UNIVERZITA KARLOVA V PRAZE

FARMACEUTICKÁ FAKULTA

KATEDRA ANALYTICKÉ CHEMIE

STABILITA VITAMÍNŮ V INTRALIPIDOVÝCH EMULZÍCH A DEGRADACE KYSELINY ASKORBOVÉ

HRADEC KRÁLOVÉ 2010 Mgr. LUCIE KHÝNOVÁ

CHARLES UNIVERSITY IN PRAGUE

FACULTY OF PHARMACY

DEPARTMENT OF ANALYTICAL CHEMISTRY

STABILITY OF VITAMINS IN INTRALIPID® EMULSIONS AND DEGRADATION OF ASCORBIC ACID

HRADEC KRÁLOVÉ 2010 Mgr. LUCIE KHÝNOVÁ

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DECLARATION

Prohlašuji, že tato práce je mým původním autorským dílem, které jsem vypracovala samostatně. Veškerá literatura a další zdroje, z nichž jsem při zpracování čerpala, jsou uvedeny v seznamu použité literatury a v práci řádně citovány.

This thesis is the result of my own investigations, except where otherwise stated. Sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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LIST OF ABBREVIATIONS

μg	Microgram
μL	Microliter
μm	Micrometer
AA	Ascorbic acid
Ag	Silver
AgCl	Silver chloride
AIO	All-in-one
Al	Aluminium
BMI	Body mass index
BP	British Pharmacopoeia
Co	Cobalt
CoA	Coenzyme A
CPN	Central parenteral nutrition
Cr	Chromium
CRC	Catheter-related complications
Cu	Copper
CVC	Central venous catheter
DNA	Deoxyribonucleic acid
EVA	Ethyl-vinyl acetate
F	Fluorine
FA	Folic acid
FAD	Flavine-adenine dinucleotide
Fe	Iron
FH_2	Dihydrofolic acid
FH_4	Tetrahydrofolic acid
FMN	Flavine-mononucleotide
FSV	Fat-soluble vitamins
g	Gram
h	Hour
HEPA	High efficiency particulate air

HPLC	High performance liquid chromatography
I	Iodine
IU	International unit
kg	Kilogram
L	Liter
LC	Liquid chromatography
LCT	Long-chain triglycerides
MCT	Medium-chain triglycerides
mEq	Milliequivalent
mg	Milligram
ml	Milliliter
mm	Millimeter
mmol	Millimol
Mn	Manganese
Mo	Molybdenum
mosm/kg	Milliosmol per kilogram
MUFA	Monounsaturated fatty acids
NA	Nicotinic acid
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NICE	National Institute for Health and Clinical Excellence
nm	Nanometer
OA	Oxalic acid
PA	Pantothenic acid
PABA	Para-aminobenzoic acid
PICC	Peripherally inserted central catheter
PN	Parenteral nutrition
PteGlu	Pteroylglutamic acid
PPN	Peripheral parenteral nutrition
PUFA	Polyunsaturated fatty acids
PVC	Poly-vinyle chloride
R^2	Correlation coefficient
RSD	Relative standard deviation
rt	Retention time

Se	Selenium
TE	Trace elements
TPN	Total parenteral nutrition
UFDW	Ultrafiltered deionised water
UV	Ultraviolet
WSV	Water-soluble vitamins
Zn	Zinc

CHAPTER ONE

INTRODUCTION AND AIMS OF PROJECT

CHAPTER ONE: INTRODUCTION AND AIMS OF PROJECT

1.1 INTRODUCTION

Malnutrition is a state in which a deficiency of energy, protein and other nutrients causes measurable adverse effects on tissue, body form, composition, function or clinical outcome. It is both a cause and a consequence of ill-health and is common nowadays. Since malnutrition increases a patient's vulnerability to ill health, providing nutrition support to patients with malnutrition should improve outcomes but decisions on the most effective and safe means to do so are complex. Parenteral nutrition (PN) refers to the administration of nutrients by the intravenous route. It is usually administered via a dedicated central or peripheral placed line[1].

1.2 AIMS OF PROJECT

The purpose of this rigorous thesis was to obtain more punctual information on stability of the vitamins in Intralipid® emulsion depending on different storage conditions and different time. There are two projects which were done.

1. Stability of the fat-soluble and the water-soluble vitamins which were added to the Intralipid® emulsion, and stored in 50 ml Luer-Lock Syringes.

Assessing the chemical and the physical stability was carried out after:

- Zero time
- 7 days in a refrigerator followed by 24 hours and 48 hours storage at ambient temperature and protected from day-light.
- 14 days in a refrigerator followed by 24 hours and 48 hours storage at ambient temperature and protected from day-light.
- 29 days in a refrigerator followed 24 hours and 48 hours storage at ambient temperature and protected from day-light.

2. Ascorbic acid (AA) is rapidly oxidised, the oxidation of AA clearly proceeds to inactive products. There is potential appearance of oxalic acid (OA) in the mixture as an end stage degradation product[2]. The purpose of my investigation was to prove the presence of OA as a degradation product of AA dissolved in water after couple-day storage at ambient temperature via HPLC analysis.

CHAPTER TWO

THEORETICAL PART

CHAPTER TWO: THEORETICAL PART

2.1 PARENTERAL NUTRITION

PN has now become the accepted method for the prevention or correction of malnutrition in patients who cannot be provided with adequate nutrition by the gastro-intestinal route. PN is an alternative method of providing nutritional support for patients via the intravenous route, the order methods being by the oral or enteral route[3].

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[4]. This guideline recommends that all hospital inpatients on admission and all outpatients at their first appointment should be screened (weight, measured and have Body Mass Index (BMI) calculated).

Malnutrition in hospitals is surprisingly common. Several studies have shown that up to 65% of patients admitted to medical and surgical wards are undernourished or become so during their stay. These patients are more likely to suffer from complications, they are more susceptible to infection and they experience delayed wound healing. Their mortality rates are higher and their stays in hospital longer. There is therefore increasing interest amongst health-care professionals in methods of providing nutritional support[5].

2.2 INDICATIONS FOR INTRAVENOUS NUTRITION

This paragraph was discussed in Diploma thesis of Khýnová[6] in detail.

2.3 COMPONENTS OF PN ADMIXTURES

Mainly three macronutrients form PN admixtures. Namely: 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. Included are the major electrolytes such as sodium, potassium, chloride, calcium, magnesium and phosphate. The addition of micronutrients, including vitamins and trace elements, make a PN complete[7]. For mixtures which contain all described nutrients is used the term total parenteral nutrition (TPN). TPN problems were specified in Diploma thesis Khýnová[6] in detail.

2.3.1 Lipids

Lipids are very important energy substances and provide caloric requirements. They also serve as a source of essential fatty acids, such as linoleic and linolenic acid, which are necessary for the functioning of all tissues[7]. Lipid requirement is about 1 – 1.5 g/ kg/ day in healthy adults[8].

Currently available intravenous emulsions consist of 10% or 20% emulsions of soybean or safflower oil. These are long-chain triglycerides (LCT), rich in omega-6 polyunsaturated fatty acids (PUFA) [7]. New lipid emulsions are created medium-chain triglycerides (MCT) derived from palm kernel and coconut oil. MCT emulsions have to be administered either at very slow rates or together with LCT because of undesirable symptoms. Administration of an MCT/LCT mixture revealed in many cases advantages over an LCT. The current interest is in the use of fish oil and olive oil lipid emulsions. Olive oil contains a high level of monounsaturated fatty acids (MUFA) and fish oil is rich in n-3 fatty acids. There is the claim that n-3 fatty acids have anti-inflammatory and immunomodulatory effects[9].

2.3.2 Proteins

Proteins are large organic compounds made of amino acids arranged in a linear chain and joined together by peptide bonds. Therefore they are a source of amino acids. Traditionally, amino acids are classified into essential and non-essential. Essential amino acids in adults include histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tyrosine, valine, tryptophan. Some of the non-essential amino acids are alanine, arginine, glycine, glutamate, glutamine, serine and taurine[10].

Essential amino acids must be provided in the diet and should provide about 40 % of the total amino acid nitrogen. A daily intake of 12 – 16 g of nitrogen has been found to be adequate for most patients[11]. Some sick patients may be unable to synthesize amino acids which are considered to be non-essential in normal subjects. For example, histidine may not be synthesized in adequate amounts in patients with renal failure[12].

2.3.3 Carbohydrates

Dietary carbohydrates usually provide between 40 – 70 % (currently recommended 50 – 55 %) of our total daily energy intake. Glucose is the main source of readily available energy[13]. Adult glucose requirements in healthy subjects can be set at 4 – 5 g/ kg/ day, equivalent to 50 % of the total energy requirement[8].

Glucose is supplied intravenously in the exact chemical form in which it is absorbed from the intestine. This glucose requirement is unique to humans and is about 145 – 160 g per day. Injured or septic patients may require more glucose than normal patients[9].

2.3.4 Fluids and Electrolytes

The intake of fluid and electrolytes is an integral part of nutritional support. The calculation of appropriate requirements is necessary because electrolytes abnormalities may cause physiological and functional problems for the patients.

Potassium, sodium, magnesium, calcium and phosphate are some of the major electrolytes required[14]. Daily requirements of each ion can vary widely, depending on the disease state and may be very high[2].

2.3.5 Micronutrients

Micronutrients are main classes of nutrient substances required in the diet in very small amount. These are the essential inorganic micronutrients otherwise the trace elements, and the vitamins or the essential organic micronutrients. The trace elements include zinc, cooper, selenium, manganese, chromium, molybdenum, iron, iodine and fluorine.

There are two main functions of micronutrients. Firstly they act as cofactors or coenzymes in enzyme catalyzed reactions. In general, the water-soluble vitamins have roles as coenzymes, taking an active part in enzyme catalyzed reactions. For both of these groups of substances, enzyme activity may be modulated by the availability of the micronutrient. In disease states, the metabolism of the major substances is significantly increased, and therefore the requirements for micronutrients are also increased. A second, micronutrient's role is as part of the free-radical scavenging system. Oxidative metabolism generates a family of reactive oxygen species (superoxide, hydroxyl) and these have the potential to cause significant chemical damage[15]. Both vitamins and trace elements are discussed under Vitamins (see 2.4).

2.4 VITAMINS

Vitamins are organic, low molecular weight substances that have key roles in metabolism. The vitamins are required in only small amounts in the diet because they are highly specific. They are classified according to their solubility into two groups - the fat-soluble vitamins and the water-soluble vitamins. The fat-soluble vitamins include vitamins A, E, D, K these are soluble in non-polar solvents and have the potential for storage in the body. The water-soluble vitamins include ascorbic acid and the vitamin B group. They are soluble in polar solvents and are

not stored in the body. They are excreted in the urine when present is excess of the body's needs[16].

Following paragraphs describe chemical structure of single vitamins and their degradation potential as well. Vitamins were discussed in Diploma thesis of Khýnová[6] in detail. See mentioned thesis for detailed information.

2.4.1. Thiamine (Vitamin B_1)

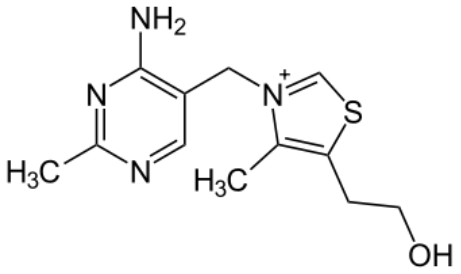
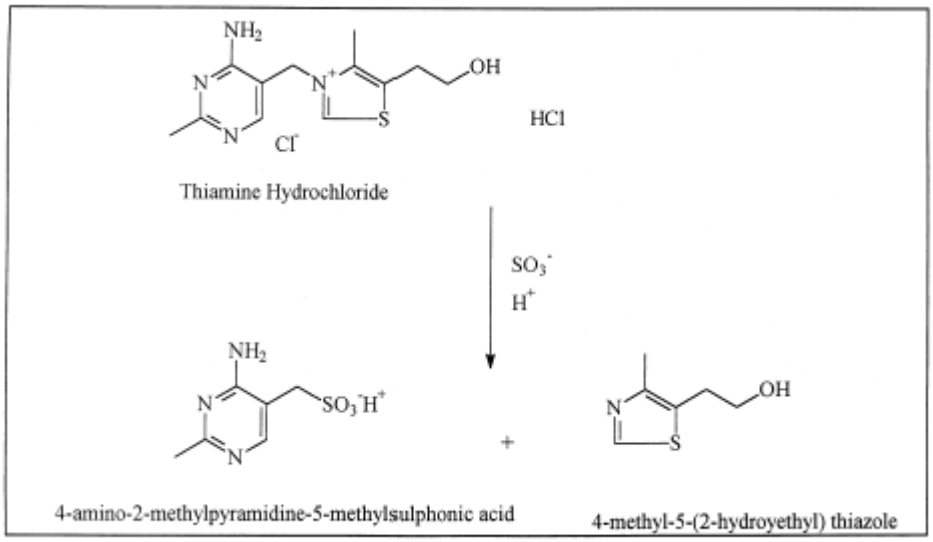


Figure 2.1: Chemical Structure of Thiamine

Vitamin B_1 is degraded by a number of mechanisms. It is increasingly unstable as the pH rises and is decomposed by oxidizing or reducing agents. The reduction is caused in particular by sodium metabisulfite used as an antioxidant in some amino acids infusions. The route of degradation is showed at Figure 2.2. The rate of this cleavage increases with increased pH to a maximum rate of pH 6. A couple studied confirmed that bisulfite at concentrations above 1 mmol/L caused thiamine degradation. There are some losses of thiamine after exposure to direct sunlight, but no significant losses after exposure to indirect daylight or fluorescent light[17].

