 7d40C simulation conditions

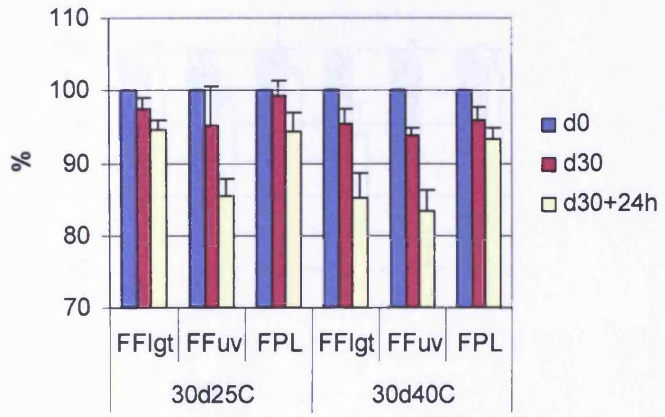


Figure 6.13: Content of thiamine in SV for 30d25C and 30d40C simulation conditions

6.3.2.1.4. Folic Acid

Table 6.11: Percentage content of folic acid in SV upon simulation conditions

Simulation condition	*Exposure condition*	*Day 0 %*	*Day 7/30 %*	*7d/30d + 24 h %*
7d25C	FFlgt	100 ± 0	96.69 ± 1.15	92.29 ± 1.97
	FFuv	100 ± 0	99.45 ± 2.47	82.38 ± 5.99
	FPL	100 ± 0	95.41 ± 1.11	97.34 ± 5.53
7d40C	FFlgt	100 ± 0	100.37 ± 1.76	99.28 ± 5.07
	FFuv	100 ± 0	100.22 ± 1.66	87.87 ± 1.94
	FPL	100 ± 0	97.12 ± 1.24	100.87 ± 1.24
30d25C	FFlgt	100 ± 0	96.39 ± 5.20	95.70 ± 6.56
	FFuv	100 ± 0	95.51 ± 4.67	85.73 ± 2.22
	FPL	100 ± 0	101.28 ± 8.79	94.12 ± 1.10
30d40C	FFlgt	100 ± 0	96.95 ± 1.82	76.83 ± 8.87
	FFuv	100 ± 0	96.40 ± 6.51	40.58 ± 6.03
	FPL	100 ± 0	96.48 ± 5.10	94.54 ± 3.79

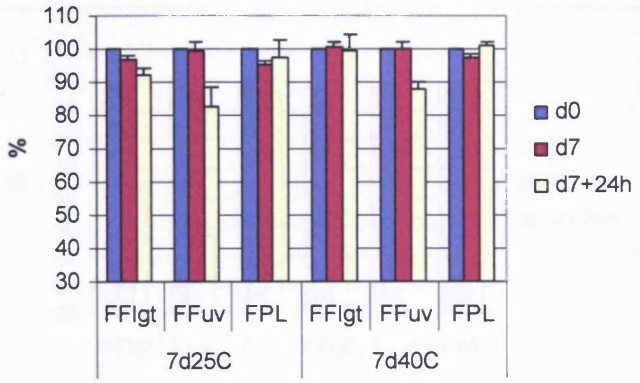


Figure 6.14: Content of folic acid in SV for 7d25C and 7d40C simulation conditions

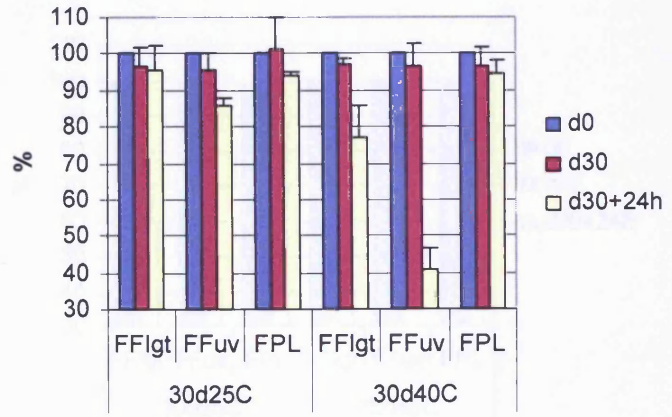


Figure 6.15: Content of folic acid in SV for 30d25C and 30d40C simulation conditions

6.3.2.1.5. Riboflavin Sodium Phosphate

Table 6.12: Percentage content of riboflavin sodium phosphate in SV upon simulation conditions

Simulation condition	*Exposure condition*	*Day 0 %*	*Day 7/30 %*	*7d/30d + 24 h %*
7d25C	FFlgt	100 ± 0	99.73 ± 4.88	62.78 ± 1.71
	FFuv	100 ± 0	95.94 ± 3.21	47.56 ± 2.75
	FPL	100 ± 0	97.54 ± 5.41	95.51 ± 1.64
7d40C	FFlgt	100 ± 0	97.26 ± 2.11	39.72 ± 3.17
	FFuv	100 ± 0	99.33 ± 1.43	20.11 ± 1.34
	FPL	100 ± 0	96.26 ± 1.61	92.75 ± 3.17
30d25C	FFlgt	100 ± 0	95.28 ± 1.99	59.99 ± 6.29
	FFuv	100 ± 0	94.20 ± 4.47	40.11 ± 3.05
	FPL	100 ± 0	96.41 ± 0.60	95.20 ± 2.14
30d40C	FFlgt	100 ± 0	93.83 ± 0.58	37.32 ± 8.27
	FFuv	100 ± 0	93.27 ± 4.16	8.63 ± 2.32
	FPL	100 ± 0	94.15 ± 0.21	92.13 ± 1.71

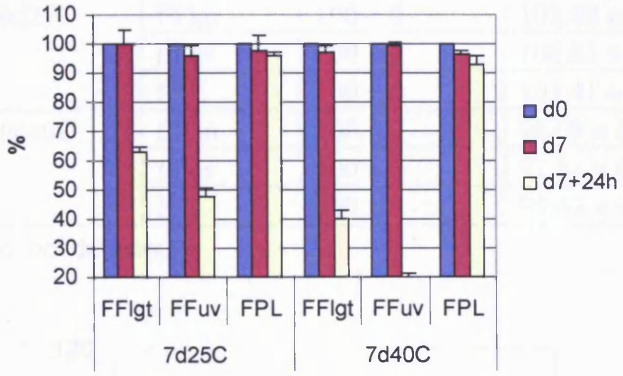


Figure 6.16: Content of riboflavine sodium phosphate in SV for 7d25C and 7d40C simulation conditions

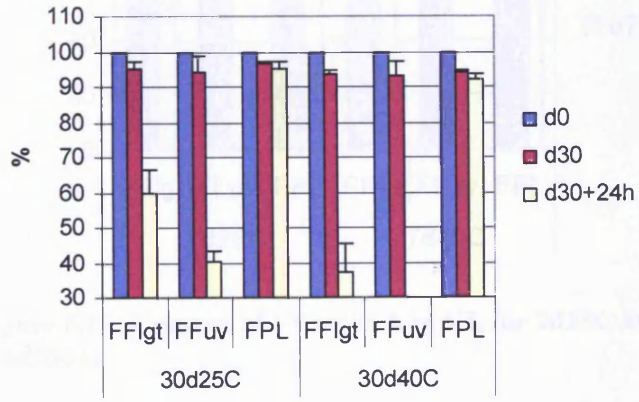


Figure 6.17: Content of riboflavin sodium phosphate in SV for 30d25C and 30d40C simulation conditions

6.3.2.2. Fat-soluble vitamins in VL

The results of Vitamin A (retinol palmitate) and Vitamin E (tocopherol) in VL emulsions are tabulated from Table 6.13 to Table 6.14, while the graphs are displayed from Figure 6.18 to Figure 6.21.

6.3.2.2.1. Vitamin A

Table 6.13: Percentage content of Vitamin A in VL upon simulation conditions

Simulation condition	*Exposure condition*	*Day 0 (N=9) %*	*Day 7/30 %*	*7d/30d + 24 h %*
7d25C	FFlgt	100 ± 0	100.55 ± 0.94	83.35 ± 3.21
	FFuv	100 ± 0	100.21 ± 1.45	1.20 ± 0.11
	FPL	100 ± 0	101.17 ± 0.62	97.09 ± 0.35
7d40C	FFlgt	100 ± 0	100.70 ± 0.70	84.35 ± 1.36
	FFuv	100 ± 0	102.03 ± 0.19	nd
	FPL	100 ± 0	100.60 ± 1.17	99.87 ± 0.23
30d25C	FFlgt	100 ± 0	103.88 ± 4.07	88.81 ± 5.42
	FFuv	100 ± 0	106.05 ± 3.10	nd
	FPL	100 ± 0	103.41 ± 1.62	98.19 ± 1.36
30d40C	FFlgt	100 ± 0	96.09 ± 0.67	78.91 ± 0.81
	FFuv	100 ± 0	92.31 ± 0.17	nd
	FPL	100 ± 0	95.63 ± 0.94	92.31 ± 1.00

nd: not detected

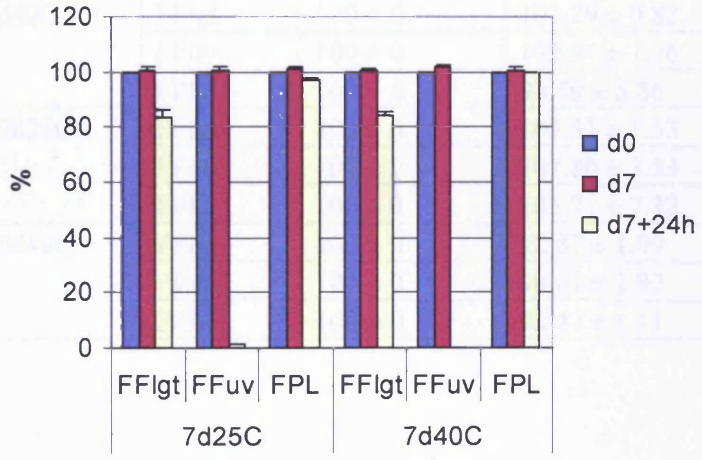


Figure 6.18: Content of Vitamin A in VL for 7d25C and 7d40C simulation conditions

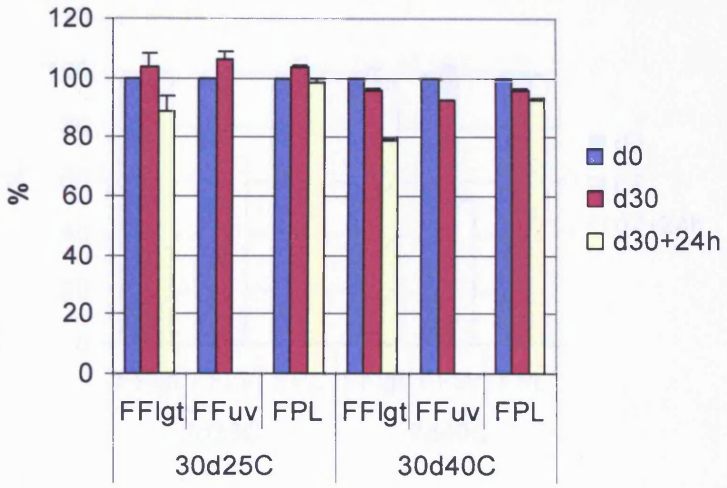


Figure 6.19: Content of Vitamin A in VL for 30d25C and 30d40C simulation conditions

6.3.2.2.2. Vitamin E

Table 6.14: Percentage content of Vitamin E in VL upon simulation conditions

Simulation condition	*Exposure condition*	*Day 0 %*	*Day 7/30 %*	*7d/30d + 24 h %*
7d25C	FFlgt	100 ± 0	99.93 ± 6.01	100.26 ± 0.55
	FFuv	100 ± 0	104.07 ± 0.39	68.65 ± 0.38
	FPL	100 ± 0	98.79 ± 6.31	100.00 ± 1.85
7d40C	FFlgt	100 ± 0	101.29 ± 0.82	93.63 ± 2.33
	FFuv	100 ± 0	102.44 ± 1.76	53.44 ± 0.36
	FPL	100 ± 0	98.59 ± 5.86	99.17 ± 0.45
30d25C	FFlgt	100 ± 0	103.51 ± 1.53	100.18 ± 6.17
	FFuv	100 ± 0	107.80 ± 3.53	65.13 ± 0.73
	FPL	100 ± 0	103.71 ± 7.32	98.70 ± 1.48
30d40C	FFlgt	100 ± 0	92.87 ± 1.09	75.95 ± 3.89
	FFuv	100 ± 0	90.61 ± 1.92	37.51 ± 4.06
	FPL	100 ± 0	92.47 ± 1.11	83.66 ± 1.74

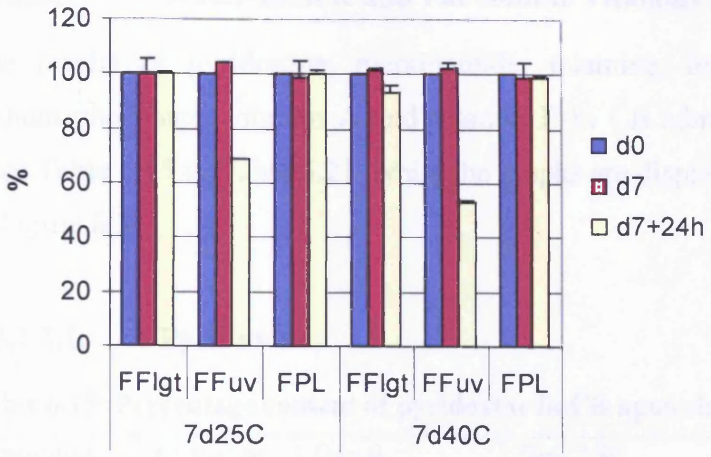


Figure 6.20: Content of Vitamin E in VL for 7d25C and 7d40C simulation conditions

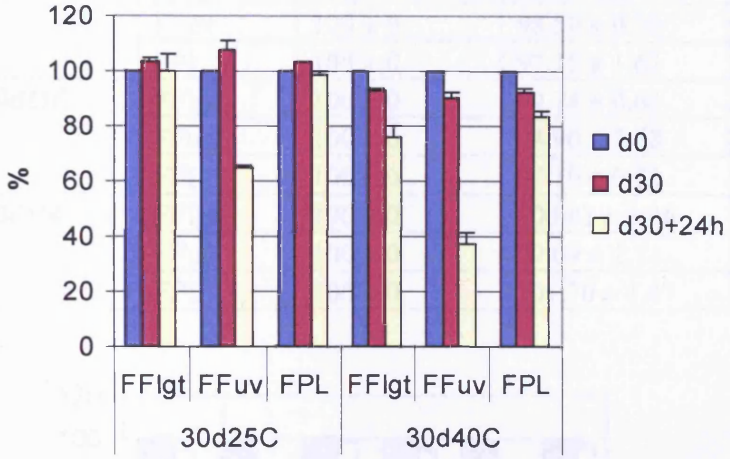


Figure 6.21: Content of Vitamin E in VL for 30d25C and 30d40C simulation conditions

6.3.2.3. Water-soluble and Fat-soluble Vitamins in CB Admixtures

The results of pyridoxine, nicotinamide, thiamine, folic acid, riboflavine sodium phosphate, vitamin A and vitamin E in CB admixtures are tabulated from Table 6.15 to Table 6.21, while the graphs are displayed from Figure 6.21 to Figure 6.34.