Figure 2.2: Thiamine Degradation Pathway



2.4.2 Riboflavin (Vitamin B_2)

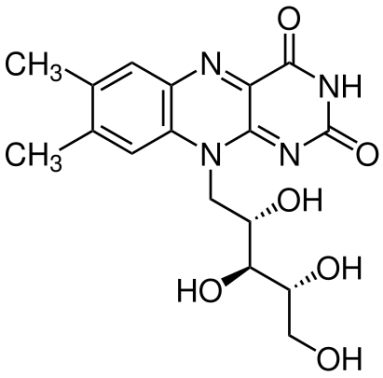


Figure 2.3: Chemical Structure of Riboflavin

Riboflavin has been reported to accelerate the photodegradation of certain amino acids. Enhanced degradation of methionine, tryptophan, proline, and tyrosine accelerated in the presence of riboflavin was reported during simulated infusion over a 24 hours period. Riboflavin is degraded by exposure to daylight, although it

is less sensitive than retinol. In contrast the vitamin was not degraded by exposure to fluorescent light[17].

2.4.3 Niacin (Vitamin B_3)

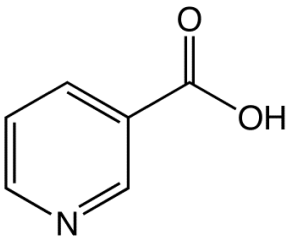


Figure 2.4: Chemical Structure of Nicotinic Acid

The term niacin is the generic descriptor for nicotinic acid (pyridine-3-carboxylic acid) (Figure 2.4) and nicotinamide (nicotinic acid amide).

Both nicotinic acid and nicotinamide are very stable in dry form, but in solution nicotinamide is hydrolyzed by acids and bases[16].

2.4.4 Pyridoxine (Vitamin B_6)

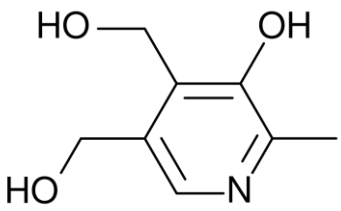


Figure 2.5: Chemical Structure of Pyridoxine

Vitamin B_6 is the generic descriptor for all 3-hydroxy-2-methylpyridine derivatives.

Allwood[2] reported that pyridoxine is also light sensitive although degradation is far less than is observed with vitamin A or riboflavin. Direct sunlight causes loss of pyridoxine, but it is stable during exposure to indirect daylight or fluorescent light.[17]

2.4.5 Folic Acid

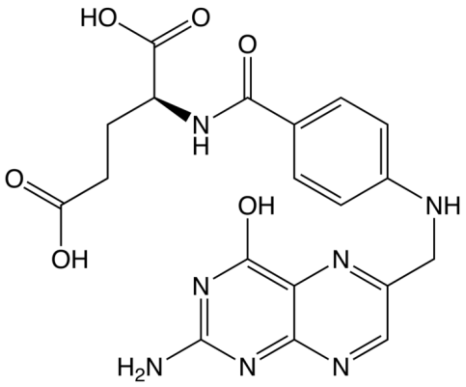


Figure 2.6: Chemical Structure of Folic Acid

Folic acid is 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[16].

Folic acid (FA) is insoluble in water and injections are formulated as sodium salt at pH above 8.0. After addition to TPN mixtures, there is a danger of free folate precipitation, especially in solutions below pH 4.5 – 5. FA in PN mixtures is compatible with plastic bags and sets. FA has been shown to be stable after exposure to indirect or direct daylight, and fluorescent light as well. Most folates are easily oxidized and, therefore, are unstable to oxidation under aerobic conditions of storage. Under such conditions tetrahydrofolic acid (FH_4) derivatives can readily be oxidized to the corresponding derivatives of dihydrofolic acid (FH_2) or folic acid[17].

2.4.6 Vitamin B_{12}

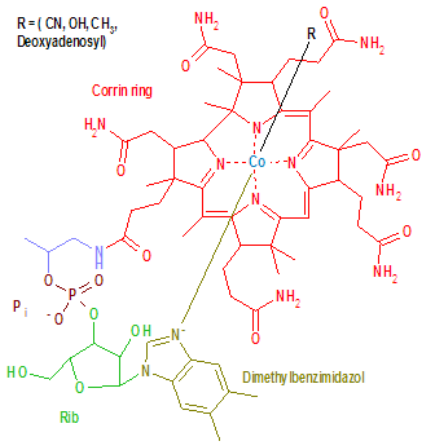


Figure 2.7: Chemical Structure of Cyanocobalamin

Vitamin B_{12} is an octahedral cobalt complex consisting of a porphyrin-like, cobalt-centered macroring (called corrin ring), a nucleotide, and a second cobalt-bound group (e.g. H_2O, CN^-, CH_3). Vitamin B_{12} is the generic descriptor for all corrinoids (i.e. compounds containing the corrin ring) exhibiting the qualitative biological activity of cyanocobalamin (Figure 2.7)[16].

Vitamin B_{12} is very stable in both crystalline form and aqueous solution. However, the cobalamins are unstable to light[16].

2.4.7 Pantothenic Acid

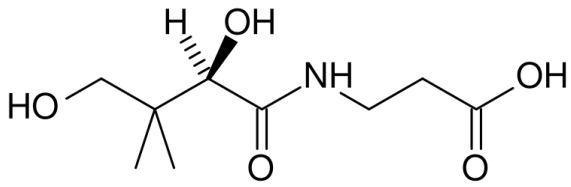


Figure 2.8: Chemical Structure of Pantothenic Acid

Pantothenic acid is yellow, viscous oil. Its calcium and other salts, however, are colourless crystalline substances. Each form is soluble in water and ethanol. Aqueous solutions of pantothenic acid are unstable to heating under acidic or alkaline conditions[16].

2.4.8 Biotin

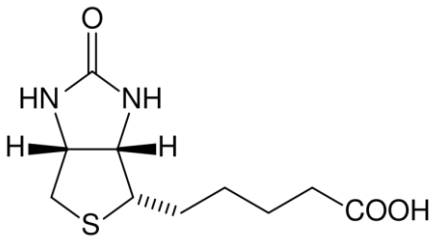


Figure 2.9: Chemical Structure of Biotin

Biotin is formerly known as vitamin H.

Biotin is unstable to oxidizing conditions and, therefore, is destroyed by heat, especially under conditions that support lipid peroxidation[16].

2.4.9 Ascorbic Acid (Vitamin C)

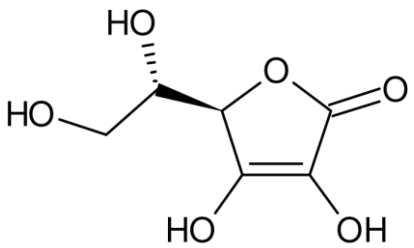


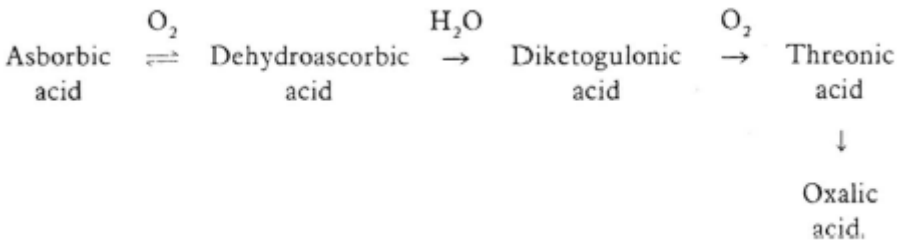
Figure 2.10: Chemical Structure of Ascorbic Acid

Vitamin C is the generic descriptor for all compounds that exhibit the biological activity of ascorbic acid (Figure 2.10). The oxidized form of this compound is called

dehydroascorbic acid. Ascorbic acid (AA) is a dibasic acid. It forms salts, the aqueous solutions of which are strongly acidic. AA is a strong reducing agent and is oxidized under mild conditions to dehydroascorbic acid via the radical intermediate semidehydroascorbic acid. The three forms (AA, semidehydroascorbic acid, dehydroascorbic acid) compose a reversible redox system. It is therefore an effective quencher of free radicals such as singlet oxygen. It reduces ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron and the superoxide radical ($O_2^{\cdot-}$) to H_2O_2 and is oxidized to monodehydroascorbic acid in the process[16].

Ascorbic acid is the least stable of all the vitamins added to TPN mixtures, although degradation of AA depends on a number of inter-related factors. AA is oxidized in aqueous solution by reaction with dissolved oxygen. The rate of degradation depends on various aspects of the solution. The most important factors are dissolved oxygen and the presence of catalysts, especially copper ions. Slow loss of vitamin C occurs during clinical administration of TPN mixtures if TE, especially copper, are excluded. If cooper is included, oxidation is accelerated. The total amount of AA degraded depends on the oxygen content of the infusion and the volume of air remaining in the bag. A second stage of vitamin C degradation occurs due to the permeation of oxygen through the plastic. Some degradation will be also occurs in the administration set for the same reasons[2]. Allwood[2] also reported that cysteine and cystine inhibit the catalytic effect of copper, therefore, if infusion includes amino acids, degradation rates can be lower.

Figure 2.11: The Degradation of AA in the Presence of Oxygen



Dehydroascorbic acid is metabolically active. Therefore if the reaction proceeds only to this point, the availability of vitamin C is unaffected. The oxidation of AA clearly proceeds rapidly to inactive products. There is the potential appearance of oxalic acid (OA) in the mixture as end stage degradation product. The potential toxicity of this reaction may be significant[2]. In vivo OA is normally excreted in urine as a waste product of intermediary metabolism, but may precipitate with calcium in urine or soft tissues. Calcium oxalate precipitation deposition plays a role in urolithiasis, renal failure and the systemic oxalosis of certain inborn errors of metabolism. In patients receiving long-term TPN has been reported hyperoxaluria, elevated serum oxalate concentration, and systemic oxalosis because of degradation of parenteral AA. Oxalogenesis from AA oxidation in TPN solutions has been reported to cause catheter occlusion. Oxalate itself is not stable in dilute solution and degrades to carbon dioxide and formic acid under UV light[18].

2.4.10 Vitamin A

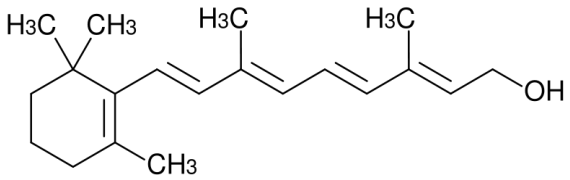


Figure 2.12: Chemical Structure of Retinol

Vitamin A is the generic descriptor for compounds with the qualitative biological activity of retinol (Figure 2.12). They are called retinoids[16].

Retinol is the most light-sensitive of the vitamins[17]. Retinol is known to be rapidly broken down by exposure to UV light. Therefore, daylight causes rapid degradation[2]. Photolysis proceeds both in the bag and during passage through the administration set. Administration in a room with only artificial lighting will lead to minimum degradation. Sorption of retinol has been reported, but it depends on the ester used. Whereas the acetate ester binds strongly to PVC bags and administration sets, the palmitate ester shows no evidence of binding to plastics used to store or administer PN[17]. The presence of amino acids and fat emulsion in the bag affords considerable protection[2].

2.4.11 Vitamin D

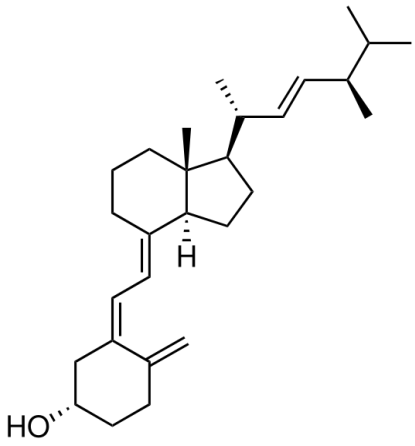


Figure 2.13: Chemical Structure of Ergocalciferol

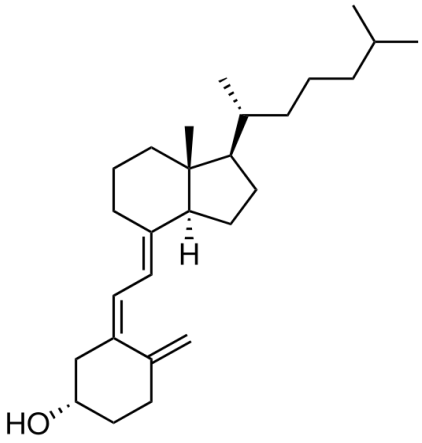


Figure 2.14: Chemical Structure of Cholecalciferol

Vitamin D is the generic descriptor for all steroids exhibiting qualitatively the biological activity of cholecalciferol. Ergocalciferol (Figure 2.13) (vitamin D_2) is found in plants, fungi, and lichens while cholecalciferol (Figure 2.14) (vitamin D_3) is widely distributed in animals[16].