6.3.2.3.1. Pyridoxine

Table 6.15: Percentage content of pyridoxine in CB upon simulation conditions

Simulation condition	*Exposure condition*	*Day 0 %*	*Day 7/30 %*	*7d/30d + 24 h %*
7d25C	FFlgt	100 ± 0	99.70 ± 1.13	94.42 ± 0.26
	FFuv	100 ± 0	99.88 ± 0.65	76.89 ± 1.10
	FPL	100 ± 0	98.49 ± 1.99	98.08 ± 1.57
7d40C	FFlgt	100 ± 0	97.77 ± 0.45	94.01 ± 1.57
	FFuv	100 ± 0	98.50 ± 0.39	65.26 ± 0.14
	FPL	100 ± 0	97.15 ± 1.62	96.51 ± 2.99
30d25C	FFlgt	100 ± 0	99.74 ± 0.64	95.67 ± 0.92
	FFuv	100 ± 0	94.90 ± 3.68	76.06 ± 1.66
	FPL	100 ± 0	97.19 ± 0.32	93.11 ± 4.52
30d40C	FFlgt	100 ± 0	100.43 ± 1.36	99.16 ± 3.90
	FFuv	100 ± 0	99.09 ± 2.71	69.97 ± 1.70
	FPL	100 ± 0	101.70 ± 1.47	99.12 ± 4.03

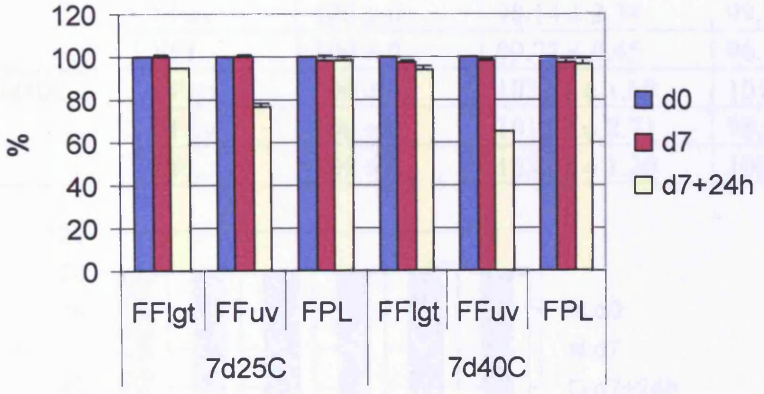


Figure 6.22: Content of pyridoxine in CB for 7d25C and 7d40C simulation conditions

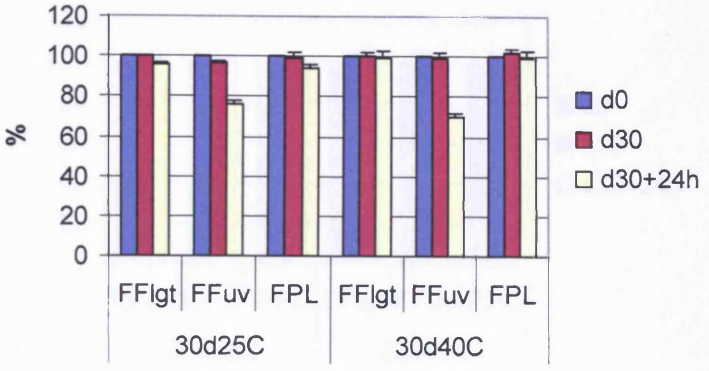


Figure 6.23: Content of pyridoxine in CB for 30d25C and 30d40C simulation conditions

6.3.2.3.2. Nicotinamide

Table 6.16: Percentage content of nicotinamide in CB upon simulation conditions

Simulation condition	*Exposure condition*	*Day 0 %*	*Day 7/30 %*	*7d/30d + 24 h %*
7d25C	FFlgt	100 ± 0	100.90 ± 1.37	98.74 ± 0.64
	FFuv	100 ± 0	100.58 ± 0.41	100.35 ± 0.80
	FPL	100 ± 0	99.83 ± 2.15	100.05 ± 1.57
7d40C	FFlgt	100 ± 0	99.38 ± 0.69	98.82 ± 1.58
	FFuv	100 ± 0	101.96 ± 3.03	102.51 ± 2.75
	FPL	100 ± 0	97.76 ± 1.77	98.21 ± 2.99
30d25C	FFlgt	100 ± 0	101.32 ± 0.60	100.67 ± 0.72
	FFuv	100 ± 0	98.14 ± 2.34	99.83 ± 1.51
	FPL	100 ± 0	99.77 ± 0.45	96.53 ± 3.75
30d40C	FFlgt	100 ± 0	102.61 ± 1.69	101.59 ± 4.11
	FFuv	100 ± 0	101.57 ± 2.71	98.00 ± 1.32
	FPL	100 ± 0	103.52 ± 1.20	100.01 ± 3.77

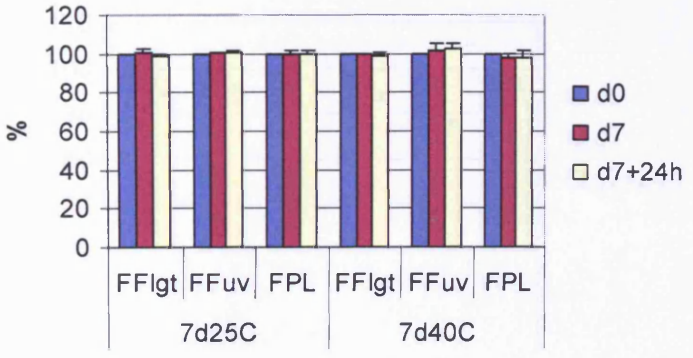


Figure 6.24: Content of nicotinamide in CB for 7d25C and 7d40C simulation conditions

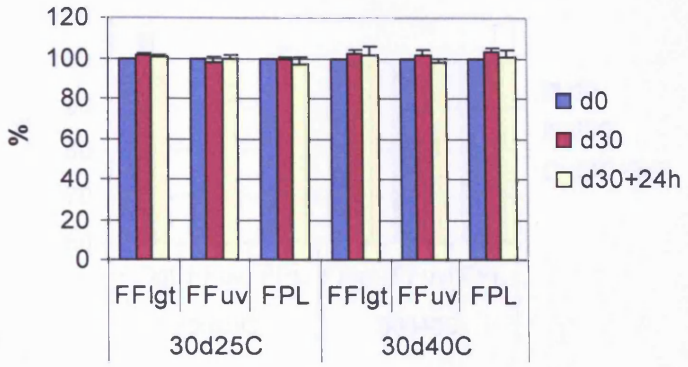


Figure 6.25: Content of nicotinamide in CB for 30d25C and 30d40C simulation conditions

6.3.2.3.3. Thiamine

Table 6.17: Percentage content of thiamine in CB upon simulation conditions

Simulation condition	*Exposure condition*	*Day 0 %*	*Day 7/30 %*	*7d/30d + 24 h %*
7d25C	FFlgt	100 ± 0	103.31 ± 0.83	94.26 ± 0.70
	FFuv	100 ± 0	100.98 ± 0.43	94.97 ± 1.49
	FPL	100 ± 0	100.17 ± 1.44	101.62 ± 1.97
7d40C	FFlgt	100 ± 0	97.13 ± 1.06	92.97 ± 1.55
	FFuv	100 ± 0	98.32 ± 0.66	87.25 ± 1.02
	FPL	100 ± 0	97.00 ± 0.88	95.06 ± 3.29
30d25C	FFlgt	100 ± 0	98.56 ± 1.15	99.19 ± 1.89
	FFuv	100 ± 0	97.24 ± 3.90	92.60 ± 1.19
	FPL	100 ± 0	101.28 ± 1.23	99.13 ± 8.99
30d40C	FFlgt	100 ± 0	102.61 ± 1.69	101.59 ± 4.11
	FFuv	100 ± 0	101.57 ± 2.71	98.00 ± 1.32
	FPL	100 ± 0	103.52 ± 1.20	100.01 ± 3.77

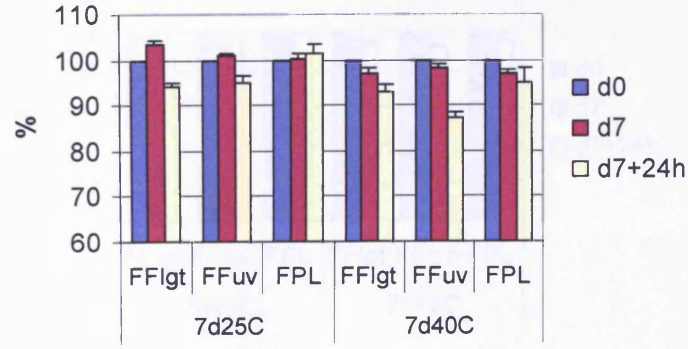


Figure 6.26: Content of thiamine in CB for 7d25C and 7d40C simulation conditions

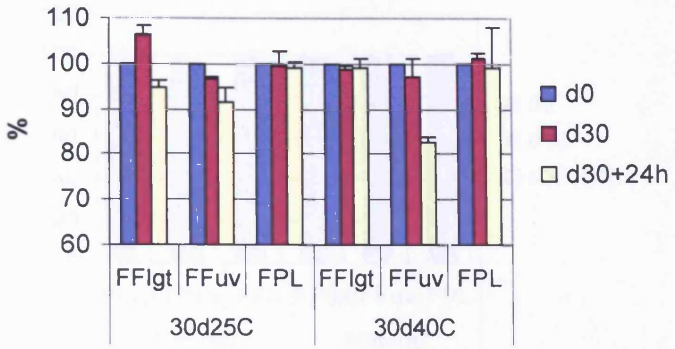


Figure 6.27: Content of thiamine in CB for 30d25C and 30d40C simulation conditions

6.3.2.3.4. Folic acid

Table 6.18: Percentage content of folic acid in CB upon simulation conditions

Simulation condition	*Exposure condition*	*Day 0* *%*	*Day 7/30* *%*	*7d/30d + 24 h* *%*
7d25C	FFlgt	100 ± 0	98.84 ± 0.73	95.25 ± 0.53
	FFuv	100 ± 0	100.10 ± 0.69	96.18 ± 0.26
	FPL	100 ± 0	98.17 ± 3.17	98.97 ± 2.00
7d40C	FFlgt	100 ± 0	97.80 ± 0.45	92.77 ± 1.82
	FFuv	100 ± 0	99.09 ± 0.56	92.01 ± 1.28
	FPL	100 ± 0	97.13 ± 1.43	95.62 ± 3.17
30d25C	FFlgt	100 ± 0	99.44 ± 2.98	97.04 ± 2.21
	FFuv	100 ± 0	97.17 ± 1.16	91.76 ± 2.75
	FPL	100 ± 0	98.78 ± 3.21	93.16 ± 3.65
30d40C	FFlgt	100 ± 0	97.67 ± 1.63	89.45 ± 0.73
	FFuv	100 ± 0	96.23 ± 2.77	68.16 ± 1.86
	FPL	100 ± 0	99.43 ± 1.29	94.23 ± 1.60

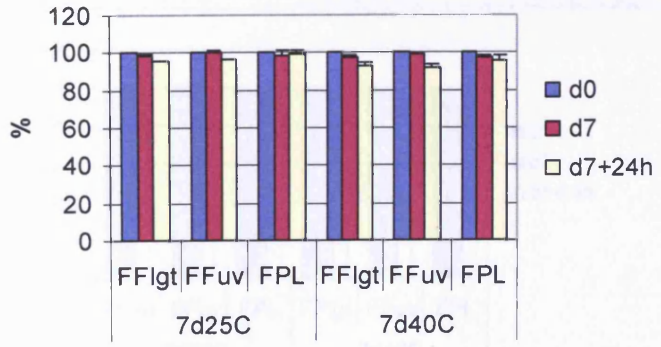


Figure 6.28: Content of folic acid in CB for 7d25C and 7d40C simulation conditions

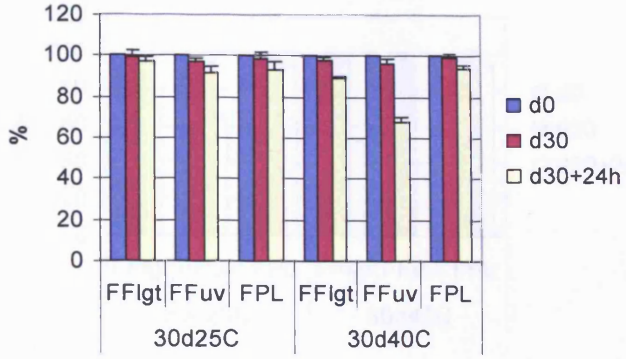


Figure 6.29: Content of folic acid in CB for 30d25C and 30d40C simulation conditions

6.3.2.3.5. Riboflavin Sodium Phosphate

Table 6.19: Percentage content of riboflavin sodium phosphate in CB upon simulation conditions

Simulation condition	*Exposure condition*	*Day 0 %*	*Day 7/30 %*	*7d/30d + 24 h %*
7d25C	FFlgt	100 ± 0	98.50 ± 1.04	73.33 ± 0.59
	FFuv	100 ± 0	101.31 ± 0.69	62.73 ± 2.02
	FPL	100 ± 0	96.70 ± 4.70	99.63 ± 1.64
7d40C	FFlgt	100 ± 0	98.34 ± 0.35	63.06 ± 3.60
	FFuv	100 ± 0	99.42 ± 0.22	43.18 ± 1.16
	FPL	100 ± 0	97.93 ± 1.41	98.90 ± 5.63
30d25C	FFlgt	100 ± 0	96.82 ± 0.93	71.14 ± 1.09
	FFuv	100 ± 0	95.29 ± 2.84	63.27 ± 0.93
	FPL	100 ± 0	96.28 ± 2.47	92.87 ± 0.97
30d40C	FFlgt	100 ± 0	98.45 ± 0.50	52.57 ± 1.45
	FFuv	100 ± 0	97.00 ± 3.17	31.42 ± 0.68
	FPL	100 ± 0	101.25 ± 1.61	93.90 ± 0.65

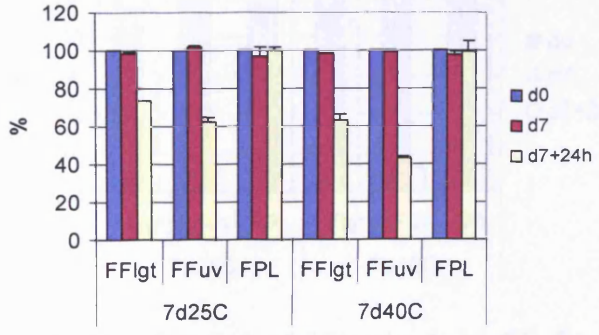


Figure 6.30: Content of riboflavin sodium phosphate in CB for 7d25C and 7d40C simulation conditions