Gillis[19] reported that vitamin D may be strongly bound to plastic. Sorption to the administration set and bag can be significant.

2.4.12 Vitamin E

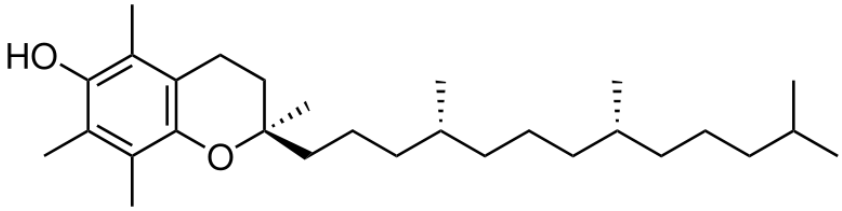


Figure 2.15: Chemical Structure of α-tocopherol

Vitamin E is the generic descriptor for all tocoretinol derivates that exhibit qualitatively the biological activity of α-tocopherol (Figure 2.15).

Tocopherol appears to be relatively stable in PN mixtures[17]. But Gillis[19] reported that vitamin E and D may be strongly bound to plastic. Sorption to the administration set and bag can be significant. Vitamin E together with Vitamin A are the most light sensitive vitamins. Vitamin E degrades by photo-oxidation[20].

2.4.13 Vitamin K

Vitamin K is the generic descriptor for 2-methyl-1,4-naphthoquinone and all of its derivatives exhibiting qualitatively the biological activity of phylloquinone. Naturally occurring forms of the vitamin have unsaturated isoprenoid side chain at C-3 of the naphthoquinone nucleus. The phylloquinone group includes forms with phytyl side chain and is called vitamin K_1 [16] (Figure 2.16). The menaquinone group have side chain composed of a varying number of isoprenoid residues. The menaquinone (Figure 2.17) is called vitamin K_2 and is synthesized by bacteria. The compound without a side chain is called menadione. It does not exist naturally and is the compound of commerce, because is made in several forms (e.g. menadione sodium bisulfite complex, menadione dimethylpyrimidinol bisulfite)[16].

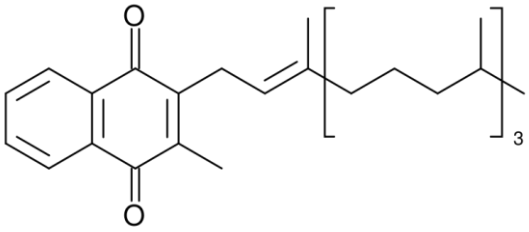


Figure 2.16: Chemical Structure of Phylloquinone

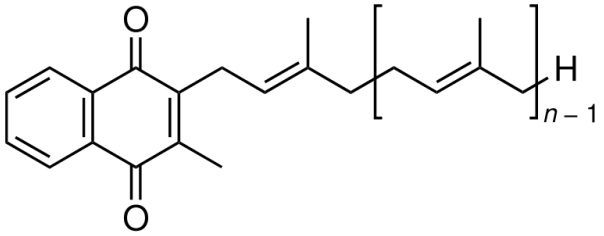


Figure 2.17: Chemical Structure of Menaquinone

Table 2.1: Daily Parenteral Vitamin Requirements in Adults[15]

Vitamins	**Adults parenteral requirements**
Vitamin A	1000 µg
Vitamin D	5 µg
Vitamin E	10 mg
Vitamin K	150 µg
Thiamine	3.0 mg
Riboflavin	3.6 mg
Pyridoxine	4.0 mg
Niacin	40 mg
B12	5 µg
Folic acid	400 µg
Biotin	60 µg
Ascorbic acid	100 mg

Commercial vitamins for PN are commonly available as multivitamin formulations such as Solvitio® N (Fresenius Kabi), Cernevit® (Baxter), Vitlipid® N Adult (Fresenius Kabi) and Vitlipid® N Infant (Fresenius Kabi) injections. Multiple TE are now available in a formulation such as Additrace® (Fresenius Kabi) and Decan® (Baxter) for adults, and Peditrace® (Fresenius Kabi) for paediatrics[7].

2.5 POTENTIAL INTERACTIONS OF COMBINED TPN MIXTURES IN BIG BAGS

Chemically unstable mixture with many possible reactions occurring may be caused by mixing of TPN components, which include glucose, lipids, amino acids, electrolytes, vitamins and TE[21]. These reactions include precipitation and degradation of particular compounds. Exposure to light may cause degradation while temperature changes can result in precipitation. Significant loss of stability or precipitation could be caused by pH changes. The filling process is important as well because causes aeration of solutions and increasing concentrations of dissolved oxygen. Poor mixing can lead to local concentration effects resulting in chemical reaction[2].

2.5.1 Amino Acid Stability

Amino acids retain their stability after dilution in TPN mixtures for at least 28 days if stored in the refrigerator. They are also stable during exposure to normal levels of artificial light or daylight. However, exposure to intense artificial light from a phototherapy unit in neonatal care may cause accelerated degradation of glycine and leucine. In the presence of riboflavin, photo-oxidation of several amino acids is accelerated[2].

2.5.2 Vitamin Stability

Vitamins are believed to be among the least stable ingredients in PN mixtures, and it is recommended that they should be added immediately before commencing infusion or that infusion should be commenced within 24 – 28 hours of addition. Some vitamins can undergo degradation during administration. The major physico-chemical considerations are exposure to light, the type of plastic used to manufacture the PN container and infusion equipment, and storage temperature. The most important cause of vitamin losses in PN mixtures is chemical degradation[17]. Stability of particular vitamins was discussed in detail under Vitamins (see 2.4).

2.5.3 Electrolytes

The major electrolytes necessary for nutritional support are sodium, potassium, calcium, magnesium, chloride, acetate and phosphate. Daily requirements of each ion can vary widely and depending on the disease state. The major physical-chemical incompatibility, causing precipitation, occurs when high concentrations of calcium and phosphate are mixed together. As the pH rises, more dibasic phosphate becomes available to react with calcium ions to form the precipitate. At low pH the monobasic ion predominates, being a far more soluble salt, precipitation is unlikely to occur. The most important factor influencing the maximum solubility of calcium + phosphate added to TPN mixtures is, therefore, pH. Practical considerations are also important. It is necessary to add and dilute the phosphate salt before adding calcium ions to maximize the solubility of the two ions of TPN mixtures. Other additives such as TE and vitamins appear to have no effect on the solubility of calcium phosphate[2].

2.5.4 Trace Elements (TE)

TE addition may lead to numerous physical, chemical and nutritional interactions especially between vitamins and TE, or amino acids and TE. But the addition of some TE does not seem to alter the stability of a parenteral mixture[22]. TE, in

particular copper and iron, may interact with complete PN mixtures leading to precipitate formation. According to Allwood[17] results, copper sulphide precipitated most rapidly in PN mixtures containing Vamin 9 ® and in mixtures stored in multilayered bags. Copper sulphide precipitation was delayed in PN mixtures containing Vamin 14 ® and was not observed in PN mixtures stored in EVA bags. Iron phosphate precipitates were observed in Synthamin®-containing PN mixtures after storage, but this was prevented in mixtures containing vitamins stored in multilayered bags. From this study result that stability of TE in TPN depends on source of amino acids and type of used container as well.

There is an important interaction between selenium (Se) and vitamin C and E. Se is combined with both of them due to its anti-oxidative role. It protects the cells against oxygen radicals, especially on the lipoprotein membranes[22]. Harraki[22] reported that the absorption of Se was much decreased when sodium selenite and ascorbic acid were provided orally together.

CHAPTER THREE

EXPERIMENTAL PART

CHAPTER THREE: EXPERIMENTAL PART

3.1 INTRODUCTION

This chapter describes materials used for tests, preparation of the samples and physical instruments used for physical analysis of the vitamins. Chemical assays of the vitamins and oxalic acid by High Performance Liquid Chromatography (HPLC), and statistical analysis are explained as well.

3.2 MATERIALS

Table 3.1: Used materials, Manufacturers and Expiry Dates

Materials	Manufacturer	Expiry Date
Intralipid® 20%, Emulsion for infusion 500ml	Fresenius Kabi Limited, Runcorn, Cheshire, U.K.	08/2009
Vitlipid® N Adult, Concentrate for emulsion for infusion, 10 ml	Fresenius Kabi Limited, Birchwood, Warrington, U.K.	10/2008
Solvitio® N, Powder for concentrate for solution for infusion	Fresenius Kabi Limited, Runcorn, Cheshire, U.K.	01/2009

Intralipid® 20%

Purified soybean oil, Emulsion for infusion, intravenous use
500 ml, 20% fat emulsion containing 4.2 MJ (1000 kcal)
500 ml contains:
Active ingredient: Purified soybean oil 100 g
Inactive ingredients: Purified egg phospholipids 6.0 g, Glycerol anhydrous 11.0 g, Water for injections to 500 ml.
Osmolality: 350 mosmol/kg water

Vitlipid® N Adult

Concentrate for emulsion for infusion

10.0 ml contains:

Active ingredients: Retinol (as palmitate) (Vitamin A) 990 μg, Ergocalciferol (Vitamin D_2) 5 μg, d1-alpha-tocopherol (Vitamin E) 9.1 mg, Phytomenadione (Vitamin K) 150μg

Inactive ingredients: Fractionated soybean oil 1.0 g, Fractionated egg phospholipids 120 mg, Glycerol 225 mg, Sodium hydroxide q.s., Water for injection to 10.0 ml

Solvitio® N

Powder for concentrate for solution for infusion

1 vial contains 484 mg powder

Active ingredients: Thiamine Nitrate 3.1 mg, Sodium riboflavine phosphate 4.9 mg, Nicotinamide 40 mg, Pyridoxine hydrochloride 4.9 mg, Sodium pantothenate 16.5 mg, Sodium ascorbate 113 mg, Biotin 60 micrograms, Folic acid 0.4 mg, Cyanocobalamin 5.0 micrograms

Inactive ingredients: Methyl parahydroxybenzoate 0.5 mg, Glycine 300 mg, Disodium edetate 0.5 mg

These correspond to: Vitamin B_1 2.5 mg, Vitamin B_2 3.6 mg, Nicotinamide 40 mg, Vitamin B_6 4.0 mg, Pantothenic acid 15.0 mg, Vitamin C 100mg, Biotin 60 μg, Folic acid 0.4 mg, Vitamin B_{12} 5.0 μg

Table 3.2: Reagents and Suppliers

Reagent	Supplier
Methanol (HPLC grade)	Fisher Scientific U.K. Loughborough, Leicestershire
Orthophosphoric acid (Analytical reagent grade)	Fisher Scientific U.K. Loughborough, Leicestershire
diPotassium hydrogen orthophosphate trihydrate $K_2HPO_4 . 3H_2O$	Fisher Scientific U.K. Loughborough, Leicestershire
Barnstead® Nanopure water	Barnstead Thermolyne Corporation, Dubuque, Iowa
pH 7 Buffer	Fisher Scientific U.K. Loughborough, Leicestershire
Potassium dihydrogen orthophosphate (HPLC grade)	Fisher Scientific U.K. Loughborough, Leicestershire
L-Ascorbic acid (Analytical reagent grade)	Fisher Scientific U.K. Loughborough, Leicestershire
Oxalic acid (Analytical reagent grade)	Fisher Scientific U.K. Loughborough, Leicestershire

Table 3.3: Sterile Equipment

Sterile equipment	Supplier
BD Microlance™ 3 Needles 19G, 21G and 23G	Becton Dickinson
BD Plastipak™ 50 ml Luer-Lock Syringes	Becton Dickinson
BD Plastipak™ Syringes 1-20 ml	Becton Dictinson
Multi-Ad Luer-Lock Syringe Cap	B/Braun

Table 3.4: Instruments

Instruments	Manufacturer
HPLC system	Thermo Spectrasystem
Orion® Model 420A pH/mV/Temperature Meter	Beverly, MA, USA
The Advanced™ Osmometer Model 3D3	Advanced Instruments, inc., Massachusetts, USA
Weighing balance Sartorius® Model A02	Sartorius AG, Goettingen, Germany
Microflow® laminar flow cabinet, horizontal	Bioquell UK Limited, Andover, Hants
Pharmaceutical refrigerator	Lec Refrigeration, Prescot, Merseyside
Light microscope Olympus® BH-2	Olympus Optical Co. Limited, Tokyo
Autosampler 2.0 ml amber vials	Fisher Scientific U.K. Loughborough, Leicestershire
Disposable Pasteur pipettes, 3 ml	Elkay Laboratory Products Ltd, Basingstoke, Hampshire
Magnetic stirrer	Fisher Scientific U.K. Loughborough, Leicestershire
Micropipettes 100-1000μL	Gilson, France
Malvern Mastersizer® X	Malvern Instruments Limited, Worcestershire
Thoma welled slides and cover slips	Weber Scientific International Limited, Teddington, Middlesex
Glassware	Fisher Scientific U.K. Loughborough, Leicestershire

3.3 EXPERIMENTAL DESIGN

3.3.1 The Preparation of Intralipid® 20% Syringes

The Intralipid® 20% emulsion was placed into a laminar flow unit. 260 ml of this emulsion were syringed out and put into the sink. 240 ml of the emulsion remained in the bag. Then 10 ml of Vitlipid® N emulsion were put into a 10ml syringe and one vial of Solvitio® N was reconstituted by this emulsion. The created mixture was syringed via the same 10ml syringe into the Intralipid® emulsion. In all, there were 60 ml of Vitlipid®-Solvitio® mixture syringed into the Intralipid® emulsion. That means six vials of Solvitio® N and six vials of Vitlipid® N emulsion were used. Total volume of the Intralipid® mixture at this point was 300 ml. There were six 50ml Luer-Lock Syringes prepared in total. Each of them was filled with 47 ml of the Intralipid® mixture and closed by Multi-Ad Luer-Lock Syringe Cap. These syringes were placed into the refrigerator for stability tests to be carried out at a different time. The sample for chemical and physical assays at time zero was taken directly from the Intralipid® mixture and was taken into Hach tube. Two autosampler vials were filled via a 3ml plastic pipette for HPLC assay; each was filled with 1 ml of the sample. Physical tests were carried out as well.