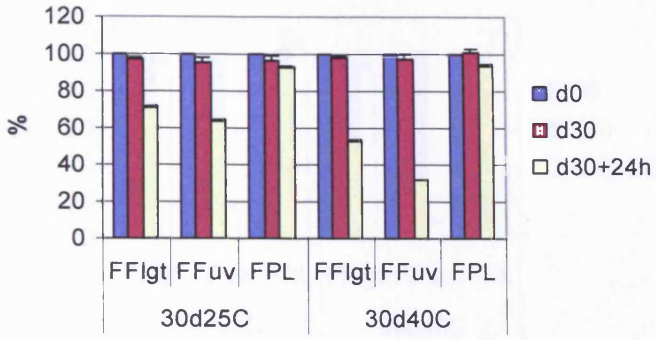


Figure 6.31: Content of riboflavin sodium phosphate in CB for 30d25C and 30d40C simulation conditions

6.3.2.3.6. Vitamin A

Table 6.20: Percentage content of Vitamin A in CB upon simulation conditions

Simulation condition	*Exposure condition*	*Day 0 %*	*Day 7/30 %*	*7d/30d + 24 h %*
7d25C	FFlgt	100 ± 0	94.49 ± 1.39	95.59 ± 1.97
	Ffuv	100 ± 0	101.41 ± 3.02	8.67 ± 0.35
	FPL	100 ± 0	96.00 ± 0.45	106.44 ± 2.22
7d40C	FFlgt	100 ± 0	103.97 ± 1.45	85.59 ± 3.11
	Ffuv	100 ± 0	105.72 ± 0.32	12.30 ± 0.42
	FPL	100 ± 0	105.92 ± 1.29	95.30 ± 0.91
30d25C	FFlgt	100 ± 0	97.94 ± 1.24	96.27 ± 0.81
	Ffuv	100 ± 0	96.99 ± 0.36	15.54 ± 3.13
	FPL	100 ± 0	102.42 ± 9.97	103.82 ± 3.32
30d40C	FFlgt	100 ± 0	96.92 ± 3.23	92.28 ± 2.00
	Ffuv	100 ± 0	99.99 ± 1.43	8.27 ± 3.17
	FPL	100 ± 0	95.49 ± 2.42	111.77 ± 2.03

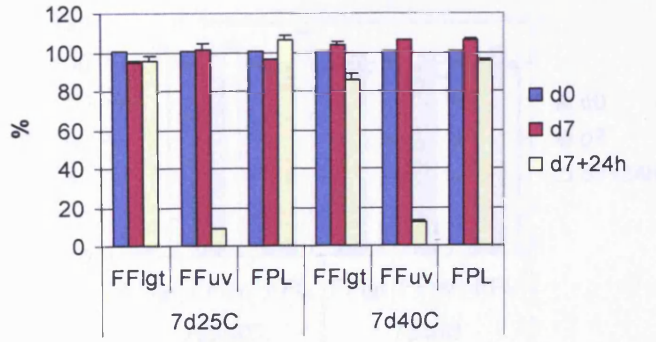


Figure 6.32: Content of Vitamin A in CB for 7d25C and 7d40C simulation conditions

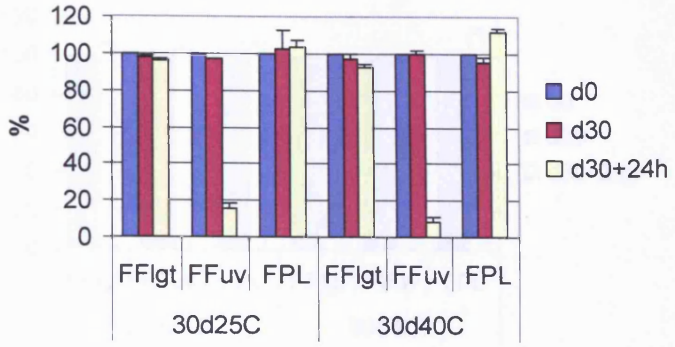


Figure 6.33. Content of Vitamin A in CB for 30d25C and 30d40C simulation conditions

6.3.2.3.7. Vitamin E

Table 6.21: Percentage content of Vitamin E in CB upon simulation conditions

Simulation condition	*Exposure condition*	*Day 0* *%*	*Day 7/30* *%*	*7d/30d + 24 h* *%*
7d25C	FFlgt	100 ± 0	95.28 ± 1.45	107.66 ± 2.81
	Ffuv	100 ± 0	101.38 ± 2.48	108.69 ± 1.11
	FPL	100 ± 0	96.28 ± 0.40	112.49 ± 2.34
7d40C	FFlgt	100 ± 0	103.39 ± 1.43	93.37 ± 0.75
	Ffuv	100 ± 0	103.13 ± 0.40	92.21 ± 0.84
	FPL	100 ± 0	105.55 ± 1.41	95.90 ± 0.89
30d25C	FFlgt	100 ± 0	97.65 ± 1.69	105.30 ± 1.33
	Ffuv	100 ± 0	97.27 ± 2.63	103.01 ± 0.78
	FPL	100 ± 0	100.75 ± 7.08	111.46 ± 2.38
30d40C	FFlgt	100 ± 0	95.99 ± 3.32	97.34 ± 2.28
	Ffuv	100 ± 0	97.56 ± 3.27	97.29 ± 2.20
	FPL	100 ± 0	95.98 ± 1.43	111.46 ± 2.38

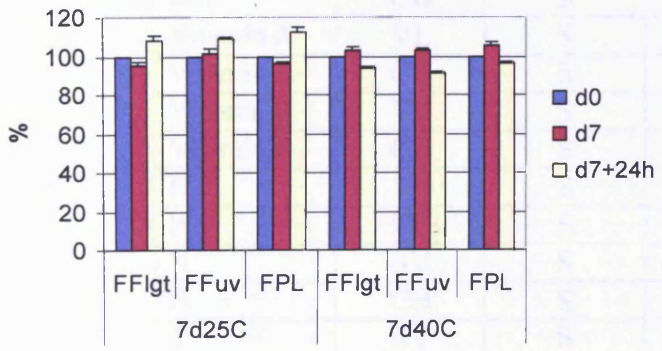


Figure 6.34. Content of Vitamin E in CB for 7d25C and 7d40C simulation conditions

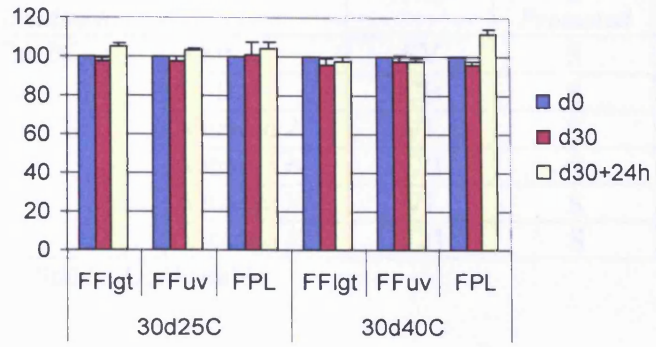


Figure 6.35. Content of Vitamin E in CB for 30d25C and 30d40C simulation conditions

6.3.3. Results Summary for Clinical Simulation Studies

The results of the clinical simulation studies are summarised in Table 6.22 and Table 6.23.

Table 6.22: Stability summary of vitamins for 7d + 24 h simulation conditions

Simulation condition	*Vitamin*	*Sample*	*Light Protected*	*Light Exposed*	*UVA Exposed*
7d25C	P	SV	S	U	U
	P	CB	S	S	U
	N	SV	S	S	S
	N	CB	S	S	S
	T	SV	S	S	U
	T	CB	S	S	S
	FA	SV	S	S	U
	FA	CB	S	S	S
	RSP	SV	S	S	U
	RSP	CB	S	S	U
	Vitamin A	VL	S	U	U
	Vitamin A	CB	S	S	U
	Vitamin E	VL	S	S	U
	Vitamin E	CB	S	S	S
7d40C	P	SV	S	U	U
	P	CB	S	S	U
	N	SV	S	S	S
	N	CB	S	S	S
	T	SV	S	S	U
	T	CB	S	S	U
	FA	SV	S	S	U
	FA	CB	S	S	S

Simulation condition	*Vitamin*	*Sample*	*Light Protected*	*Light Exposed*	*UVA Exposed*
7d40C	RSP	SV	S	U	U
	RSP	CB	S	U	U
	Vitamin A	VL	S	U	U
	Vitamin A	CB	S	U	U
	Vitamin E	VL	S	S	U
	Vitamin E	CB	S	S	S

S: Stable; U: Unstable

Table 6.23: Stability summary of vitamins for 30 d + 24 h simulation conditions

Simulation condition	*Vitamin*	*Admixture*	*Light Protected*	*Light Exposed*	*UVA Exposed*
30d25C	P	SV	S	U	U
	P	CB	S	S	U
	N	SV	S	S	S
	N	CB	S	S	S
	T	SV	S	S	U
	T	CB	S	S	S
	FA	SV	S	S	U
	FA	CB	S	S	S
	RSP	SV	S	U	U
	RSP	CB	S	U	U
	Vitamin A	VL	S	U	U
	Vitamin A	CB	S	S	U
	Vitamin. E	VL	S	S	U
	Vitamin E	CB	S	S	S
30d40C	P	SV	S	S	U
	P	CB	S	S	U
	N	SV	S	S	S
	N	CB	S	S	S
	T	SV	S	U	U
	T	CB	S	S	S
	FA	SV	S	U	U
	FA	CB	S	U	U
	RSP	SV	S	U	U
	RSP	CB	S	U	U
	Vitamin A	VL	S	U	U
	Vitamin A	CB	S	S	U
	Vitamin E	VL	U	U	U
	Vitamin E	CB	S	S	S

S: Stable; U: Unstable

6.4. Discussion

In all the three different samples tested, there was no obvious physical sign of degradation when stored for up to 7 days. Physical appearance remained unchanged and physical assessments remained within specified limits. Upon storage for 30 days, physical appearance appeared stable with no colour change observed and, for VL and CB samples, there was no change in the size of the lipid globules observed.

Subject to the exposure conditions after storage in the refrigerator, the samples showed different physical profiles. There was colour change in the SV samples when exposed to 40°C without light protection. The turbidity of SV samples decreased from its baseline at 0 hour, as expected due to the dissolution of the lyophilised powder upon time and aided by the increase in temperature and light.

In all samples, there was a decrease in pH from baseline value at 0 hour, possibly due to some degradation to a more acidic product taking place. It could be argued that this pH change could be due to a normal fall associated with absorbed carbon dioxide in solution, from the possible increase of carbon dioxide with time from the headspace. In the time available, the actual cause could not be identified, but it would be thought that the amount of carbon dioxide absorbed was negligible as the headspace was very small, besides the fact that all the samples would have consistently the same headspace. The varying pH results of the samples from the dark controls would indicate that the decrease in pH was more likely due to vitamin degradation.

No colour change, phase separation, or lipid globules size greater than 7.5 μm was observed for VL samples, but a pH change of more than 0.5 units for samples exposed to UVA at 40°C was observed. For the CB samples, colour change and an increase in the size of the lipid globules were observed when UVA exposed at 40°C after 7 days and after 30 days.

Based on the physical analysis, 30d40C samples exposed to UVA were the most unstable in all the samples, with colour change observed in SV and CB samples, a change in pH of greater than 0.5 units for all samples, and in CB, lipid globules greater than 5 μm size were observed by light microscopy and laser diffraction.

DO measurements showed the DO content to increase upon storage in the refrigerator and decrease when samples were subjected to higher temperatures. These results are similar in trend to those obtained in the earlier chapters. A study of the DO content of the base vehicle, 0.9% sodium chloride, in the Freeflex® minibags and in 100 ml glass bottles, having the same experimental design as in this chapter has also been conducted (see 3.2.1.2). These results serve as baseline information for the DO content present in the minibag.

Upon exposure to the simulation conditions, different stability profiles of the vitamins were gathered. Chemical analysis revealed all vitamins examined to be stable at 7d25C and 7d40C, provided samples were light-protected. The stability of the vitamins at these simulation conditions is summarised in Table 6.22. A study by Dahl et al (1986) also found the chemical stability of the vitamins to be acceptable during the one week storage and administration at ambient temperature.

For the 30d25C and 30d40C simulation studies, the stability of the vitamins examined is summarised in Table 6.23. The long stability of Vitamin A at 4°C was comparable to the study by Allwood and Plane (1984) who found no degradation of Vitamin A when stored for 28 days in the refrigerator.

Upon exposure to the simulation temperatures after 30 days storage, all vitamins analysed, with the exception of Vitamin E, were stable, provided that they were light protected. As for Vitamin E, at 30d40C study in VL with light protection, the content left was about 84%, so even though Vitamin E is regarded as unstable based upon the 30 days simulation study, there was still a

considerable amount of the vitamin available. It may be suggested that the content of vitamin E be increased to accommodate for this loss, but the stability at a higher concentration would require further investigation.

These experiments also highlighted the effects of the vitamins to different light exposures. From Table 6.22 (7d25C), it can be seen that while thiamine, folic acid and riboflavin sodium phosphate in SV solutions were stable under fluorescent white light, they became unstable when exposed to UVA light. Similarly with Vitamin E in VL emulsion, whilst stable under fluorescent light, it was unstable under UVA light.

It can be seen too that the vitamins are somewhat affected by UVA to varying degree compared to artificial fluorescent light, as shown from the reduced concentrations of the vitamins when exposed to UVA. The reduction of the contents of the vitamins varies from slight to high. For 7d25C study, while nicotinamide is not affected by UVA (see Table 6.9), thiamine is slightly affected to 88% (see Table 6.10) and Vitamin A is greatly affected to the extent that it was not detected after exposure to UVA (see Table 6.13).

Comparison between the 7 days and 30 days simulation studies revealed that some vitamins may degrade even more after prolonged storage and subjecting to the same extreme exposure conditions. For example, with folic acid, the experiment at 7d40C exposed to UVA (see Table 6.11) resulted in a percentage content of 88%, but at 30d40C exposed to UVA, its percentage content was reduced to 41%. Another example is the riboflavin sodium phosphate where the content was 20% when UVA exposed at 7d40C but was reduced to 9% when UVA exposed at 30d40C.

From the results, it is interesting to find that the combined CB admixture gives some protection from light to some vitamins. In the combined CB admixture, although riboflavin sodium phosphate was degraded when the admixture was irradiated by UVA, it was found that the degradation was less as compared to

SV solution. For 7d25C simulation study, the content of riboflavin sodium phosphate in clear Freeflex® bag in CB emulsion was 63% while its content in SV solution was 48% (refer Table 6.12 and Table 6.19). This was suggested by Smith et al (1988) to be due to the fact that riboflavin was protected by emulsification whereby the opacity of the emulsion will reduce to some extent the light transmission.

Some protection from light was conferred to Vitamin A, Vitamin E and folic acid in the CB admixture too. As an example, for Vitamin A in VL and exposed to UVA irradiation, Vitamin A could not be detected (Table 6.13), however in CB, slight concentrations of between 8% - 15 % could still be detected (Table 6.20). When vitamin A in CB sample is protected from light, it was stable throughout the 30 days simulation conditions.

6.5. Conclusions

All vitamins analysed were stable when stored refrigerated for up to 30 days. When subjected to administration temperatures of 25°C following the next 24 hours, the vitamins remained stable provided they were protected from light.

CHAPTER SEVEN

PHOTOSTABILITY OF VITAMINS - EFFECTS OF ULTRAVIOLET LIGHT UPON DIFFERENT DELIVERY CONDITIONS

7.1. Introduction

Photostability testing in recent years has gained much attention partly due to the introduction of the ICH Guideline Q1B, to regulate the registration of pharmaceutical products (ICH Steering Committee 1996; Tonnesen 1996). Artificial light sources have negligible UV irradiation and only sunlight causes photodegradation by absorption of UV light (Allwood 2000). For sunlight, influencing factors include the time of day, the weather condition, and the season (Allwood 1982; Allwood 2000). The photodegradation of a light sensitive product may vary too, depending on a number of factors, such as the packaging material, the light source, the intensity of the light, the proximity of the infusion to the light source, the infusion time and infusion rate (Allwood and Martin 2000).

UV radiation can be divided into three bands, UVC, UVB and UVA. UVC is shortwave radiation with wavelengths of between 200 nm – 290 nm. It is also called far UV and is absent at the earth surface because of absorption by ozone in the upper atmosphere. UVB has wavelengths between 280 nm – 320 nm, and is the UV band which causes sunburn and skin cancer. UVA has long wavelengths of between 320 nm – 400 nm, is also called near UV, because of its close proximity to the visible spectrum. Sunlight also emits radiation in the visible region (400 – 800 nm) and infrared region (800 – 3200 nm) (Tonnesen 2004).

A commercially prepared PN formulation is packaged with a sheet of plastic overwrap for protection. This package overwrap is removed so that the PN

components can be mixed and the addition of the vitamins, trace elements and other additives can be made. The degree of photodegradation is difficult to predict, therefore during storage and administration of the infusion, photolabile products such as the PN formulations, are recommended to be protected from light (Allwood 2000). It has also been shown in the earlier chapters that some vitamins are degraded by light. For home patients, PN is administered on a cyclical basis during the night, however, if infused during the day, it is inevitable that some exposure to light occurs, therefore the potential problem of photoinstability should be considered (Allwood 1999; Wormleighton and Catling 1998).

Penetration of UV radiation depends on the degree of transparency of the packaging material, based on their light transmission characteristics. Plastics, for example, block UV penetration differently according to their composition (Tonnesen 2004). Red plastic bags offer good protection between 190 and 590 nm (Dahl et al. 1986). Black plastic bags give a good protection against UV light (Webber 1979) while aluminium foil is a light-proof material.

Covering the PN formulation totally with aluminium foil has the disadvantage of decreasing visibility since it is not possible to check the bag during delivery, such as for any sign of precipitates, air inline or creaming. Therefore different types of coloured protective covers are used to give some protection from light. Red plastic covers are usually supplied by pharmaceutical manufacturers while the black plastic cover is usually the improvised cover used in practice by nursing staff during PN administration.

In this chapter, the photostability of the vitamin minibag formulations was assessed in the presence of different types of protective covers, to examine the degree of UVA protection conferred by the covers.

7.2. Methods

Three different test samples, SV, VL and CB, were evaluated. Test samples SV were prepared as in Chapter Four (see 4.2.2), test samples VL were prepared as in Chapter Five (see 5.2.1), and test samples CB were prepared as in Chapter Six (see 6.2.2).

7.2.1. Experimental Design

The samples were stored at either 25°C or at 40°C in the pharmaceutical stability chamber for 24 hours. The minibags were, in addition, exposed to UVA irradiation of 3.5 W/m^2 intensity, this exposure being equivalent to about 10 – 20 hours daylight exposure of the samples placed on a sunny window sill (Anderson and Byard 2004).

The types of protective covers used for the minibags were as follows:

i) Without any protective covers

ii) With red plastic covers (from Fresenius Kabi)

iii) With black plastic covers (black plastic bin liners)

iv) Wrapped with aluminium foil

Samples wrapped with aluminium foil acted as controls. Another different set of samples were prepared and exposed to the same condition for physical analysis. The test samples were prepared in triplicate.

7.2.2. Sampling

For physical analysis, samples were taken at 0 hour and at 24 hours after exposure in the pharmaceutical stability chamber. For chemical analysis, samples were taken at 0 hour, and after 3, 6 and 24 hours of exposure. Peak areas of all the 12 samples at 0 hour were averaged and the content analysis was regarded as 100%.

The sampling methods and the physical and chemical analyses performed are described in Chapter Four for SV and Chapter Five for VL.

The sampling method and the analysis for CB are described in Chapter Six (see 6.2.3).

7.3. Results

The following abbreviations are used to denote the exposure conditions of the minibags:

FF25C /40C	Without any protective covers, stored at 25°C or 40°C
Red25C /40C	With red FK plastic covers, stored at 25°C or 40°C
Black25C/40C	With black plastic covers, stored at 25°C or 40°C
Al25C /40C	Wrapped with aluminium foil, stored at 25°C or 40°C

7.3.1. Physical Analysis

7.3.1.1. Water-soluble Vitamin Solutions SV

7.3.1.1.1. Appearance

There was no apparent change in appearance of SV solutions from their initial light yellow colour and a light green haze when viewed with the fibreoptic light, except those of clear minibags kept at 40°C (FF40C) where the colour turned to a dull yellow.

7.3.1.1.2. Turbidity

The results of NTU measurements are tabulated in Table 7.1. All samples showed a decrease in NTU units by the end of the study period. The clear minibags without any protective cover, kept at 40°C (FF40C) showed the highest change in NTU units.

Table 7.1: NTU measurements of SV solutions with different protective covers

Minibags	0 h (n=12)	24 h
FF25C	4.74 ± 0.07	4.28 ± 0.08
Red25C	4.74 ± 0.07	4.80 ± 0.06
Black25C	4.74 ± 0.07	4.73 ± 0.09
Al25C	4.74 ± 0.07	4.74 ± 0.21
FF40C	4.75 ± 0.13	2.81 ± 0.07 *
Red40C	4.75 ± 0.13	4.66 ± 0.07
Black40C	4.75 ± 0.13	4.65 ± 0.09
Al40C	4.75 ± 0.13	4.65 ± 0.06

*: values with change in NTU of more than 0.5 units

7.3.1.1.3. Dissolved Oxygen

The results of DO content of SV minibags are tabulated in Table 7.2. Graphical illustrations are shown in Figure 7.1 and Figure 7.2. The graphs clearly showed that DO content of solutions decreased upon subjection to high temperatures, and that the DO content of the clear minibags was lowest.

Table 7.2: DO content in mg/l of SV minibags with different protective covers

Minibags	*0 h (n=12)*	*24 h*
FF25C	4.38 ± 0.45	0.87 ± 0.03
Red25C	4.38 ± 0.45	1.78 ± 0.17
Black25C	4.38 ± 0.45	2.78 ± 0.40
Al25C	4.38 ± 0.45	3.41 ± 0.09
FF40C	4.54 ± 0.38	1.53 ± 0.12
Red40C	4.54 ± 0.38	2.19 ± 0.07
Black40C	4.54 ± 0.38	2.17 ± 0.26
Al40C	4.54 ± 0.38	2.26 ± 0.05

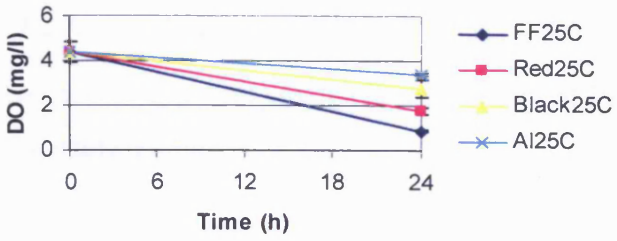


Figure 7.1: DO content of SV minibags with various protective covers at 25°C

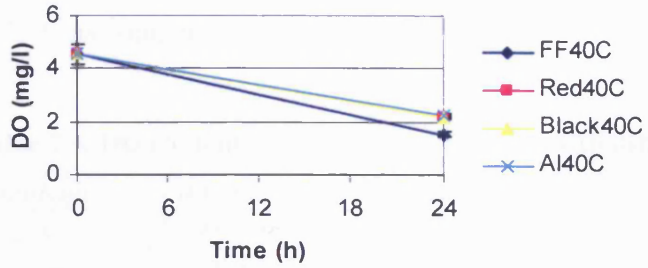


Figure 7.2: DO content of SV minibags with various protective covers at 40°C

7.3.1.1.4. pH

The results of pH measurements of SV samples are tabulated in Table 7.3. Clear samples without any protective covers showed pH changes of more than 0.5 units after exposure to UVA. The higher the exposure temperature, the greater the pH change.

Table 7.3: pH measurements of SV minibags with different protective covers

Minibags	*0 h (n=12)*	*24 h*
FF25C	6.00 ± 0.04	5.31 ± 0.06 *
Red25C	6.00 ± 0.04	5.59 ± 0.06
Black25C	6.00 ± 0.04	5.77 ± 0.10
Al25C	6.00 ± 0.04	6.00 ± 0.04
FF40C	5.95 ± 0.04	4.81 ± 0.05 *
Red40C	5.95 ± 0.04	5.34 ± 0.02 *
Black40C	5.95 ± 0.04	5.56 ± 0.05
Al40C	5.95 ± 0.04	5.74 ± 0.03

*: change in pH of more than 0.5 units

7.3.1.2. Fat-soluble vitamins VL

7.3.1.2.1. Appearance

There was no apparent change in appearance of VL emulsions from their initial milky white colour.

7.3.1.2.2. Dissolved Oxygen

The results of DO measurement of VL minibags are tabulated in Table 7.4 with the graphs shown in Figure 7.3 and Figure 7.4. DO change was not remarkable at 25°C as compared to 40°C.

Table 7.4: DO content in mg/l of VL minibags with different protective covers

Minibags	*0 h (n=12)*	*24 h*
FF25C	5.80 ± 0.45	4.66 ± 0.09
Red25C	5.80 ± 0.45	5.97 ± 0.07
Black25C	5.80 ± 0.45	5.98 ± 0.07
Al25C	5.80 ± 0.45	6.19 ± 0.06
FF40C	6.06 ± 0.26	3.75 ± 0.13
Red40C	6.06 ± 0.26	4.35 ± 0.17
Black40C	6.06 ± 0.26	4.50 ± 0.04
Al40C	6.06 ± 0.26	4.61 ± 0.22

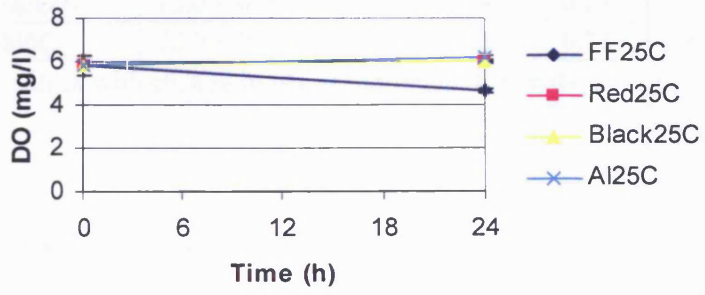


Figure 7.3: DO content of VL minibags with various protective covers at 25°C

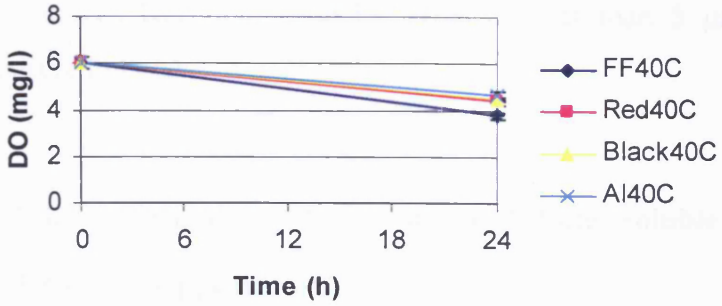


Figure 7.4: DO content of VL minibags with various protective covers at 40°C

7.3.1.2.3. pH

The results of pH of VL minibags are shown in Table 7.5. From the table, only VL minibags without any protective covers exposed to UVA at 40°C during administration produced pH changes of more than 0.5 units.

Table 7.5: pH measurements of VL minibags with different protective covers

Minibags	0 h (n=12)	24 h	ΔpH
FF25C	6.90 ± 0.20	6.74 ± 0.02	0.16
Red25C	6.90 ± 0.20	6.99 ± 0.02	0.09
Black25C	6.90 ± 0.20	7.05 ± 0.08	0.15
Al25C	6.90 ± 0.20	6.99 ± 0.02	0.08
FF40C	7.20 ± 0.04	6.64 ± 0.06	0.56*
Red40C	7.20 ± 0.04	6.93 ± 0.03	0.27
Black40C	7.20 ± 0.04	6.93 ± 0.08	0.27
Al40C	7.20 ± 0.04	6.98 ± 0.02	0.21

*: values with change in pH of more than 0.5 units

7.3.1.2.4. Laser Diffraction

The maximum diameter for all samples was less than 4.3 μm with D[4,3] of between 0.33 to 0.36 μm throughout the study durations.

7.3.1.2.5. Light Microscopy

All samples had lipid globules sizes of less than 5 μm before and after exposure to UVA.

7.3.1.3. Combined Fat-soluble and Water-soluble Vitamins (CB)

7.3.1.3.1. Appearance

The CB emulsions were homogenous milky yellow with a milky white meniscus. There was no change in appearance upon subjecting the samples to the treatment conditions.

7.3.1.3.2. Dissolved Oxygen

The results of DO measurement for CB minibags are tabulated in Table 7.6. DO content decreased when minibags were subjected to the treatment conditions.

Table 7.6: DO content in mg/l of CB minibags with different protective covers

Minibags	*0 h (n=12)*	*24 h*
FF25C	4.27 ± 0.47	0.61 ± 0.05
Red25C	4.27 ± 0.47	2.32 ± 0.21
Black25C	4.27 ± 0.47	2.75 ± 0.13
Al25C	4.27 ± 0.47	2.69 ± 0.24
FF40C	4.30 ± 0.38	1.14 ± 0.15
Red40C	4.30 ± 0.38	1.84 ± 0.38
Black40C	4.30 ± 0.38	1.90 ± 0.35
Al40C	4.30 ± 0.38	2.18 ± 0.29

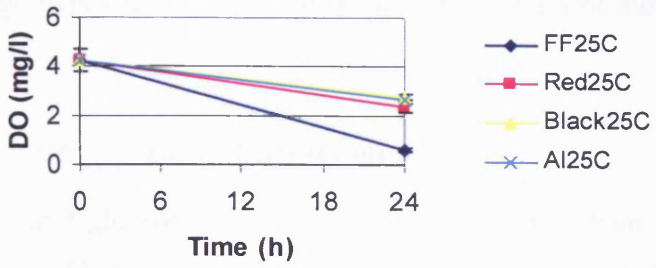


Figure 7.5: DO content of CB minibags with various protective covers at 25°C

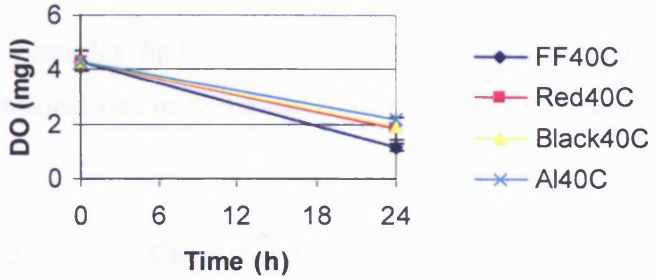


Figure 7.6: DO content of CB minibags with various protective covers at 40°C

7.3.1.3.3. pH

The results of pH for CB minibags are shown in Table 7.7.