Figure 3.1: Intralipid® Syringes

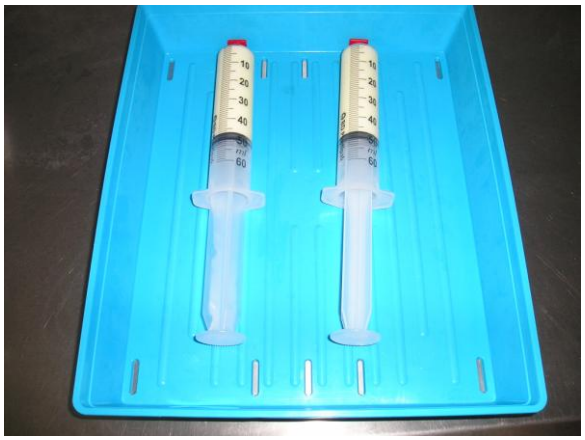


Figure 3.2: Hach tube

3.3.2. Sampling technique of Intralipid® 20% Syringes

Chemical assays by HPLC and physical tests were carried out after:

- 7 days storage in the refrigerator followed 24 h and 48 h storage at ambient temperature and protected from day-light. Two syringes were used and each day 15 ml of the volume was used for analysis.
- 14 days storage in the refrigerator followed 24 h and 48 h storage at ambient temperature and protected from day-light. Another two syringes were used and each day 15 ml of the volume was used for analysis.
- 29 days storage in the refrigerator followed 24 h and 48 h storage at ambient temperature and protected from day-light. The last two syringes were used and each day 15 ml of the volume was used for analysis.

3.3.3 Sampling Technique of AA and OA Analysis

Three different samples were prepared. Firstly 100 mg AA reconstituted with UFDW in 100ml volumetric flask and diluted 30 times into 10ml volumetric flask. Secondly 100 mg OA filled with UFDW in a 100ml volumetric flask and diluted 30 times in 10ml volumetric flask. The third sample was prepared by mixing 100 mg AA with 100 mg OA, dissolving in a 100 ml volumetric flask in UFDW and solution prepared this way was diluted 30 times in a 10ml volumetric flask. Each sample was assayed by HPLC in time zero and after 1, 4, 5, 6 and 7 days storage at ambient temperature. The solutions were not protected against daylight.

3.4 PHYSICAL ANALYSIS

The following analyses were carried out. Emulsions were visually checked with the naked eye as well as by light microscopy. The lipid particle size was measured by Malvern Mastersizer® X, and the osmolality was measured with The Advanced™ Osmometer. The pH of the emulsions was determined by using Orion® pH meter.

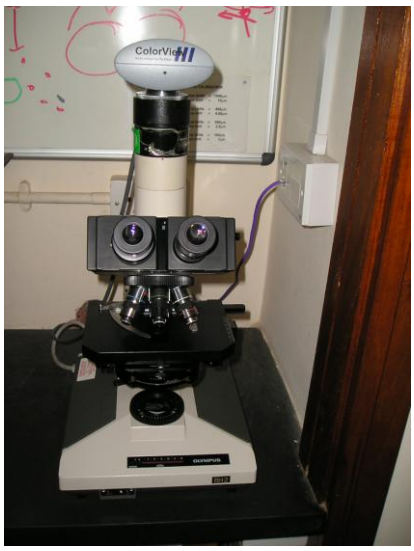
3.5 INSTRUMENTS USED FOR PHYSICAL TESTS

Instruments which were used are listed in Table 3.4.

3.5.1 Light Microscope

Microscopy was used for observation of lipid particle size in tested emulsion samples. A pre-calibrated light microscope (Olympus® BH–2) was used.

Figure 3.3: Olympus® BH-2



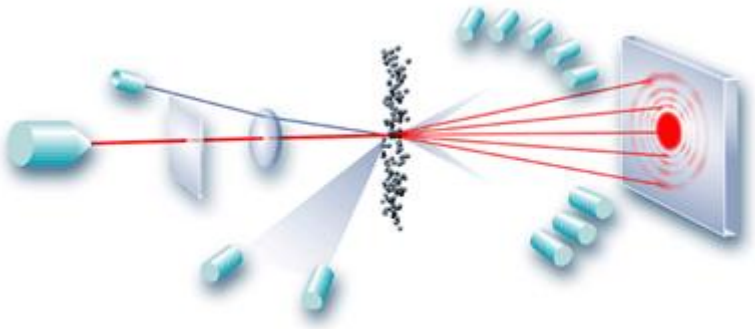
One drop of the emulsion was placed in the centre of a Thoma-welled counting chamber slide of a height of 0.2 mm, and gridlines of a total of 256 squares. A 1ml syringe was used and the sample was then covered with a glass cover slip. It is important to avoid creating air bubbles, which may cause a problem, because their appearance is similar to lipid globules.

The samples were examined at 100-time magnification. One unit gridline of the scale is equivalent to 10 μm in size. The biggest particle size, the amount of particles bigger than 10 μm and particles between 7.5-10 μm, were recorded. The particles that were visible outside of the grid were not included.

3.5.2 The Mastersizer® X Laser Diffraction Particle Size Analyser

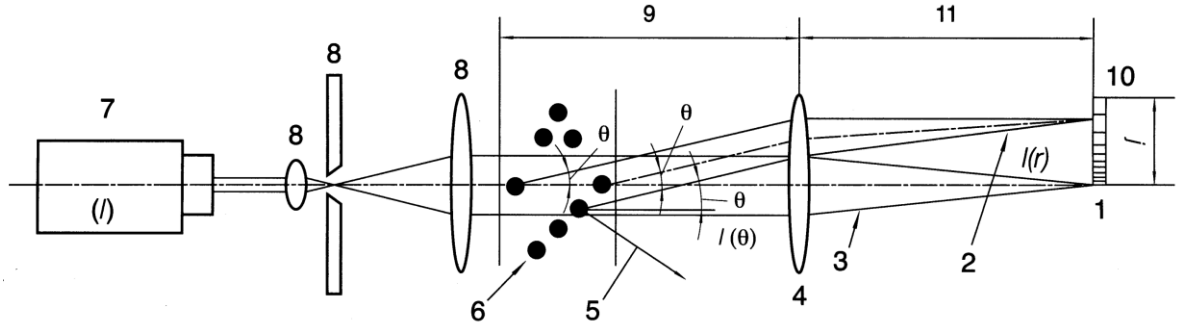
The Malvern Mastersizer® particle size analyzer uses the laser diffraction technique which is based on the analysis of the diffraction pattern produced when particles are exposed to a beam of monochromatic light.

Figure 3.4: Laser diffraction system



A representative sample, dispersed at an adequate concentration in a suitable liquid or gas, is passed through a beam of monochromatic light, usually a laser. The light scattered by the particles at various angles is measured by a multi-element detector. Numerical values representing the scattering pattern are then recorded for subsequent analysis. These scattering pattern values are then transformed, using an appropriate optical model and mathematical procedure, to yield the proportion of total volume to a discrete number of size classes, forming a volumetric particle-size distribution.

Figure 3.5: Laser diffraction instrument



1. Obscuration detector
2. Scattered beam
3. Direct beam
4. Fourier lens
5. Scattered light not collected by lens (4)
6. Particle ensemble
7. Light source laser
8. Beam processing unit
9. Working distance of lens (4)
10. Multi-element detector
11. Focal distance of lens (4)

The particles can enter the laser beam in 2 positions. In the conventional case the particles enter the parallel beam before the collecting lens and within its working distance. In so-called reversed Fourier optics the particles enter behind the collecting lens and thus, in a converging beam. The advantage of the conventional set-up is that a reasonable path length for the sample is allowed within the working distance of the lens. The second set-up allows only small path lengths but enables measurement of scattered light at larger angles, which is useful when submicron particles are present. Traditionally, the measurement of particle size using laser diffraction has been limited to particles in the range of approximately 0.1 µm to 3 mm (British Pharmacopoeia 2008)[23].

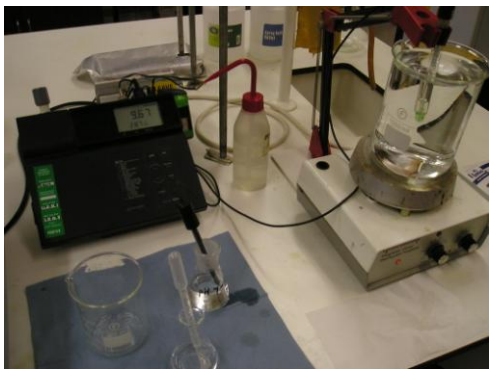
The size of the lipid globules is measured by passing the laser beam through the emulsion samples dispersed in ultrapure deionised water. The sampler was filled with ultrapure deionised water and stirred at a constant flow rate. Firstly the background was measured and then the sample was added. It was necessary to add the sample slowly. The amount of sample required was adjusted to an obscuration value between 19% - 21%. Initial tests carried out with Intralipid®

emulsions required to add about 3 drops of this emulsion to obtain this obscuration concentration. The particle size data was analysed using the Malvern Mastersizer® software version 2.19.

3.5.3 The pH Meter

The pH is a number which represents conventionally the hydrogen ion concentration of an aqueous solution (BP 2008)[24]. Orion® Model 420A pH meter was used for measuring my samples. This measuring system comprises of two electrodes, the sensitive glass electrode and a combined reference electrode.

Figure 3.6: Orion® pH meter Model 420A



The glass electrode consists of a glass tube with a sealed membrane of glass at its base. The membrane glass bulb is filled with buffer into which a silver wire (Ag) coated with silver chloride (AgCl) is placed (A Guide to Electrochemistry). When a pH glass electrode comes in contact with an aqueous measuring solution a gel layer develops on the pH-sensitive glass membrane and inside of this membrane as well. The H^+ ions either diffuse out of the layer or into the gel layer, depending on the pH value of the measured solution. The total membrane potential is a result of the difference between the inner and outer charge. In the combination electrode the glass electrode is concentrically surrounded by the reference electrolyte[25].

The pH meter was calibrated before use with pH 7 phosphate buffer (Fisher Scientific U.K., Loughborough, Leicestershire). The glass electrode was inserted into the sample using the holder. It is necessary to wash the glass electrode with distilled water between measuring each sample to prevent contamination.

3.5.4 Osmometer

Osmometers are devices designed for extremely precise measurement of the osmotic concentration of body fluids. The Advanced™ Model 3D3 Osmometer determines solute concentration by the extremely precise freezing point method (User's Guide, Advanced Instruments 1996).

Osmometer is the apparatus for measuring osmolality. The unit of osmolality is osmole per kilogram (osmol/kg), but the submultiple milliosmole per kilogram (mosmol/kg) is usually used. Osmolality ξ_m is determined by measurement of the depression of freezing point. The following relationship exists between the osmolality and the depression of freezing point ΔT (BP 2008)[26]:

$$\xi_m = \frac{\Delta T}{1.86} \times 1000 mosmol/kg$$

Figure 3.7: The Advanced™ Osmometer

A pre-calibrated The Advanced™ Osmometer Model 3D3 was used. The amount of 250 µl of the sample was given into sample tubes for in vitro diagnostic with Gilson micropipette. Osmolality was determined at each sample at time zero, after 29 days of storage in the refrigerator followed by 24 hours and 48 hours of storage at ambient temperature and under light protected conditions.

3.5.5 Laminar Flow Cabinet

The horizontal laminar flow cabinet, Microflow®, is fitted with high efficiency particulate air (HEPA) filter with 99.97% efficiency. The HEPA filter provides ultra-filtered air and allows a clean air work environment (Said 2006b)[27].