Table 7.7: pH measurements of CB minibags with different overwraps

Minibags	*0 h (n=12)*	*24 h*
FF25C	6.31 ± 0.06	5.37 ± 0.22 *
Red25C	6.31 ± 0.06	5.86 ± 0.01
Black25C	6.31 ± 0.06	6.02 ± 0.03
Al25C	6.31 ± 0.06	6.20 ± 0.03
FF40C	6.21 ± 0.04	4.90 ± 0.01 *
Red40C	6.21 ± 0.04	5.56 ± 0.01 *
Black40C	6.21 ± 0.04	5.63 ± 0.03 *
Al40C	6.21 ± 0.04	5.72 ± 0.02

*: change in pH of more than 0.5 units

From the table, CB minibags without any protective covers exposed to UVA at 25°C and 40°C produced pH changes of more than 0.5 units, the change in pH

was greater for the FF40C minibag. Even minibags with red and black plastic wraps exposed to UVA at 40°C had pH changes of more than 0.5 units.

7.3.1.3.4. Laser Diffraction

The lipid globule size for all samples was less than 4.30 μm with a value of D[4,3] of between 0.32 to 0.36 μm throughout the study durations tested.

7.3.1.3.5. Light Microscopy

The samples had lipid globule sizes of less than 5 μm diameter with an occasional one or two 5 μm lipid globule observed.

7.3.2. Chemical Analysis

7.3.2.1. Water-soluble Vitamins SV

The results of pyridoxine, nicotinamide, thiamine, folic acid, and riboflavin sodium phosphate in SV solutions are tabulated from Table 7.8 to Table 7.14, while the graphs are displayed from Figure 7.7 to Figure 7.14.

7.3.2.1.1. Pyridoxine

Table 7.8: Percentage content of Pyridoxine in SV minibags with different protective covers

Minibags	*0 h*	*3 h*	*6 h*	*24 h*
FF25C	100 ± 0	84.33 ± 0.23	74.54 ± 1.66	56.95 ± 1.00
Red25C	100 ± 0	96.45 ± 1.88	97.88 ± 1.72	94.48 ± 0.66
Black25C	100 ± 0	95.69 ± 0.65	96.93 ± 1.01	95.11 ± 1.23
Al25C	100 ± 0	97.25 ± 2.99	97.98 ± 2.12	97.81 ± 2.01
FF40C	100 ± 0	84.90 ± 1.99	76.78 ± 1.07	46.40 ± 3.55
Red40C	100 ± 0	98.18 ± 2.60	95.28 ± 1.26	93.82 ± 1.13
Black40C	100 ± 0	95.82 ± 4.11	95.26 ± 3.98	96.68 ± 0.08
Al40C	100 ± 0	97.93 ± 1.84	99.39 ± 0.40	92.67 ± 0.04

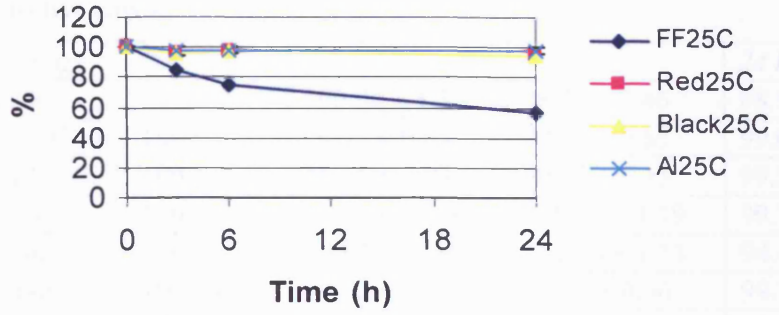


Figure 7.7: Pyridoxine content in SV minibags with different protective covers, exposed to UVA at 25°C

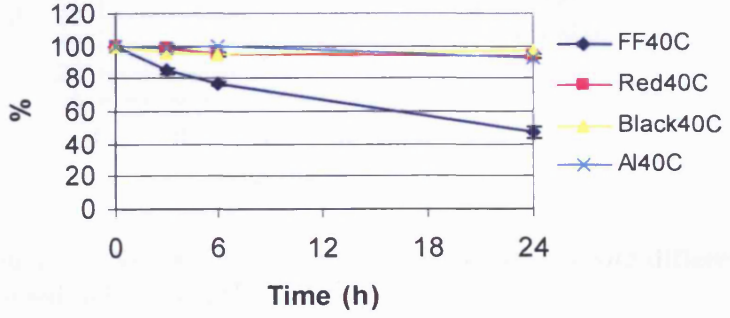


Figure 7.8: Pyridoxine content in SV minibags with different protective covers, exposed to UVA at 40°C

There was significant difference ($p<0.001$) between clear minibags and all the protective covers at both temperatures. There was no significant difference between red, black or aluminium foil covers at either temperature.

7.3.2.1.2. Nicotinamide

Table 7.9: Percentage content of nicotinamide in SV minibags with different protective covers

Minibags	*0 h*	*3 h*	*6 h*	*24 h*
FF25C	100 ± 0	100.04 ± 1.58	96.30 ± 3.46	98.59 ± 1.54
Red25C	100 ± 0	99.17 ± 0.35	99.29 ± 0.65	99.07 ± 0.04
Black25C	100 ± 0	99.35 ± 0.94	99.52 ± 0.15	99.23 ± 0.40
Al25C	100 ± 0	101.20 ± 2.50	100.01 ± 1.19	99.75 ± 1.35
FF40C	100 ± 0	100.33 ± 0.94	101.06 ± 1.33	94.44 ± 4.35
Red40C	100 ± 0	99.10 ± 1.68	99.75 ± 0.36	99.34 ± 1.61
Black40C	100 ± 0	97.51 ± 3.63	99.24 ± 2.80	101.12 ± 0.88
Al40C	100 ± 0	99.78 ± 2.41	100.51 ± 0.23	99.68 ± 1.93

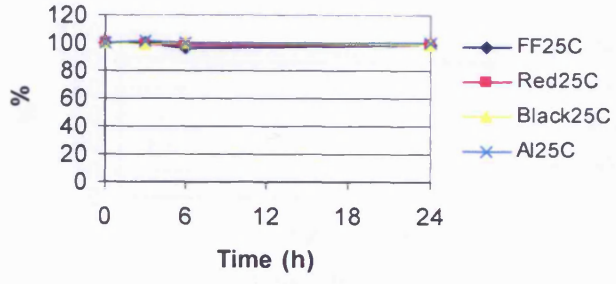


Figure 7.9: Nicotinamide content in SV minibags with different protective covers, exposed to UVA at 25°C

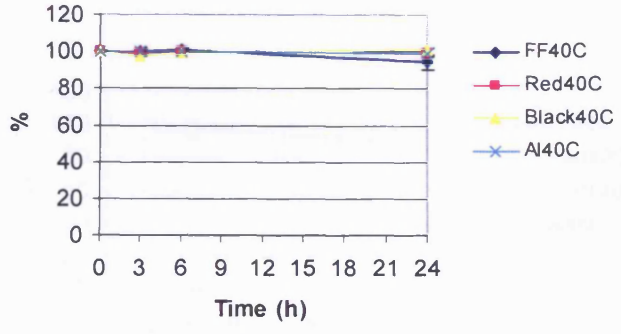


Figure 7.10: Nicotinamide content in SV minibags with different overwraps, exposed to UVA and 40°C

There was no significant difference between the groups tested at either temperature.

7.3.2.1.3. Thiamine

Table 7.10: Percentage content of thiamine in SV minibags with different protective covers

Minibags	*0 h*	*3 h*	*6 h*	*24 h*
FF25C	100 ± 0	103.74 ± 2.20	97.61 ± 2.56	97.09 ± 1.44
Red25C	100 ± 0	103.67 ± 0.60	104.19 ± 1.02	105.41 ± 2.10
Black25C	100 ± 0	102.10 ± 3.63	105.53 ± 0.40	106.95 ± 1.35
Al25C	100 ± 0	103.89 ± 4.35	107.08 ± 2.54	101.15 ± 6.52
FF40C	100 ± 0	97.26 ± 2.05	97.75 ± 1.47	86.05 ± 4.19
Red40C	100 ± 0	96.35 ± 5.59	95.30 ± 3.29	91.65 ± 2.26
Black40C	100 ± 0	96.96 ± 3.46	97.72 ± 2.96	98.67 ± 0.15
Al40C	100 ± 0	99.47 ± 1.69	100.67 ± 1.65	94.78 ± 3.23

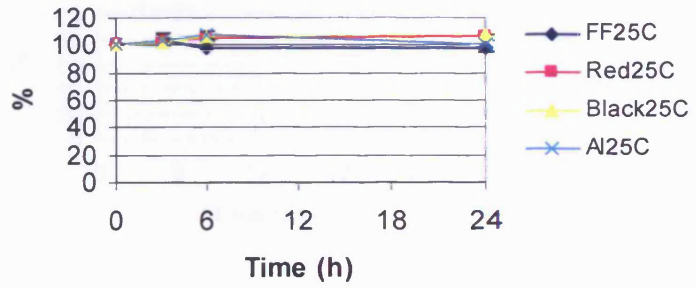


Figure 7.11: Thiamine content in SV minibags with different protective covers, exposed to UVA at 25°C

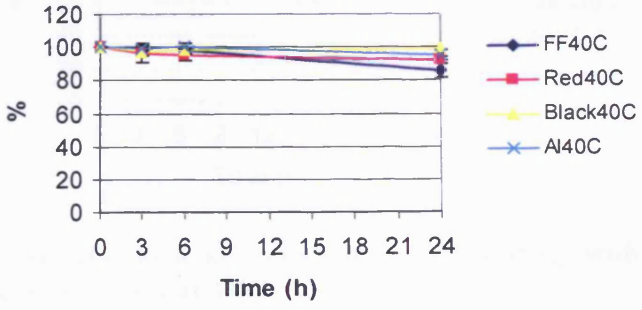


Figure 7.12: Thiamine content in SV minibags with different protective covers, exposed to UVA at 40°C

There was no significant difference between the groups tested at either temperature.

7.3.2.1.4. Folic acid

Table 7.11: Percentage content of folic acid in SV minibags with different protective covers

Minibags	*0 h*	*3 h*	*6 h*	*24 h*
FF25C	100 ± 0	104.37 ± 1.03	99.95 ± 3.43	101.83 ± 1.31
Red25C	100 ± 0	103.22 ± 0.85	103.78 ± 0.89	103.17 ± 0.39
Black25C	100 ± 0	102.94 ± 1.27	104.01 ± 0.62	103.59 ± 0.81
Al25C	100 ± 0	105.28 ± 2.84	104.75 ± 1.84	104.17 ± 1.63
FF40C	100 ± 0	101.08 ± 1.66	100.87 ± 2.14	90.25 ± 4.07
Red40C	100 ± 0	98.59 ± 1.70	97.77 ± 0.66	97.34 ± 1.41
Black40C	100 ± 0	97.52 ± 4.08	97.64 ± 3.37	99.67 ± 0.50
Al40C	100 ± 0	98.59 ± 2.02	100.17 ± 0.39	96.95 ± 1.17

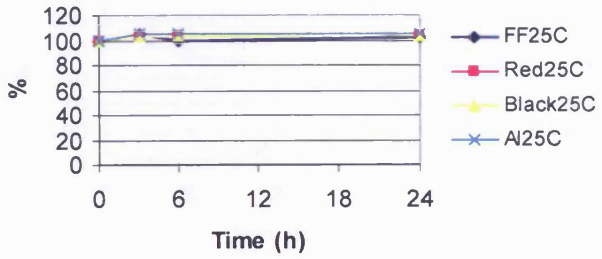


Figure 7.13: Folic acid content in SV minibags with different protective covers, exposed to UVA at 25°C

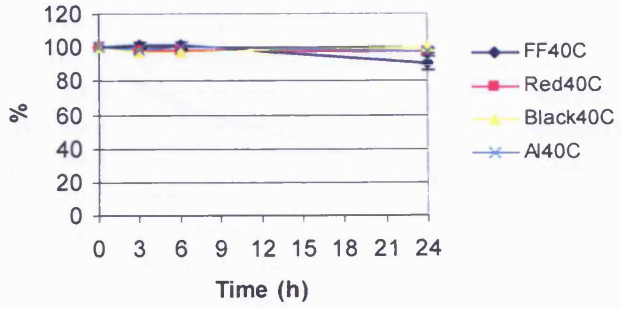


Figure 7.14: Folic acid content in SV minibags with different protective covers, exposed to UVA at 40°C

There was no significant difference between the groups tested at either temperature.

7.3.2.1.5. Riboflavin Sodium Phosphate

Table 7.12: Percentage content of riboflavin sodium phosphate in SV minibags with different protective covers

Minibags	*0 h*	*3 h*	*6 h*	*24 h*
FF25C	100 ± 0	84.58 ± 0.92	74.29 ± 2.14	54.01 ± 0.48
Red25C	100 ± 0	95.93 ± 1.84	97.47 ± 1.30	94.18 ± 0.72
Black25C	100 ± 0	95.52 ± 1.32	96.45 ± 1.60	95.10 ± 1.32
Al25C	100 ± 0	97.82 ± 2.83	98.14 ± 2.02	97.71 ± 2.11
FF40C	100 ± 0	82.65 ± 2.26	71.09 ± 2.28	29.45 ± 5.56
Red40C	100 ± 0	98.43 ± 2.29	97.86 ± 4.18	92.51 ± 1.20
Black40C	100 ± 0	96.18 ± 3.96	96.05 ± 3.94	96.31 ± 0.80
Al40C	100 ± 0	97.86 ± 1.56	100.05 ± 0.66	92.05 ± 1.55

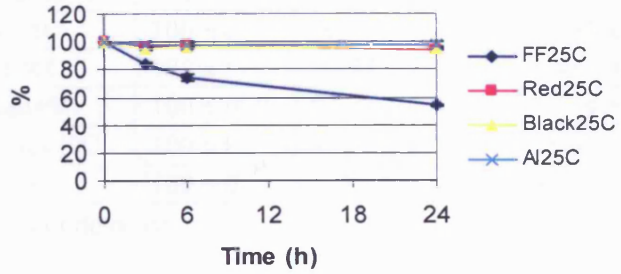


Figure 7.15: Riboflavin sodium phosphate content in SV minibags with different protective covers, exposed to UVA at 25°C

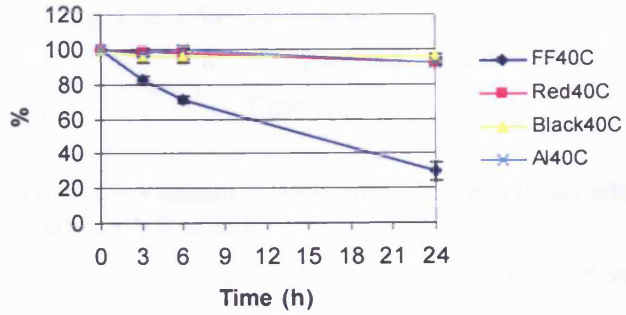


Figure 7.16: Riboflavin sodium phosphate content in SV minibags with different protective covers, exposed to UVA at 40°C

The mean difference was significant at $p<0.001$ between the clear minibags and all the protective covers at both temperatures. There was no significant difference between red, black or aluminium foil covers at either temperature.

7.3.2.2. Fat-soluble Vitamins VL

The results of Vitamin A and Vitamin E in VL minibags are tabulated in Table 7.13 and 7.14 with their corresponding graphs depicted in Figures 7.17 to 7.20.

7.3.2.2.1. Vitamin A

Table 7.13: Percentage content of Vitamin A in VL minibags with different protective covers

Minibags	*0 h*	*3 h*	*6 h*	*24 h*
FF25C	100 ± 0	24.24 ± 3.44	4.87 ± 1.20	nd
Red25C	100 ± 0	94.67 ± 1.60	90.50 ± 2.07	72.51 ± 0.44
Black25C	100 ± 0	98.86 ± 1.87	96.93 ± 2.93	92.08 ± 0.47
Al25C	100 ± 0	96.26 ± 4.27	95.94 ± 3.57	91.71 ± 2.91
FF40C	100 ± 0	11.97 ± 2.75	1.48 ± 0.13	nd
Red40C	100 ± 0	97.73 ± 1.46	91.14 ± 1.46	65.83 ± 1.25
Black40C	100 ± 0	97.00 ± 3.50	91.03 ± 2.20	71.06 ± 9.17
Al40C	100 ± 0	100.64 ± 2.14	96.75 ± 1.97	95.14 ± 3.98

nd: not detected

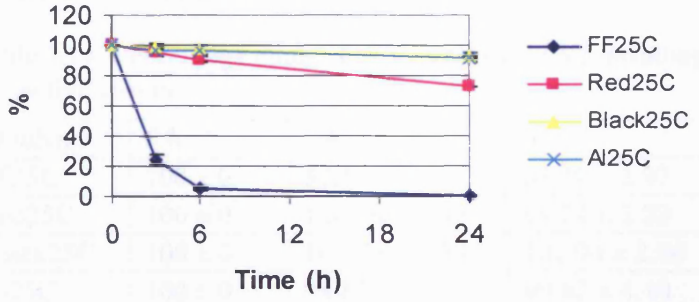


Figure 7.17: Vitamin A content in VL minibags with different protective covers, exposed to UVA at 25C

There was significant difference at $p<0.001$ between the clear FF25C and the red, black or aluminium foil covers, and at $p<0.05$ between the red and the black or aluminium foil covers. There was no significant difference in the means between the aluminium foil covers and the black plastic covers.

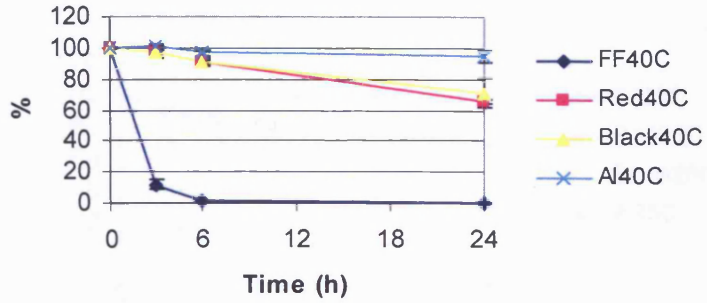


Figure 7.18: Vitamin A content in VL minibags with different protective covers, exposed to UVA at 40C

The difference between group means was significant at $p<0.001$ between the clear FF40C minibags and the red, black or aluminium foil covers. The aluminium foil cover was significantly different from the red and black covers at $p<0.05$. There was no group difference between the black and red protective covers.

7.3.2.2.2. Vitamin E

Table 7.14: Percentage content of Vitamin E in VL minibags with different protective covers

Minibags	*0 h*	*3 h*	*6 h*	*24 h*
FF25C	100 ± 0	89.55 ± 1.74	83.40 ± 2.97	68.35 ± 0.91
Red25C	100 ± 0	100.36 ± 1.99	99.24 ± 2.23	97.22 ± 1.34
Black25C	100 ± 0	101.76 ± 3.59	101.94 ± 2.06	100.79 ± 2.26
Al25C	100 ± 0	98.82 ± 3.10	99.62 ± 4.40	97.95 ± 3.64
FF40C	100 ± 0	90.35 ± 1.30	78.24 ± 7.65	51.69 ± 1.10
Red40C	100 ± 0	104.56 ± 1.47	93.01 ± 12.55	95.24 ± 4.83
Black40C	100 ± 0	100.48 ± 4.74	96.02 ± 1.23	90.53 ± 8.47
Al40C	100 ± 0	100.22 ± 4.50	91.83 ± 4.44	86.77 ± 8.26

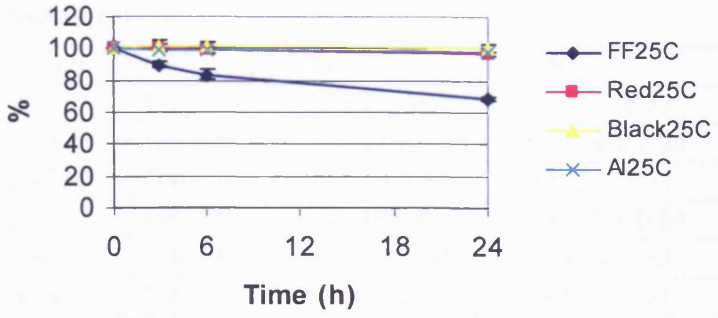


Figure 7.19: Vitamin E content in VL minibags with different protective covers, exposed to UVA at 25C

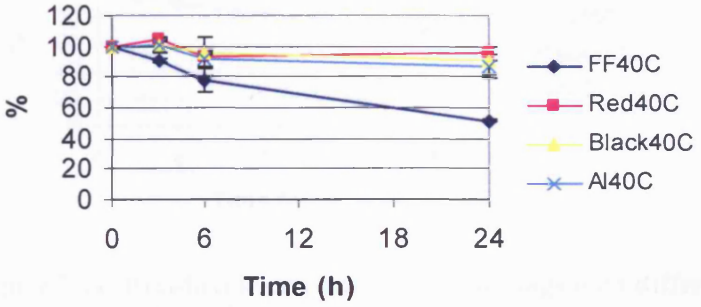


Figure 7.20: Vitamin E content in VL minibags with different protective covers, exposed to UVA at 40C

There was significant difference at $p<0.001$ between the clear minibags and all the protective covers. There was no significant difference between red, black or aluminium foil covers at either temperature.

7.3.2.3. Fat-soluble and Water-soluble Vitamin Admixtures CB

The results of pyridoxine, nicotinamide, thiamine, folic acid, and riboflavin sodium phosphate, Vitamin A and Vitamin E in CB emulsions are tabulated from Table 7.15 to Table 7.21, while the graphs are displayed from Figure 7.21 to Figure 7.34.

7.3.2.3.1. Pyridoxine

Table 7.15: Percentage content of pyridoxine in CB minibags with different protective covers

Minibags	*0 h*	*3 h*	*6 h*	*24 h*
FF25C	100 ± 0	96.13 ± 3.52	89.17 ± 1.27	70.25 ± 3.78
Red25C	100 ± 0	101.87 ± 0.86	100.39 ± 1.24	98.14 ± 0.41
Black25C	100 ± 0	99.62 ± 3.95	99.18 ± 1.73	96.00 ± 4.44
Al25C	100 ± 0	101.98 ± 0.59	100.21 ± 0.84	98.88 ± 2.33
FF40C	100 ± 0	87.19 ± 0.46	79.86 ± 0.53	57.64 ± 2.78
Red40C	100 ± 0	97.51 ± 2.01	93.02 ± 0.31	91.81 ± 1.92
Black40C	100 ± 0	93.87 ± 2.01	93.43 ± 0.62	89.88 ± 0.74
Al40C	100 ± 0	92.94 ± 1.95	91.7 ± 1.76	91.60 ± 0.28

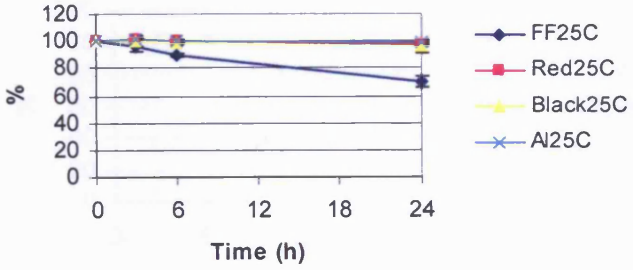


Figure 7.21: Pyridoxine content in CB minibags with different protective covers, exposed to UVA at 25C

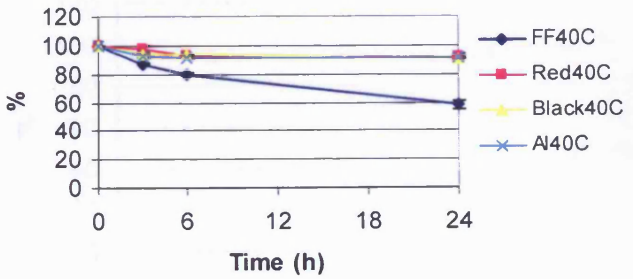


Figure 7.22: Pyridoxine content in CB minibags with different overwraps, exposed to UVA at 40C

In Figure 7.21 and Figure 7.22, there was significant difference at $p<0.001$ between the clear minibags and all the protective covers at both temperatures. The group means between red, black and aluminium covers were not significantly different.

7.3.2.3.2. Nicotinamide

Table 7.16: Percentage content of nicotinamide in CB minibags with different protective covers

Minibags	*0 h*	*3 h*	*6 h*	*24 h*
FF25C	100 ± 0	99.92 ± 3.70	100.87 ± 1.03	99.55 ± 3.68
Red25C	100 ± 0	102.15 ± 0.40	100.80 ± 1.26	100.40 ± 0.18
Black25C	100 ± 0	99.61 ± 3.88	99.17 ± 1.79	99.65 ± 5.95
Al25C	100 ± 0	101.56 ± 1.11	100.79 ± 0.72	99.41 ± 1.64
FF40C	100 ± 0	97.54 ± 0.59	94.52 ± 0.42	93.37 ± 1.49
Red40C	100 ± 0	98.87 ± 1.78	95.00 ± 0.18	94.54 ± 1.84
Black40C	100 ± 0	94.84 ± 1.98	94.70 ± 0.23	92.75 ± 0.86
Al40C	100 ± 0	94.38 ± 2.05	93.37 ± 1.84	94.00 ± 0.23

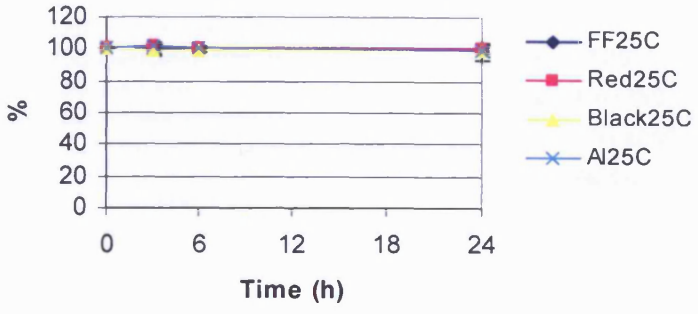


Figure 7.23: Nicotinamide content in CB minibags with different overwraps, exposed to UVA at 25C

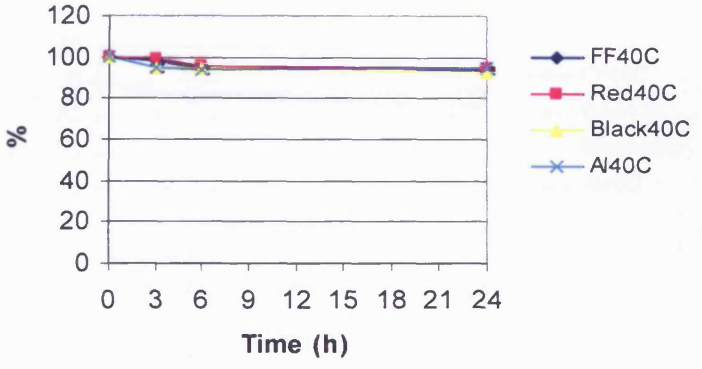


Figure 7.24: Nicotinamide content in CB minibags with different overwraps, exposed to UVA at 40C

There was no significant difference between the protective covers at 25°C and 40°C for nicotinamide.

7.3.2.3.3. Thiamine

Table 7.17: Percentage content of thiamine in CB minibags with different protective covers

Minibags	*0 h*	*3 h*	*6 h*	*24 h*
FF25C	100 ± 0	99.51 ± 4.07	103.92 ± 2.12	99.61 ± 4.40
Red25C	100 ± 0	104.77 ± 2.50	106.16 ± 0.81	100.09 ± 5.32
Black25C	100 ± 0	101.70 ± 4.27	105.49 ± 1.95	102.04 ± 4.69
Al25C	100 ± 0	105.26 ± 0.60	106.49 ± 0.81	104.59 ± 4.41
FF40C	100 ± 0	93.64 ± 0.28	91.57 ± 2.24	86.73 ± 2.26
Red40C	100 ± 0	95.83 ± 1.58	92.91 ± 0.67	95.19 ± 1.83
Black40C	100 ± 0	92.70 ± 2.39	95.27 ± 0.43	92.76 ± 0.95
Al40C	100 ± 0	92.40 ± 1.04	94.17 ± 1.84	95.55 ± 0.02

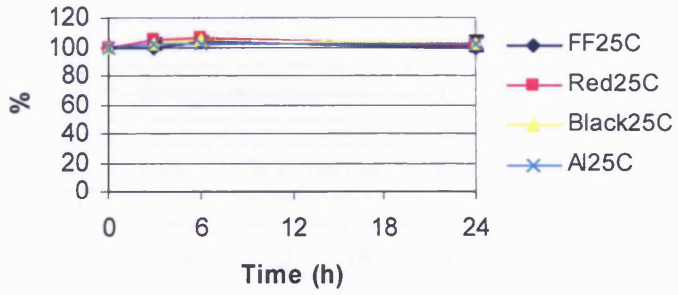


Figure 7.25: Thiamine content in CB minibags with different protective covers, exposed to UVA at 25°C

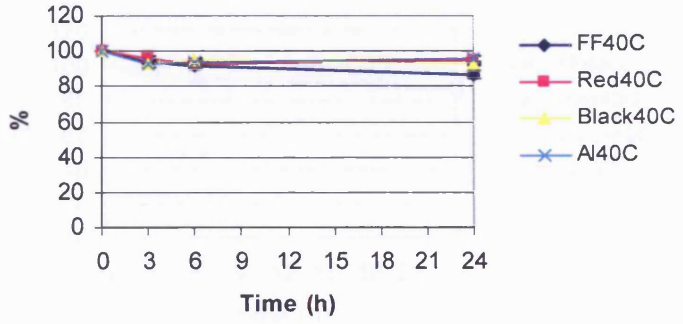


Figure 7.26: Thiamine content in CB minibags with different protective covers, exposed to UVA at 40°C

There was no significant difference between the groups tested at either temperature for thiamine.