Figure 3.8: Laminar flow workstation

3.6 CHEMICAL ASSAYS BY HPLC

HPLC is becoming a standard method in vitamin assay, especially for routine work[28]. Liquid chromatography (LC) is a method of chromatographic separation based on the difference in the distribution of species between two non-miscible phases, in which the mobile phase is a liquid which percolates through a stationary phase contained in a column. The apparatus consists of a pumping system, an injector, a chromatographic column, a detector and a data acquisition system. The mobile phase is supplied from one or several reservoirs and flows through the column, usually at a constant rate, and then through the detector[29].

3.6.1 HPLC Instrument System Used for Analysis of Intralipid® Syringes

Thermo Spectra System P2000 gradient pump, UV detector 6000LP and Spectra Series AS1000 autosampler with 20µl injection loop were used for the HPLC analysis. The data acquisition system software was called Chromquest 4.1. The column used was Varian® C18 stainless steel column, 150 mm × 3 mm ID, 5 µm

particle size, fitted with a ChromStep® C18 stainless steel guard column, 10 mm × 2 mm ID.

3.6.2 HPLC Analysis of Water-soluble Vitamins Assay

Solvitio® N Adult is an injectable multivitamin, in the form of a yellow powder for reconstitution (Said 2006b) [27].

The separation method from Khýnová[30] was adapted for the water-soluble vitamins. All samples were analysed at ambient temperature, a reversed phase HPLC analysis with a gradient elution method was employed. Methanol and phosphate buffer 0.05M pH 7 was used as the mobile phase. The following gradient run was used:

1% methanol from 0 to 5 minutes,
1% - 30% methanol from 5 to15 minutes,
30% methanol from 15 to 20 minutes,
30% - 1% methanol from 20 to 20.1 minutes,
1% methanol from 20.1 to 25 minutes

Table 3.5: Gradient Run for Water-soluble Vitamins Assays

Time (minutes)	Buffer pH 7	MeOH
	A%	B%
0	99	1
5	99	1
15	70	30
20	70	30
20.1	99	1
25	99	1

20 µl sample was injected via autosampler. The flow rate was set 1 ml/min with UV detection 265 nm.

3.6.2.1 Preparation of Phosphate Buffer for Intralipid® Syringes Analysis

The mobile phase used consists of phosphate buffer and methanol. Fresh solution of 0.05M phosphate buffer pH 7 was prepared by dissolving 11.41 g 0.05M $K_2HPO_4.3H_2O$ in ultrafiltered deionised water (UDW). This solution was put into a 1000ml volumetric flask and filled with UDW. Amount of weighed $K_2HPO_4.3H_2O$ (formula mass FM= 228.22) was counted via the following figure: 0.05M $\times$ 228.22 = 11.41 g ($\pm$ 0.05 g). The solution was put in a beaker, stirred with a magnetic stirrer and pH of this solution was measured. pH was adjusted to pH 7 $\pm$ 0.05 units by using orthophosphoric acid. Finally the buffer was filtered under vacuum through a 0.2 μm polyamide filter.

3.6.3 HPLC Analysis of Fat-soluble Vitamins Assay

For the fat-soluble vitamins, the UV absorbance found was 292 nm for vitamin E and 325 nm for vitamin A. Therefore methods that use two different wavelengths were carried out (Said 2006b)[27]. According to Said 2006 results, using the stability-indicating method described helps determine two vitamins, namely Vitamin A and Vitamin E. The other fat-soluble vitamins were omitted in this thesis. Methanol was used as the mobile phase.

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. Two wavelengths were used, 292 nm for the detection of vitamin E and 325 nm for the detection of vitamin A. A 20 µl sample was injected via the autosampler.

3.6.4 HPLC Instrument System Used for AA and OA Analysis

Thermo Spectra System P2000 gradient pump, UV detector 6000LP and Spectra Series AS1000 autosampler with 20μl injection loop were used for the HPLC

analysis. The data acquisition system software was called Chromquest 4.1. The column used was Synergi™ 4 μ Hydro-RP 80A, 250 x 4.60 mm, Phenomenex.

3.6.4.1 Preparation of Phosphate Buffer for AA and OA Analysis

The mobile phase used was 0.02M phosphate buffer pH 2.5. The fresh buffer was prepared by dissolving 2.72 g KH_2PO_4 in UDW. The amount of weighed KH_2PO_4 (formula mass FM= 136.086) was counted via the following figure: 0.02M × 136.086= 2.72 g (± 0.05 g). This solution was put into a 1000ml volumetric flask and filled with UDW, and after mixing was put into a beaker with magnetic stirrer. The pH of the solution was adjusted with orthophosphoric acid to pH 2.5 ± 0.05 units. Finally the buffer was filtered under vacuum through a 0.2 μm polyamide filter.

3.7 STATISTICAL ANALYSIS

Microsoft Excel 2003 software for Windows® was used for data analysis. All results throughout the project were expressed as the mean ± standard deviation (SD) from triplicate samples.

CHAPTER FOUR

RESULTS AND DISCUSSION

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 HPLC ANALYSIS OF INTRALIPID® SYRINGES

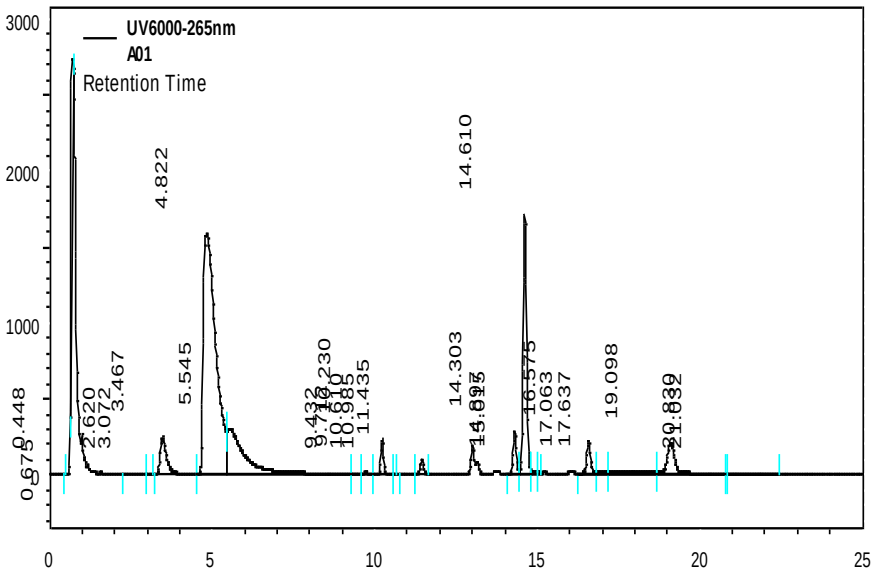
Firstly it was necessary to find an optimal method for vitamin assay by HPLC. The initial step was reading the diploma thesis of mine, Khýnová[30], paragraph 4.1: HPLC Analysis of Kabiven® Peripheral Bag. Mentioned project was similar to the one discussed in this rigorous thesis. Literature about vitamin assays by HPLC was consulted as well (For example PhD thesis of Said).

The separation method from Khýnová[30] was adapted for the water-soluble and the fat-soluble vitamins analysis. The same column and HPLC system, like Khýnová[30] used, was employed.

4.1.1 Results and Discussion for Water-soluble Vitamins

The chromatogram of Solvitio® N standard solution is shown at Figure 4.1. Peaks of the Solvitio® N solution were identified according to Khýnová[30] results.

Figure 4.1: Chromatogram of Solvitio® N Standard Solution



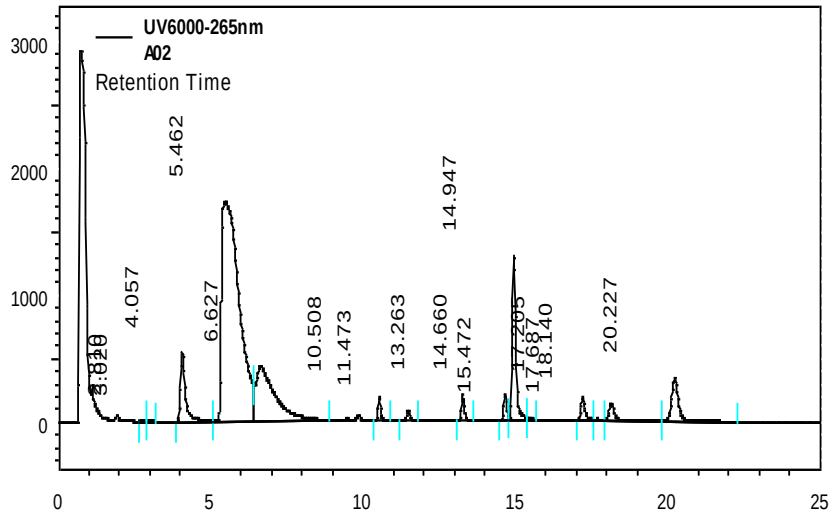
The chromatogram peaks with the approximate retention times were:

Ascorbic acid	rt. 0.67 minutes
Pyridoxine hydrochloride	rt. 3.46 minutes
Nicotinamide	rt. 4.82 minutes
Thiamine hydrochloride	rt. 5.54 minutes
Folic acid	rt. 10.23 minutes
Riboflavin sodium phosphate	rt. 14.61 minutes
Pantothenic acid	rt. 19.09 minutes

Five peaks for riboflavin sodium phosphate were identified according to Said's 2006 results, which may be the isomers of riboflavin phosphate, riboflavin diphosphate and free riboflavin. The highest peak identified at about 14.61 minutes was used for analysis. Ascorbic acid was eluted very early in the gradient run, in less than one minute. The peak for cyanocobalamin was also identified, but its concentration in the multivitamin solution was too minute and the peak was hardly noticeable. The biotin peak could not be identified in this gradient run, and the peak for pantothenic acid was detected after 20 minutes of the gradient run. In Said's 2006 thesis the peaks of degraded products were identified, mostly at retention times of less than 2 minutes. Therefore it was decided that this method was not suitable for analysing the ascorbic acid, because the peaks of the degraded products would interfere with the ascorbic acid peak.

Six vitamins were found to be suitable for assay by this method, which are pyridoxine, nicotinamide, thiamine, folic acid, riboflavin sodium phosphate and pantothenic acid (Said 2006b)[27]. All these vitamins were analysed in different times in Intralipid® syringes (Figure 4.2).

Figure 4.2: Chromatogram of Intralipid® Syringe – Detection of Water-soluble Vitamins. Separation of Pyridoxine (rt. 4.05 minutes), NA (rt. 5.46 minutes), Thiamine (rt. 6.62 minutes), FA (rt. 10.50 minutes), Riboflavin sodium phosphate (rt. 14.94 minutes), PA (rt. 20.22 minutes).



4.1.1.1 Results of HPLC for Water-soluble Vitamins (WSV) in Intralipid® Syringes

Amount of vitamin B6 was not less than 94 % during the storage period. Almost 100 % of the vitamin left after 7 days and 14 days storage in the refrigerator followed two days storage at ambient temperature. There was detected over 94 % of the vitamin at the day 29+2 (see Table 4.1).

Amount of Nicotinic acid (NA) was not less than 85 % of its initial amount during storage period. Around 86 % of the vitamin left after 7 days storage in the fridge followed two days storage at ambient temperature. Over 99 % of NA left after 14 days storage, but during two days storage at ambient temperature amount of NA decreased to 89 % of its initial amount. Similar amount of this vitamin like at the day 14 was detected at the day 29. Over 85 % of NA was presented at the day 29+2 in the sample (see Table 4.1).

Amount of vitamin B1 was not less than 94 % during storage period. Almost 100 % of the vitamin was presented after 7 days storage in the fridge. During storage at ambient temperature the amount of vitamin B1 was decreasing. Losses of the vitamin were small after 14 and 29 days storage, around 3 % (see Table 4.1).

Amount of Folic acid (FA) was not less than 68 % of its initial amount during storage period. There was detected only 84 % of FA at the day 7, 82 % at the day 14 and 94 % at the day 29. Amount of this vitamin after two days storage at ambient temperature was decreasing at all time points (see Table 4.1).

Amount of vitamin B2 was not less than 63 % during storage period. Around 63 % of the vitamin left after 7 days storage in the fridge followed two days storage at ambient temperature. There was detected around 76 % of vitamin B2 at the day 14 and only 65 % of the vitamin at the day 29+2.

Amount of Pantothenic acid (PA) was not less than 82 % of its initial amount during storage period. Almost 90 % of PA left after 7 days storage. There was detected around 96 % of this vitamin after 14 days storage and almost 83 % at the day 29 (see Table 4.1).

There were detected also values over 100 % of the initial amount of the vitamins. It might be caused by measure error. Some results may be influenced by changing ambient temperature during stability studies, because the room where HPLC system was, was not equipped by air-condition. Exactly the sensitivity of the detector may be influenced by big temperature difference between single assays and column could be influenced as well.

All results are expressed via graphs (see Figures 4.3, 4.4 and 4.5) and Table 4.1.