7.3.2.3.4. Folic acid

Table 7.18: Percentage content of folic acid in CB minibags with different protective covers

Minibags	*0 h*	*3 h*	*6 h*	*24 h*
FF25C	100 ± 0	98.52 ± 3.37	99.65 ± 1.13	96.74 ± 4.19
Red25C	100 ± 0	103.92 ± 0.54	99.02 ± 1.06	98.41 ± 0.05
Black25C	100 ± 0	98.04 ± 3.63	97.58 ± 1.76	94.28 ± 3.33
Al25C	100 ± 0	97.08 ± 5.84	95.01 ± 7.29	97.92 ± 2.23
FF40C	100 ± 0	96.04 ± 0.46	92.59 ± 1.22	86.41 ± 3.45
Red40C	100 ± 0	96.07 ± 2.24	92.22 ± 0.43	91.52 ± 3.35
Black40C	100 ± 0	92.61 ± 2.19	93.23 ± 0.23	89.69 ± 0.88
Al40C	100 ± 0	92.07 ± 2.20	91.52 ± 1.30	91.78 ± 0.31

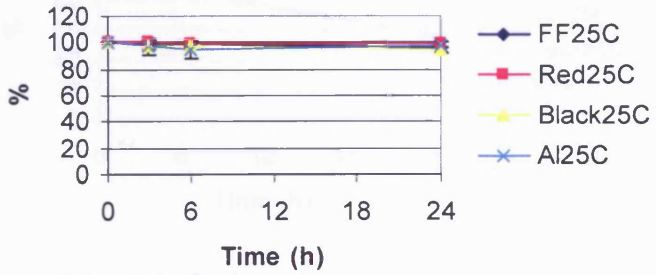


Figure 7.27: Folic acid content in CB minibags with different protective covers, exposed to UVA at 25°C

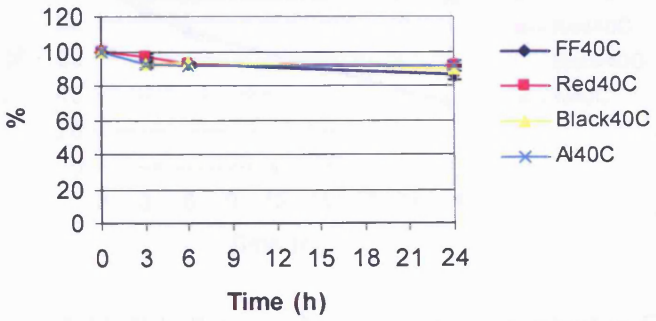


Figure 7.28: Folic acid content in CB minibags with different protective covers, exposed to UVA at 40°C

There was no significant difference between the protective covers at either 25°C and 40°C for folic acid.

7.3.2.3.5. Riboflavin Sodium Phosphate

Table 7.19: Percentage content of riboflavin sodium phosphate in CB minibags with different protective covers

Minibags	*0 h*	*3 h*	*6 h*	*24 h*
FF25C	100 ± 0	90.59 ± 3.37	85.15 ± 1.48	57.41 ± 2.90
Red25C	100 ± 0	99.71 ± 0.83	97.37 ± 0.90	97.25 ± 1.69
Black25C	100 ± 0	97.35 ± 3.65	96.45 ± 1.75	94.29 ± 4.26
Al25C	100 ± 0	99.26 ± 0.66	97.61 ± 0.56	96.22 ± 1.61
FF40C	100 ± 0	85.75 ± 0.42	74.95 ± 0.94	35.00 ± 2.92
Red40C	100 ± 0	96.32 ± 1.50	93.61 ± 0.49	91.28 ± 2.11
Black40C	100 ± 0	92.80 ± 2.12	93.86 ± 0.73	89.88 ± 1.11
Al40C	100 ± 0	92.31 ± 1.77	93.19 ± 1.82	91.78 ± 0.27

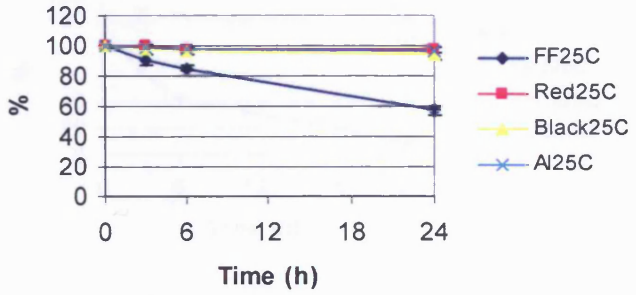


Figure 7.29: Riboflavin sodium phosphate content in CB minibags with different protective covers, exposed to UVA at 25°C

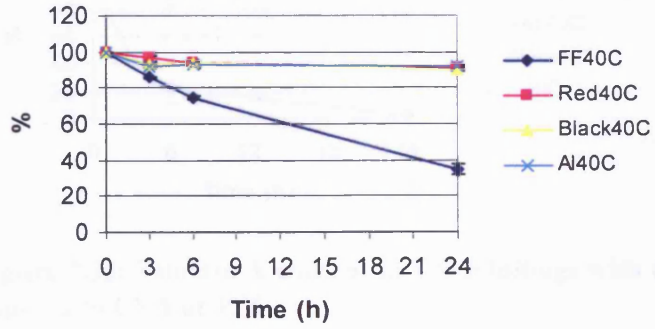


Figure 7.30: Riboflavin sodium phosphate content in CB minibags with different protective covers, exposed to UVA at 40°C

There was significant different at $p<0.001$ between the clear minibags and all the protective covers at both temperatures. There was no significant difference between the red, black and aluminium foil covers at either temperature.

7.3.2.3.6. Vitamin A

Table 7.20: Percentage content of Vitamin A in CB minibags with different protective covers

Samples	*0 h*	*3 h*	*6 h*	*24 h*
FF25C	100 ± 0	62.31 ± 1.36	42.30 ± 2.05	8.53 ± 0.84
Red25C	100 ± 0	95.52 ± 4.01	102.90 ± 2.90	83.04 ± 1.84
Black25C	100 ± 0	98.00 ± 2.70	102.90 ± 1.34	91.45 ± 3.96
Al25C	100 ± 0	100.28 ± 7.98	101.76 ± 2.10	99.19 ± 1.88
FF40C	100 ± 0	45.68 ± 3.48	21.93 ± 2.36	2.62 ± 0.10
Red40C	100 ± 0	101.84 ± 2.09	91.71 ± 8.72	79.93 ± 5.02
Black40C	100 ± 0	102.35 ± 5.70	94.59 ± 3.68	85.81 ± 6.58
Al40C	100 ± 0	100.40 ± 6.73	102.08 ± 4.41	93.53 ± 12.25

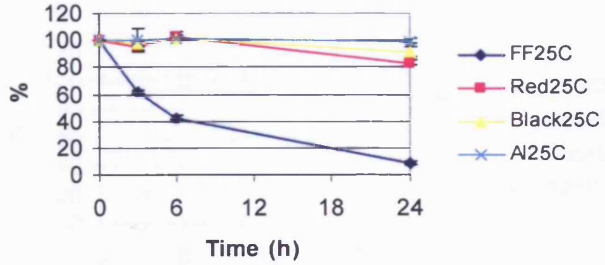


Figure 7.31: Vitamin A content in CB minibags with different protective covers, exposed to UVA at 25°C

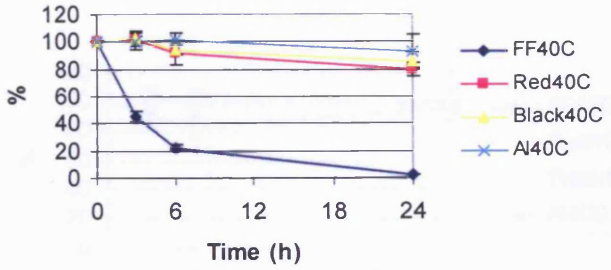


Figure 7.32: Vitamin A content in CB minibags with different protective covers, exposed to UVA at 40°C

The group mean difference was significant at $p<0.001$ between the clear minibags and all the protective covers at both temperatures. The group means between red, black and aluminium covers were not significantly different.

7.3.2.3.7. Vitamin E

Table 7.21: Percentage content of Vitamin E in CB minibags with different protective covers

Minibags	*0 h*	*3 h*	*6 h*	*24 h*
FF25C	100 ± 0	94.37 ± 0.61	92.48 ± 2.16	88.19 ± 2.23
Red25C	100 ± 0	97.67 ± 4.17	108.18 ± 2.40	95.18 ± 1.89
Black25C	100 ± 0	99.15 ± 2.78	104.54 ± 1.94	98.04 ± 2.14
Al25C	100 ± 0	101.51 ± 7.78	103.58 ± 2.60	103.14 ± 1.52
FF40C	100 ± 0	90.35 ± 5.74	82.51 ± 7.31	95.17 ± 4.05
Red40C	100 ± 0	103.95 ± 2.15	97.83 ± 8.96	96.33 ± 5.24
Black40C	100 ± 0	104.92 ± 6.05	106.12 ± 3.31	100.93 ± 4.82
Al40C	100 ± 0	100.68 ± 0.06	104.71 ± 3.96	97.31 ± 11.30

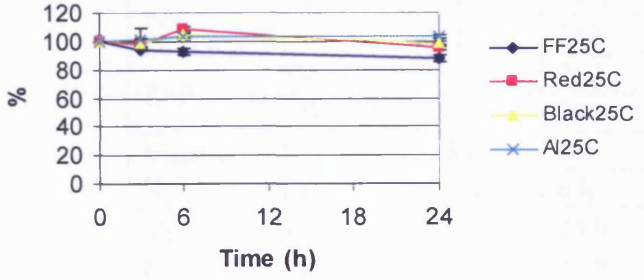


Figure 7.33: Vitamin E content in CB minibags with different protective covers, exposed to UVA at 25°C

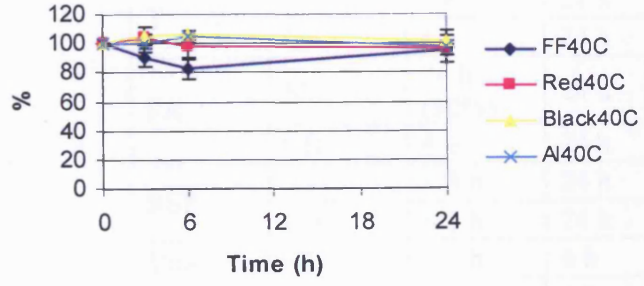


Figure 7.34: Vitamin E content in CB minibags with different overwraps, exposed to UVA at 40°C

There was significant difference at 25°C ($p<0.05$) and at 40°C ($p<0.001$) between the clear minibags and the protective covers. The results between red, black and aluminium foil covers were not significantly different at either temperature.

7.3.3. Results Summary

The results of this study is summarised in Table 7.22.

Table 7.22: Summary of the stability duration of vitamin minibags with different protective covers exposed to UVA at 25°C and 40°C

Temperature	*Vitamin*	*Minibag*	*Protective covers*			
			None	*Red*	*Black*	*Al foil*
25°C	P	SV	< 3 h	24 h	24 h	24 h
		CB	3 h	24 h	24 h	24 h
	N	SV	24 h	24 h	24 h	24 h
		CB	24 h	24 h	24 h	24 h
	T	SV	24 h	24 h	24 h	24 h
		CB	24 h	24 h	24 h	24 h
	FA	SV	24 h	24 h	24 h	24 h
		CB	24 h	24 h	24 h	24 h
	RSP	SV	< 3 h	24 h	24 h	24 h
		CB	3 h	24 h	24 h	24 h
	Vitamin A	VL	< 3 h	6 h	24 h	24 h
		CB	< 3 h	6 h	24 h	24 h
	Vitamin E	VL	3 h	24 h	24 h	24 h
		CB	6 h	24 h	24 h	24 h
40°C	P	SV	< 3 h	24 h	24 h	24 h
		CB	< 3 h	24 h	24 h	24 h
	N	SV	24 h	24 h	24 h	24 h
		CB	24 h	24 h	24 h	24 h
	T	SV	6 h	24 h	24 h	24 h
		CB	6 h	24 h	24 h	24 h
	FA	SV	24 h (90%)	24 h	24 h	24 h
		CB	6 h	24 h	24 h	24 h
	RSP	SV	< 3 h	24 h	24 h	24 h
		CB	< 3 h	24 h	24 h	24 h
	Vitamin A	VL	< 3 h	6 h	6 h	24 h
		CB	< 3 h	6 h	6 h	24 h
	Vitamin E	VL	3 h	24 h	24 h	24 h
		CB	3 h	24 h	24 h	24 h

S: Stable; U: Unstable

7.4. Discussion

Visual examination of the samples showed that all samples remained clear for SV and homogenous for VL and CB, with lipid globules sizes of less than 5 μm. NTU measurements of SV solutions showed a decrease in NTU units. As for pH, the minibags without the protective covers had pH change of more than 0.5 units, while the dissolved oxygen contents decreased upon subject to higher temperatures. These physical assessments have been discussed in the earlier chapters and will not be reiterated in this chapter.

As can be seen from the summary in Table 7.22, at 25°C and 40°C with the plastic or aluminium covers, all the water-soluble vitamins examined, and the fat-soluble Vitamin E were stable for at least 24 hours in the SV or VL, and the CB minibags. When exposed without any protective covers, the stability of pyridoxine, riboflavin sodium phosphate, Vitamin A and Vitamin E were reduced to 6 hours or less at 25°C, while at 40°C, the stability of thiamine was also reduced to 6 hours in SV, VL or CB minibags. Folic acid stability in clear minibags was reduced to 6 hours in CB emulsion at 40°C although its percentage content at 24 hours was only slightly reduced to 86%.

Wrapping the vitamin minibags during delivery with red or black plastics, or with aluminium foil hence gives some protection of the vitamins from degradation by UVA, where significant protection at $p<0.001$ was conferred by the protective covers for pyridoxine and riboflavin sodium phosphate in SV and CB minibags, and for Vitamin A and Vitamin E in VL and CB minibags. There was some UVA protection by the protective covers for thiamine and folic acid although this degree of protection was not shown to be statistically significant from the unprotected minibag.

With Vitamin A, it can be seen that it is greatly affected when exposed to UVA with its stability reduced to 6 hours even when wrapped with red plastic cover at 25°C, while without any protective cover, the stability was reduced to less than 3 hours in both formulations VL and CB. These results again confirm the

results of Chapter Five that Vitamin A is greatly affected by light, in this case by UVA. Greatest protection is obtained when the minibag was wrapped with aluminium foil. There was insufficient protection of Vitamin A with the black or red plastic bags at 40°C, with the stability reduced to 6 hours.

The result is comparable to the study conducted by Drott et al (1991) where they found that the use of red plastic bags was inadequate to preserve the Vitamin A in solution but sufficient to protect the Vitamin E. Another study by Shenai et al (1981) suggested that the amount of Vitamin A delivered to be increased by three to fourfold to compensate for the losses or that Vitamin A to be administered by an alternative method such as by intramuscular injection. Having said this, if a red plastic protective cover was used, it may be sufficient to infuse the vitamin minibag formulation for up to 6 hours since its stability duration is within this period of time. Alternatively, the infusion may be started during the night when UVA radiation is negligible.

As for the CB admixtures, some protection from light was noted for Vitamin A. In VL emulsion with FF25C exposure condition (see Table 7.13), Vitamin A content was 24% at 3 hours and could not be detected at 24 hours. However, in CB emulsion and with the same exposure condition (see Table 7.20), Vitamin A content at 3 hours was 62% and there was still about 8% left at 24 hours. Slight protection was conferred with riboflavin sodium phosphate in CB emulsions too. In SV solution at FF40C (see Table 7.12), the riboflavin sodium phosphate content was 29% at 24 hours, while in CB emulsion at FF40C (see Table 7.19), the content was 35% at 24 hours. As discussed in Chapter Six, these are possibly due to emulsification which reduced the light transmission. There was no obvious protective effect for the other vitamins in CB emulsion.