Table 4.1: Peak Area and RSD for WSV in Intralipid® Syringes throughout the Investigation

Vitamin	B6		NA		B1		FA		B2		PA	
Day	%	RSD	%	RSD	%	RSD	%	RSD	%	RSD	%	RSD
0	100	0,30	100	1,40	100	0,19	100	1,39	100	1,42	100	4,55
7	101,7	0,47	87,0	1,28	99,4	4,40	84,8	5,18	63,3	3,94	89,7	1,08
7+1	101,4	4,24	86,6	1,21	95,7	3,29	110,5	4,48	91,4	8,49	97,3	2,80
7+2	99,9	1,26	98,3	1,78	94,9	5,19	73,4	3,71	65,7	5,43	96,6	1,33
	B6		NA		B1		FA		B2		PA	
Day	%	RSD	%	RSD	%	RSD	%	RSD	%	RSD	%	RSD
0	100	0,30	100	1,40	100	0,19	100	1,39	100	1,42	100	4,55
14	101,6	0,64	99,2	2,09	96,5	4,07	82,5	5,17	76,6	3,81	96,1	1,22
14+1	100,4	0,37	92,3	2,08	100,7	3,58	79,2	5,85	71,1	2,37	97,9	1,92
14+2	96,8	0,80	89,7	2,34	100,3	5,23	74,4	6,09	68,1	2,69	100,7	1,95
	B6		NA		B1		FA		B2		PA	
Day	%	RSD	%	RSD	%	RSD	%	RSD	%	RSD	%	RSD
0	100	0,30	100	1,40	100	0,19	100	1,39	100	1,42	100	4,55
29	104,9	0,63	99,6	1,83	98,8	4,65	94,4	4,81	98,2	9,78	82,9	4,41
29+1	98,9	0,82	94,3	2,06	102,7	4,07	76,6	4,96	81,1	11,2	92,6	1,23
29+2	94,5	1,28	85,4	1,99	103,5	3,29	68,2	5,23	65,3	7,04	98,7	0,85

Figure 4.3: Peak Area for WSV in the Syringes after 7 Days Storage in the Fridge

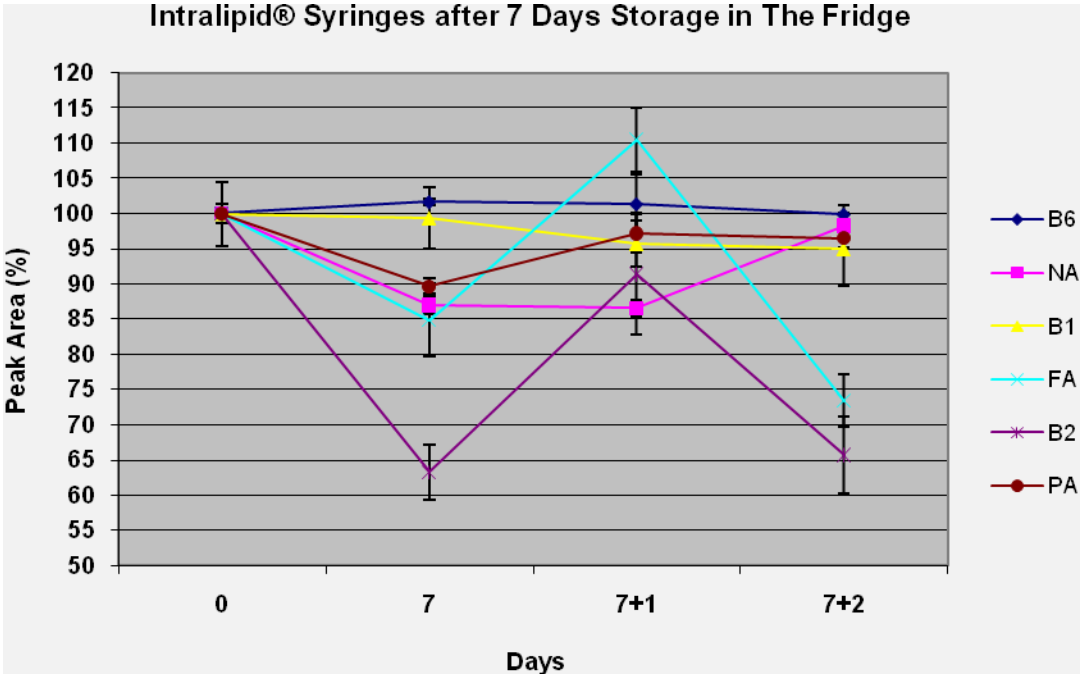


Figure 4.4: Peak Area for WSV in the Syringes after 14 Days Storage in the Fridge

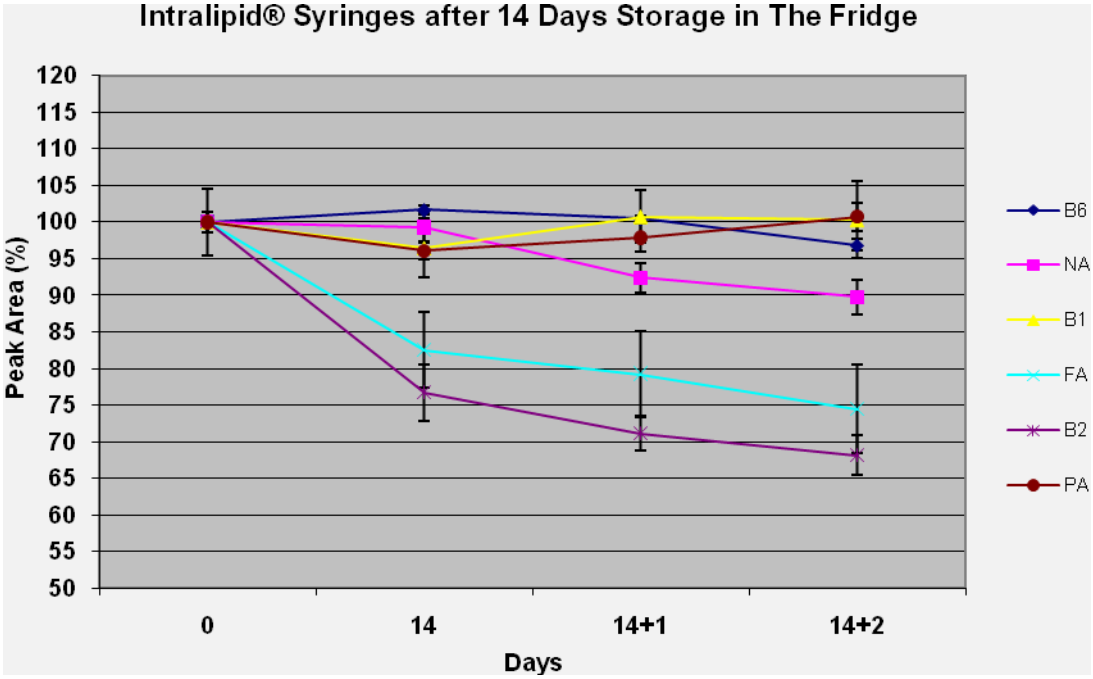
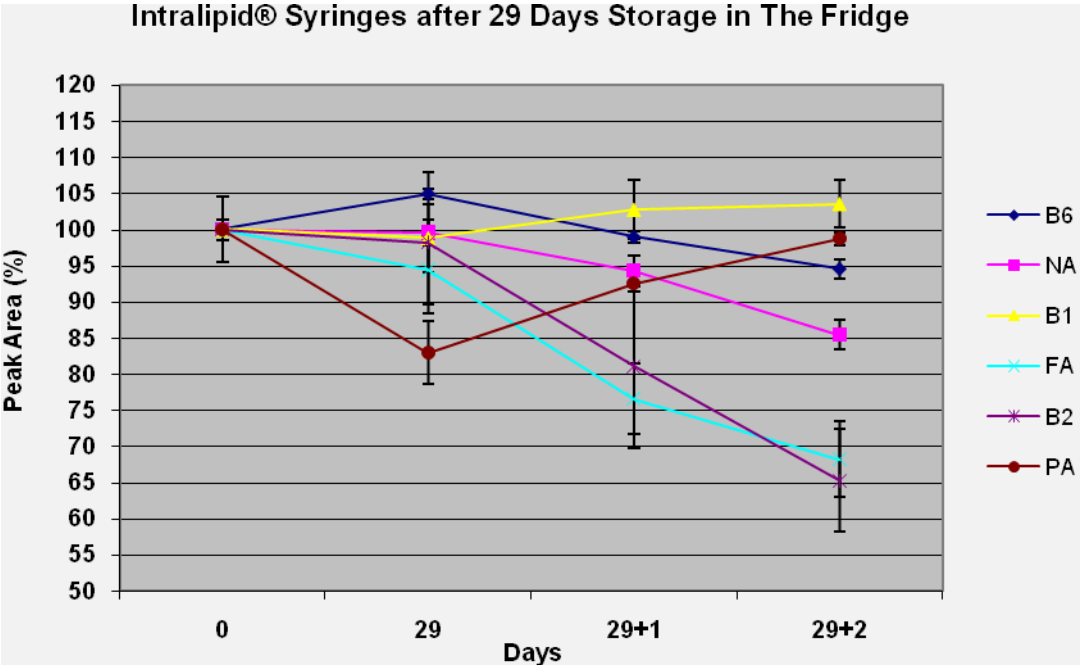


Figure 4.5: Peak Area for WSV in Syringes after 29 Days Storage in the Fridge



4.1.1.2 Discussion

Stability of vitamins is discussed in previous paragraph 2.4 Vitamins. Pyridoxine is light-sensitive vitamin and its losses were reported more than 80 % when exposed to daylight[2]. Previous HPLC results have shown that degradation of pyridoxine is negligible when the samples are light protected. Pyridoxine was shown as a stable vitamin because its losses were small during whole storage period. HPLC results of NA have shown that its losses during storage in the fridge were small. More significant degradation of NA was noticed after two days storage at ambient temperature. Therefore samples containing NA should be stored in a refrigerator before administration to patients. Thiamine was shown to be stable in the mixture with fat emulsion. Almost no losses were detected when thiamine containing samples were stored in the fridge. Previous HPLC results have shown that FA was rapidly degraded when the samples were stored out of the fridge. Losses of FA in the mixture with fat emulsion were less if the samples stored in the fridge, around 17 % of its initial amount. FA degradation is rapid so mixtures containing FA should be administered to patients after preparation and not stored. Riboflavin losses were significant throughout investigation, especially when samples were stored at ambient temperature for two days. Riboflavin containing mixtures should be administered after preparation as well as mixtures with FA. Previous HPLC results have shown that PA degradation is small even after long time storage period. Losses of this vitamin were negligible if samples stored in the fridge as well as at ambient temperature.

4.1.2 Results and Discussion for Fat-soluble Vitamins Assay

Peaks for vitamin A and vitamin E were identified by comparing chromatograms of fat-soluble sample emulsion from Said 2006 thesis with chromatograms obtained from analysis of 10 ml of Vitlipid® N Adult injection diluted in a 100ml volumetric flask with ultrafiltered deionised water. Peaks were identified at 2.65 minutes for vitamin E (Figure 3.14) and at 8.53 minutes for vitamin A (Figure 3.15). Peaks for vitamin K and vitamin D were observed too, but according to Said 2006 results these two vitamins are unsuitable for analysis by this stability-indicating method.

Vitamin K peak was too small with lack of sensitivity and from the calibration curve of vitamin D less linearity and therefore less precision was found.

For the fat-soluble Vitlipid® N Adult vitamin assay the stability-indicating method described was able to determine two vitamins, namely Vitamin A and Vitamin E in single run[27].