In terms of the degree of UVA protection of the vitamins by different types of protective covers, it can be seen that different coloured plastic covers have different degree of protection. From this study, the red plastic cover seemed to have less UVA protective effect when compared to the black plastic cover, as

shown by the reduced stability of Vitamin A at 25°C. This may be due to the limited wavelength range of UV protection provided by the red plastic bag of between 190 and 590 nm as compared to black plastic bag which generally gives good protection against UV light over a much wider wavelength range. The protective effects also depend on the type of dyes used (Weber 1979) and the thickness of the plastic bags. Having said this, Vitamin A was also affected when covered with the black plastic bag at 40°C, with its stability reduced to 6 hours too. In determining the choice of protective covers in clinical practice, other factors such as the cost, availability, and ease of use by nursing staff, like when carrying out visual checks on the minibags during infusion to patients, will all ultimately have to be considered.

7.5. Conclusions

All the vitamins analysed were stable for 24 hours, provided they were light protected. Aluminium foil provided the best protection from UVA for all the vitamins, while the red and black plastic bags provided partial protection of Vitamin A from UVA.

CHAPTER EIGHT

CONCLUSIONS

8.1. Conclusions

Long shelf life macronutrient PN bags are available commercially as triple chamber bags in which the seals of the amino acids, glucose and lipid components can be opened and the components mixed as an all-in-one admixture. For complete nutrition support of parenterally fed patients however, these still require the addition of micronutrients into the final bag just before administration because of stability issues with the vitamins and incompatibility and precipitation issues with the trace elements.

The problems associated with the provision of micronutrients to PN patients in regards to stability issues and differing stability data have been outlined in Chapter One. The recent NICE guidelines (NICE 2006a) recommended that micronutrients should be added to PN just before administration. For a ward based inpatient requiring PN in the hospital, the micronutrient additions are carried out by the pharmacy aseptic compounding unit, although these additions are possibly omitted for the weekend supply as any additions are only made aseptically by the pharmacy. For home PN patients, either the patient or their care provider is taught to do these micronutrient additions. There is a possibility that the micronutrients are inadvertently omitted due to additional task involved. In a national audit carried out by the British Pharmaceutical Nutrition Group in 2003, the audit revealed that for hospitals with a PN service, 33% of the patients did not receive vitamins or trace elements in accordance with recommendations (Sizer 2004). Problems of personnel handling of sterile preparations arise here too as in relation to the Audit Commission's 'A Spoonful of Sugar' report (Audit Commission 2001), which recommends that aseptic manipulations should be carried out by the pharmacy

as part of improvement in the quality of care. This is also in line with the NICE guidelines that the micronutrients additions into PN bag be made under a pharmaceutically controlled environmental condition (NICE 2006a). Therefore there was an opportunity to develop a micronutrient formulation with a longer shelf life and a stability profile that need not require reconstitution and/or any further handling.

In considering the micronutrient formulation, in theory, it would be most practical to have a single micronutrient formulation consisting of both the vitamins and trace elements. Knowing the fact that both the vitamins and trace elements are multi-components by their own category, having a complete micronutrient formulation would require an extensive length of study, due to many other influencing factors such as interaction problems between the vitamins and trace elements (Harraki et al. 1995). Hence it was decided that either the vitamins or the trace elements could be investigated at any one time. The vitamin formulation was decided upon because the equipment needed to investigate its stability, such as the HPLC, was already available in the present laboratory and the readily available multivitamin injections on the market such as Solivito® N Adult and Vitlipid® N Adult injections would be a good start point since end-users would be familiar with their composition and some stability data would be available for comparison.

Another point of consideration was that the base vehicle used should be an aqueous 0.9% sodium chloride intravenous solution. Presently, no stability studies have been carried out with fat-soluble vitamins in aqueous systems or combined water-soluble and fat-soluble vitamins in such aqueous admixtures.

The development of validated stability indicating HPLC methods in this thesis enables only the investigation of the vitamins that could be determined in a single run as described in Chapter Two. Thus, the stability of five water-soluble B vitamins, namely, pyridoxine, nicotinamide, thiamine, folic acid and riboflavin sodium phosphate contained in Solivito® N Injection, and the fat-

soluble vitamins, Vitamin A and Vitamin E, contained in Vitlipid® N Adult Injection in 100 ml 0.9% sodium chloride was investigated. A test run in glucose 5% intravenous solution was also tested with the same peaks obtained. The five water-soluble vitamins were identified by separating Solivito® N Adult solution by reversed phase HPLC and gradient run method while the two fat-soluble vitamins were identified from an assay method developed by the changing of detection wavelength in a single run. Forced degradation of the samples showed no interference of the degradation products with the peaks of interest and the methods developed were found to be repeatable and stability indicating.

Henceforth, their basic stability profile upon different storage conditions at 4, 25, 30 and 40°C was investigated as described in Chapter Four for the water-soluble vitamins and Chapter Five for the fat-soluble vitamins, as well as upon subjection to simulation conditions encountered in clinical practice as described in Chapter Six. Stability studies assessed included delivery conditions with different protective covers at 25°C and 40°C as described in Chapter Seven. A preliminary study also investigated the vitamins stability in glass containers as background data in the initial phase of the project work and as comparison but was phased out in view of the fact that the most used packaging nowadays is plastic bags as discussed in Chapter Three.

Initial assessment of the five water-soluble vitamins upon different storage conditions showed that the vitamins were stable for 168 hours at 4°C, with more than 90% remaining. For solutions stored at 25°C, they remained stable for 168 hours if they were light-protected, in this case from artificial fluorescent light having an illuminance equivalent to general ward lighting condition. Interestingly, for the fat-soluble vitamins examined, they were also found to be stable for 168 hours at 4°C and at 25°C, provided the emulsions were protected from the artificial light.

It has been implicated that only the UV light affects the photostability of vitamins (Allwood 1982a; Allwood and Kearney 1998; Dahl et al. 1986) and not fluorescent light as it emits insignificant UV radiation. However, this study has shown that extended exposure to artificial light affects the vitamin stability. Therefore, the precautionary measure of light protection for the vitamins is still required.

In Chapter Six, when the vitamins were investigated simulating clinical conditions, they were found to be stable for up to 30 days storage at 4°C, and remained stable when the solutions were then subjected to 25°C for the next 24 hours, provided they were light-protected. Because it would be most convenient to administer the water-soluble and the fat-soluble vitamins together at the same time, an assessment of the combined admixture of the water-soluble and fat-soluble vitamins simulating clinical conditions were also carried out. The results were promising with all vitamins examined remaining stable for up to 30 days in the refrigerator, followed by 25°C for the next 24 hours, provided they were light protected. These stability data could be practically useful for pharmacy aseptic compounding unit preparing multivitamin infusion for both hospital ward inpatients and home PN patients.

The photostability study during delivery of the vitamin minibags with the different protective covers in Chapter Seven showed that different coloured plastic protective covers have varying protection against UVA radiation, depending among others, on their degree of light penetration and the type of coloured dye used (Webber 1979). The study showed that the protective covers provide protection from UVA for 24 hours for most of the vitamins investigated except for Vitamin A when the stability was reduced to 6 hours with the red plastic cover. One way of minimising photodegradation during delivery is by infusing the vitamin minibag at night as an alternative time of delivery or to infuse the vitamin minibag for up to 6 hours when using a red plastic cover which may provide sufficient protection.

In conclusion, a simple ready-to-use micronutrient minibag formulation containing water-soluble and/or fat-soluble vitamins in an aqueous normal saline solution was studied for its possible development, with baseline physical and chemical data gathered. Extensive data on the vitamins investigated in different storage and clinical condition of administration showed promising results with the vitamins being stable during its application in simulated clinical practice if light protected. This longer shelf life vitamin minibag developed would be useful and of benefit when made available in the market, for its application to PN patients in the hospital especially for the weekend supply and for home PN patients where a ready-to-use vitamin minibag frees the patient or their care provider from additional reconstitution and dilution of sterile preparations.

8.2. Limitations

There are a number of limitations to this study. Firstly, not all the vitamins in the formulation, such as ascorbic acid, Vitamin B_{12}, Vitamin D and Vitamin K have been assayed, as in the time available, suitable stability indicating assay methods that could be used alongside those developed for the majority of vitamins could not be found and validated.

The studies carried out were not controlled for oxygen as these studies investigated the real clinical set-up when prepared in the aseptic compounding unit. For commercial purposes, the effect of oxygen on the stability of the vitamins in the minibags requires further investigation.

When investigating the vitamins' stability in the minibag, its use in clinical practice was simulated from its storage right through to its administration to the patient. However, when investigating the protective covers in the delivery study in Chapter Seven, it needs to be pointed out that investigation of the vitamins stability does not mimic delivery through the infusion tubing set.

In terms of time and workable scope, because there was only one pharmaceutical stability chamber to test for the different temperatures, studies were not run concurrently, therefore analyses such as HPLC assays were carried out according to temperature settings, thus this prolonged the duration of the project study.

8.3. Future Work

Future work would investigate the stability of the other component vitamins in this multivitamin infusion minibag which are ascorbic acid, biotin, pantothenic acid, Vitamin B_{12}, Vitamin D and Vitamin K. For the stability study of ascorbic acid, the total ascorbic acid concentration which includes dehydroascorbic acid would require investigation too as dehydroascorbic acid is also a biologically active component of Vitamin C.

Another area would be to investigate the vitamins' photostability through the infusion set during administration to patients. This study would include investigation of the protective effects of different coloured tubings during infusion.

For commercial production, the stability of these vitamins in multilayered or gas impermeable bags, together with the overwraps to exclude the permeation of oxygen would possibly need to be carried out. A controlled stability study with oxygen degassed from the minibag by using an inert gas such as helium or nitrogen could be investigated. The control for this study would also need to be light-protected.

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APPENDIX 1

Some known nutritional functions of selenium as selenoproteins (adapted from Rayman 2000)

Selenoprotein	*Function*
Glutathione peroxidises	Antioxidant enzymes; remove hydrogen peroxide, and lipid and phospholipid hydroperoxides (thereby maintaining membrane integrity, modulating eicosanoid synthesis, modifying inflammation and likelihood of ropagation of further oxidative damage to biomolecules such as lipids, lipoproteins, and DNA.
(Sperm) mitochondrial capsule selenoprotein	Form of glutathione peroxidise (GPx4); shields developing sperm cells from oxidative damage and later polymerises into structural protein required for stability / motility of mature sperm.
Iodothyronine deiodinases	Production and regulation of level of active thyroid hormone, T3, from thyroxine, T4.
Thioredoxin reductase	Reduction of nucleotides in DNA synthesis; regeneration of antioxidant systems; maintenance of intracellular redox state, critical for cell viability and proliferation; regulation of gene expression by redox control of binding of transcription factors to DNA.
Selenophosphate synthetase	Required for synthesis of selenophosphate, the precursor of selenocysteine, and therefore for selenoprotein synthesis.
Selenoprotein P	Found in plasma and associated with endothelial cells. Appears to protect endothelial cells against damage from peroxynitrite.
Selenoprotein W Prostate epithelial selenoprotein (15kDa)	Needed for muscle function. Found in epithelial cels of ventral prostate. Seems to have redox function (resembles GPx4), perhaps protecting secretory cells against development of carcinoma.
DNA bound spermatid selenoprotein (34 kDa)	Glutathione peroxidise-like activity. Found in stomach and spermatozoa. May protect developing sperm.
18 kDa selenoprotein	Important selenoprotein, found in kidney and large number of other tissues. Preserved in selenium deficiency.

APPENDIX 2

Stability characteristics of vitamins (adapted from DeRitter 1982)

Vitamin A	Sensitive to atmospheric oxygen (retinol less stable than its esters); trace metals catalyse decomposition; inactivated by UV light; isomerises at acid pH; stable in alkali; in aqueous dispersions palmitate esters more stable to heat than retinol<pH 5.5; palmitate most stable ester under moisture stress; stabilise by antioxidants and protective coatings.
Cholecalciferol (Vitamin D)	Sensitive to atmospheric oxygen; trace metals and carriers with acid surface activity catalyse isomerisation or decomposiyion; generally more stable than vitamin A; stabilised by antioxidants and protective clothings.
Vitamin E	Tocopherol sensitive to atmospheric oxygen, especially to alkali and sensitive to UV light; esters very stable.
Vitamin K	Fairly stable to heat; decomposed to sunlight and alkali.
Ascorbic acid (Vitamin C)	Stable when dry; readily oxidised in solution; decomposition catalysed by metal ions (copper and iron); greatest instability at about pH 4.
Biotin	Stable to air and acid and at neutral pH; slightly unstable in alkali.
Cyanocobalamin (Vitamin B_{12})	Decomposed by oxidising and reducing agents; slightly unstable in acid and alkaline solution; ascorbic acid and thiamine-niacinamide combination accelerate the decomposition; sensitive to light in very dilute solutions for assay.
Folic acid	Unstable <pH 5; decomposed by sunlight and riboflavin; decomposed by reducing agents.
Niacin and niacinamide	Very stable.
Calcium pantothenate	Unstable in acid (<pH 5) and alkali; maximum stability at pH 6-7.
Panthenol	More stable than calcium pantothenate at ≤ pH 5.
Pyridoxine (Vitamin B_6)	Normally very stable; metal ions can catalyse decomposition; dilute solutions for assay are very sensitive to light.
Riboflavin (Vitamin B_2)	Stable in acid solution; unstable in alkaline solution; sensitive to light, especially in alkaline solution or in very dilute acid solutions for assay; decomposed by reducing agents.
Thiamine (Vitamin B_1)	Increasingly unstable in solution as pH rises; decomposed by oxidising or reducing agents; cleaved by sulphite very rapidly at high pH; hydrochloride more hygroscopic than mononitrate.

PUBLICATIONS

Recent Publications (Abstracts)

Cosslett, A. G. and Said, S. N. 2004. Problems associated with the provision of vitamins to parenteral nutrition(PN) patients. *Clinical Nutrition* 23(4), pp. 823-824.

Cosslett, A. G. and Said, S. N. 2005. A Clinical Simulation Study of a Micronutrient Minibag to Home Parenteral Nutrition (HPN) Patients. *Clinical Nutrition* 24(4), pp. 700-701.

Said, S. N. and Cosslett, A. G. 2004. Development of a micronutrient minibag for parenteral nutrition (PN) patients. *Journal of Pharmacy and Pharmacology* 56(supplement), pp. S-54.

Said, S. N. and Cosslett, A. G. 2005a. Development of a micronutrient minibag formulation for parenteral nutrition (PN) patients - effects of light and temperature on stability. *Journal of Pharmacy and Pharmacology* 57(September Supplement), pp. S-87.

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Said, S. N. and Cosslett, A. G. 2006. Stability of Vitamins A and E in an Aqueous Minibag Formulation Exposed to a Variety of Different Storage Conditions. *e-SPEN, the European e-Journal of Clinical Nutrition and Metabolism* 1(2), p. 84.