Figure 4.6: Chromatogram of Vitlipid® N Injection - Detection of Vitamin E (rt. 2.65 minutes)

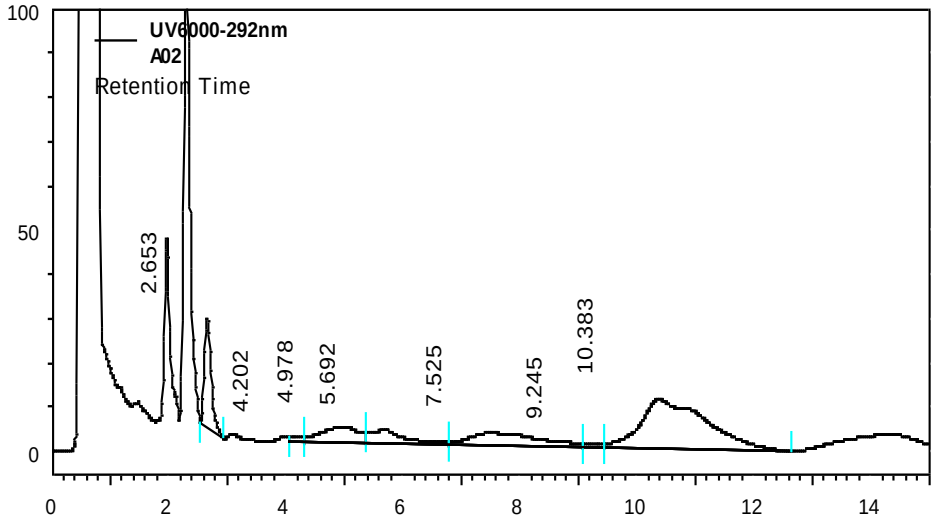


Figure 4.7: Chromatogram of Vitlipid® N Injection- Detection of Vitamin A (rt. 8.53 minutes)

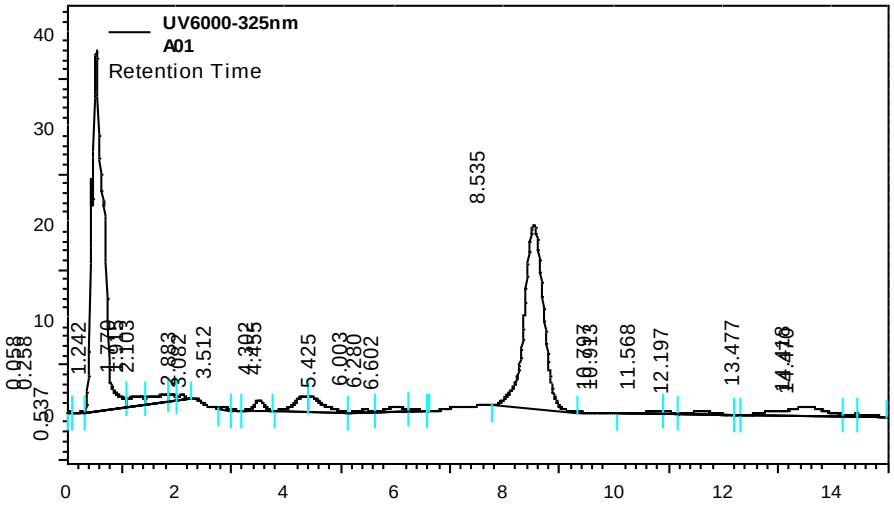


Figure 4.8: Chromatogram of Intralipid® Syringe – Detection of Vitamin E (rt. 2.44 minutes)

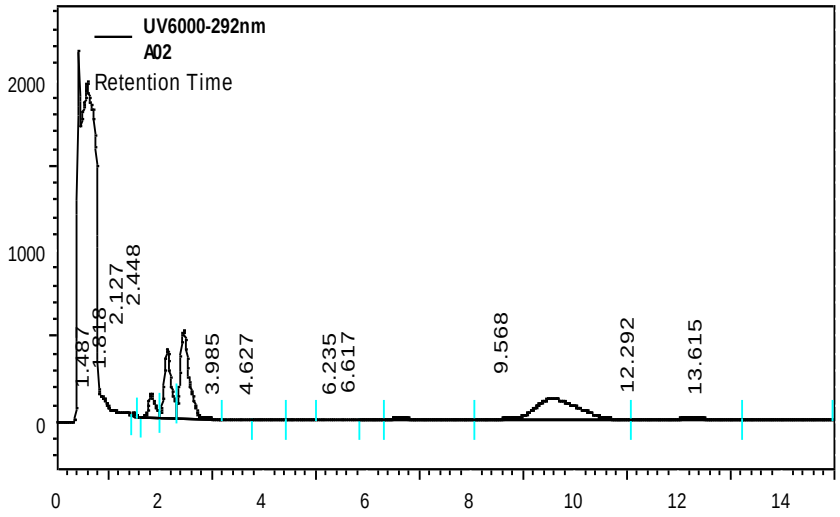
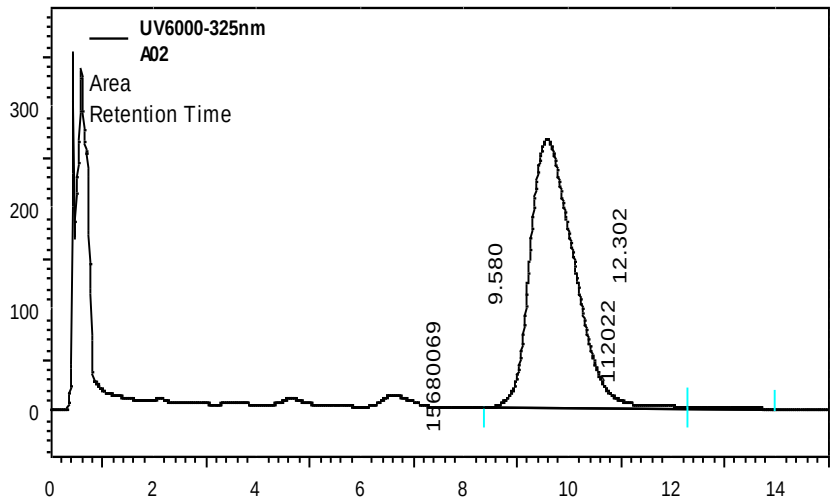


Figure 4.9: Chromatogram of Intralipid® Syringe- Detection of Vitamin A (rt. 9.58)



4.1.2.1 Results of HPLC for Fat-soluble Vitamins (FSV) in Intralipid® Syringes

Amount of vitamin A was not less than 79 % of its initial amount during storage period. There was detected around 95 % of the vitamin after 7 days storage in the fridge followed two days storage at ambient temperature. At the day 14 there was presented almost 94 % of vitamin A in the sample and almost 80 % of the vitamin was detected at the day 29 (see table 4.2). Amount of vitamin E was not less than 76 % during storage period. This value was detected after 29 days storage in the refrigerator. Amount of vitamin E at the day 7 and 14 was around 90 % of its initial amount (see table 4.2).

There were detected values over 100 % of initial amount of the vitamins. Some results may be influenced by changing ambient temperature during stability studies, because the room where HPLC system was, was not equipped by air-condition. Exactly the sensitivity of the detector may be influenced by big temperature difference between single assays and column could be influenced as well.

All results are expressed via graphs (see Figures 4.10, 4.11 and 4.12) and Table 4.2.

Table 4.2: Peak Area and RSD of FSV in the Syringes throughout the Investigation

Vitamin	A		E	
Day	Peak Area (%)	RSD	Peak Area (%)	RSD
0	100	0,19	100	2,43
7	93,8	0,79	89,5	1,23
7+1	98,2	0,74	92,5	1,45
7+2	95,9	2,39	105,4	1,08
	A		E	
Day	Peak Area (%)	RSD	Peak Area (%)	RSD
0	100	0,19	100	2,43
14	93,7	0,43	91,3	2,10
14+1	96,4	2,08	97,1	1,80
14+2	107,2	0,19	101,5	0,52
	A		E	
Day	Peak Area (%)	RSD	Peak Area (%)	RSD
0	100	0,19	100	2,43
29	79,8	0,11	76,3	0,93
29+1	94,6	3,12	96,1	1,12
29+2	121,7	0,11	114,9	0,37

Figure 4.10: Peak Area for FSV in the Syringes after 7 Days Storage in the Fridge

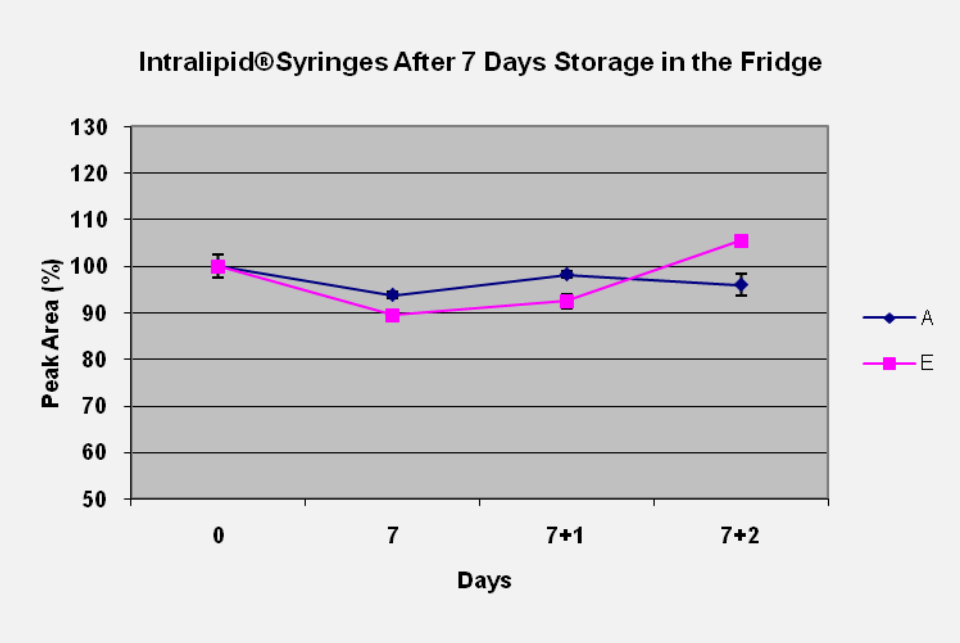


Figure 4.11: Peak Area for FSV in the Syringes after 14 Days Storage in the Fridge

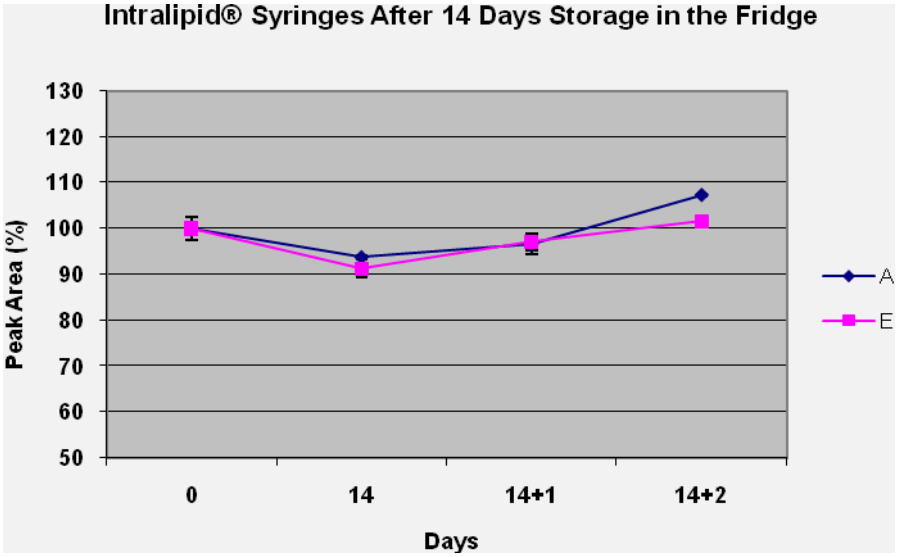
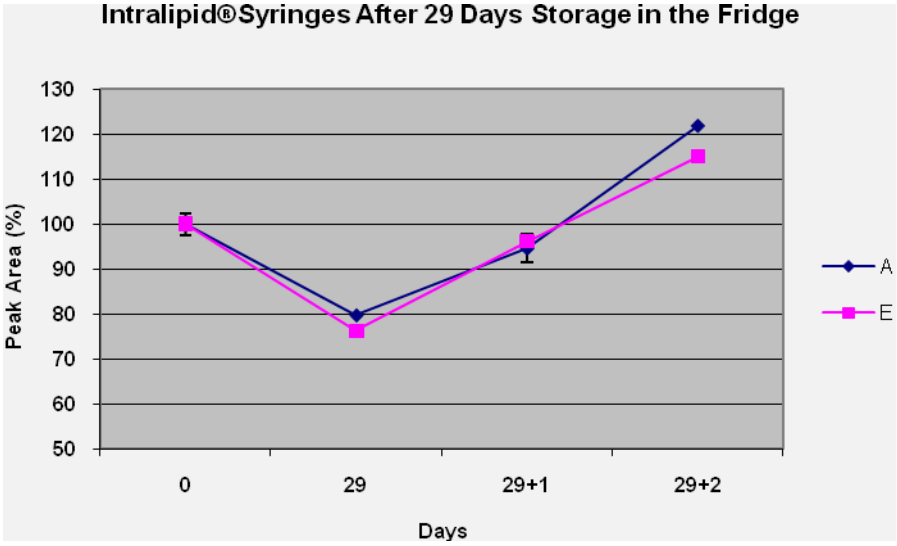


Figure 4.12: Peak Area for FSV in the Syringes after 29 days Storage in the Fridge



4.1.2.2 Discussion

Stability of vitamins A and E was discussed in previous paragraph 2.4. Vitamins. Retinol is the most light-sensitive of the vitamins and is known to be rapidly broken down by exposure to UV light[2]. Intralipid® Syringes were daylight-protected throughout the investigation, therefore degradation was not rapid. Vitamin A in the mixture with fat emulsion was shown quite stable if its samples stored in the fridge. An influence of the temperature was not possible to evaluate beacause the peak area values after storage at ambient temperature were inapplicable. Gillis[19] reported that vitamin E may be strongly bound to plastic. According to previous results vitamin E losses were not rapid in the mixture with fat emulsion. Allwood[2] reported that presecne of a fat emulsion affords considerable protection.

4.2 RESULTS AND DISCUSSION OF pH FOR INTRALIPID® SYRINGES

4.2.1 pH Results of Intralipid® Syringes

The measured pH was in range between 6.16 at day zero and 6.61 at day seven. There is not any difference bigger than 0.5 units among all results. The biggest difference is between day zero and day seven, exactly 0.45 units (see Table 4.3). The changes of pH after 24 and 48 hours storage at ambient temperature are quite small and do not differ from the day when they were take out from the fridge to much. The biggest difference is between day seven and seven plus two, exactly 0.24 units. The graphical results are expressed in Figures 4.13, 4.14 and 4.15 in differen time.

Table 4.3: pH of Intralipid® Syringes throughout The Investigation

day	0	7	7+1	7+2	14	14+1	14+2	29	29+1	29+2
pH	6.16	6.61	6.48	6.37	6.55	6.45	6.45	6.40	6.31	6.34

Figure 4.13: pH of The Syringes after 7 Days Storage in The Fridge

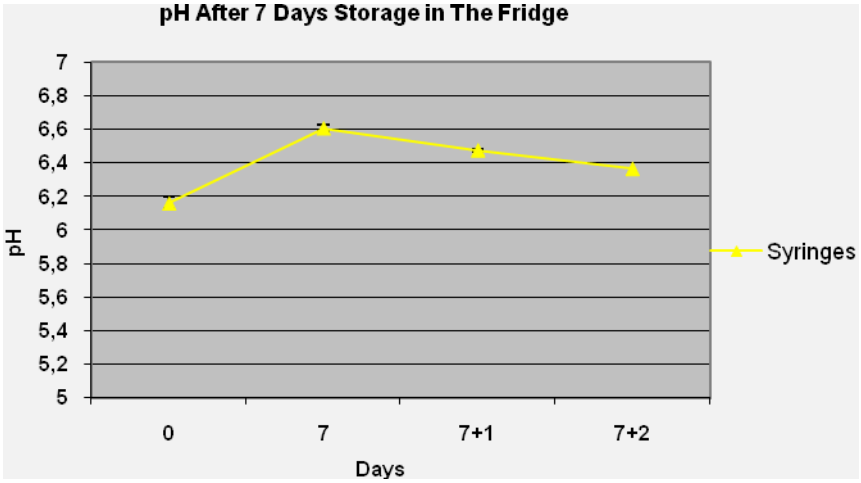


Figure 4.14: pH of The Syringes after 14 Days Storage in The Fridge

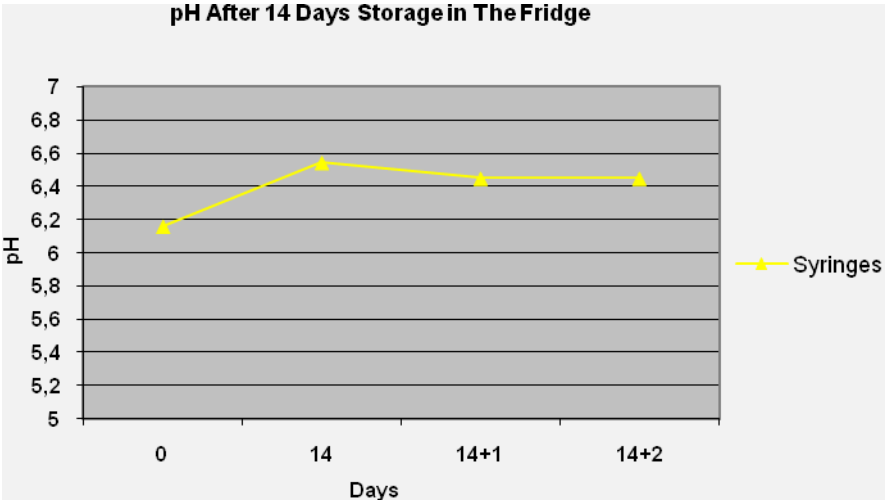
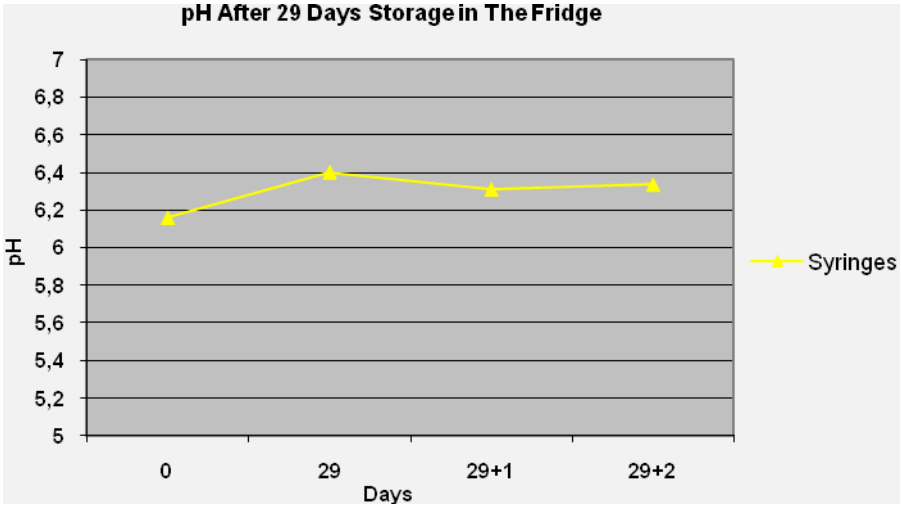


Figure 4.15: pH of The Syringes after 29 Days Storage in The Fridge



4.2.2 Discussion

Allwood[2] has reported that pH changes can lead to significant loss of stability or to precipitation. That is the reason why observation of pH is very important. Changes of pH which are less than 0.5 units are negligible. Only changes of pH bigger than 0.5 units may cause dangerous precipitation. According to the results all pH changes were in norm. The biggest difference in pH values was between initial pH value and the pH values after longer storage period in the fridge (7, 14 and 29 days). Storage of syringes at ambient temperature was not manifest any significant pH changes. Therefore, investigated admixtures were suitable for intravenous delivery.

4.3 RESULTS AND DISCUSSION OF INTRALIPID® SYRINGES MICROSCOPY

4.3.1 Microscopy Results of Intralipid® Syringes

Many enlarged lipid particles were seen in Intralipid® admixtures. The biggest one of 15 μm was observed at day twenty nine plus one (see Table 4.4). One particle bigger than 10 μm was observed at day seven plus one, seven plus two, fourteen plus two and twenty-nine plus one. Four particles bigger than 10 μm were observed at day fourteen plus one. Particles in range 7.5 and 10 μm are showed in Table 4.5. All other lipid globules were 5μm or smaller at all time points.

Table 4.4: Results of Microscopy of Intralipid® Syringes

Intralipid® Syringes			
Day	**Biggest Particle Size (µm)**	**Amount of Particles >10µm**	**Particles Between 7.5-10 µm**
0	10	0	3
7	10	0	3
7+1	12,5	1	2
7+2	12,5	1	3
14	10	0	3
14+1	12,5	4	4
14+2	12,5	1	3
29	10	0	2
29+1	15	1	4
29+2	10	0	4

4.3.2 Discussion

Potential interactions of combined TPN mixtures were discussed in paragraph 2.5. Allwood[2] reported that precipitation may be a result of temperature changes, pH changes or poor mixing. The results have showed that the most stable admixtures are those stored in refrigerator. If they are stored out of refrigerator they become unstable because of higher amount of enlarged lipid globules. Higher amount of lipid particles is presented when investigated admixtures are stored for period longer than 7 days. It means that stability of discussed admixtures is decreasing when storage period is prolonged and admixtures are stored at ambient temperature.

4.4 RESULTS AND DISCUSSION OF LASER DIFFRACTION OF INTRALIPID® SYRINGES

4.4.1 Intralipid® Syringes Laser Diffraction Results

No lipid droplets greater than 5.29 µm were detected in any Syringes (see Figures 4.16 and 4.17). Many samples presented a maximum particle size of just 3.49 µm.

Figure 4.16: Particle Size Distribution of Globules in Lipid Admixtures (Syringes-Day Zero)

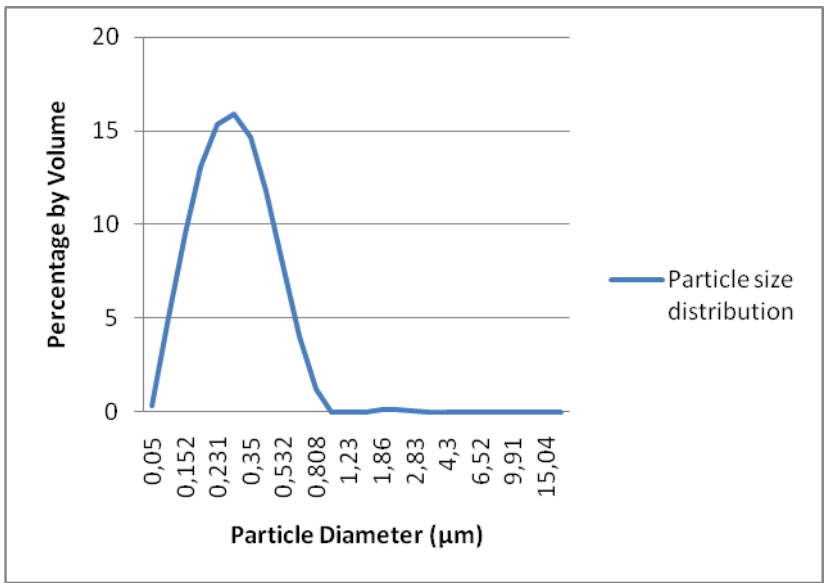
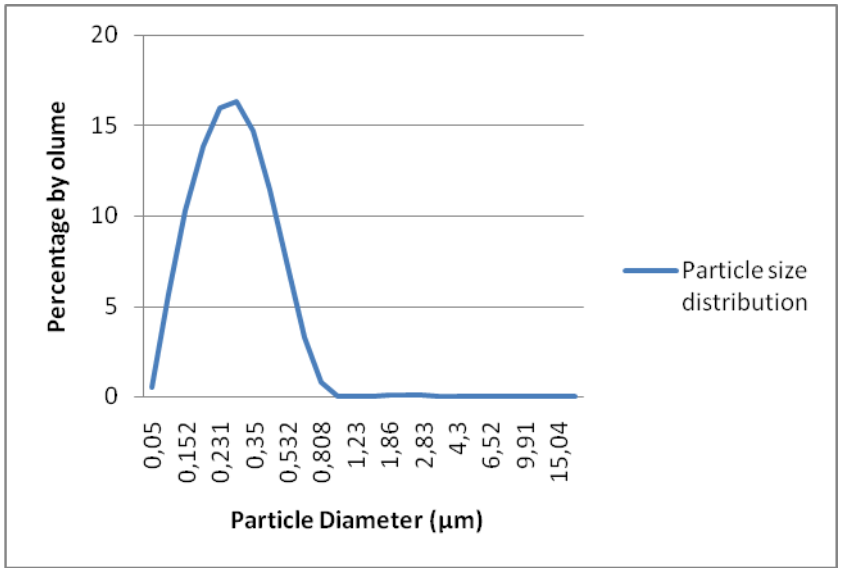


Figure 4.17: Particle Size Distribution of Globules in Lipid Admixtures (Syringes-Day twenty-nine)



4.4.2 Discussion

Intravenously administered fat droplets exceeding 5 µm in diameter are believed to cause adverse reactions, in particular emboli of the lungs. There was found a relationship between particle size and toxicity. The toxicity increases with higher average particle size and wider particle size distribution. Many autors take 5 µm as the upper limit, since larger particles pose a risck of lung embolism[31].

According to the results no lipid droplets in Intralipid® Syringes samples were enlarged. Emulsions in which > 0.4 % of the fat particles are above 5 µm, are likely to become unstable[31]. In discussed trials the volume of droplets bigger than 5 µm was 0 %. Concerning the lipid globules size this Intralipid® admixture could be used for intravenou delivery during whole storage period.

4.5 RESULTS AND DISCUSSION OF OSMOLALITY OF INTRALIPID® SYRINGES

Osmolality was measured at day zero, day twenty-nine, day twenty-nine plus one and day twenty-nine plus two. The values are expressed in Table 4.5.

Table 4.5: Osmolality of The Syringes

Day	Osmolality (mOsm/kg)
0	**469**
29	**467**
29+1	**475**
29+2	**472**

There was not noticed any significant difference among osmolality results during storage period.

4.6 HPLC ANALYSIS OF OXALIC ACID AND ASCORBIC ACID

4.6.1 Determination of Optimal HPLC Method

Firstly it was necessary to develop an optimal method of analysing OA as degradation product of AA. Previously published literature about HPLC analysis of OA was consulted, the sources deal with the HPLC method for analysing organic acids (Tormo and Izco 2004)[32], as well as the analysis of L-AA by HPLC (Novakova et al. 2008[33]; Burini 2007[34]) were red.

A special HPLC column for analysing polar compounds Synergi™ 4 μ Hydro-RP 80A, 250 x 4.60 mm, Phenomenex was recommended and employed. Information about this column and the method convenient for analysing OA was retrieved from the Phenomenex 05/06 Catalog. This column can provide improved retention of extremely polar compounds when 100% aqueous mobile phase on C18 column is used. Separation method recommended for OA analysis by this column was

investigated. 20mM Potassium phosphate pH 2.9 as a mobile phase was employed, flow rate was set up to 0.7 mL/min and UV detection used was 220 nm. Obtained chromatograms showed suitable separation of AA and OA, but OA peak was not sharp enough, so the used method was slightly changed. Flow rate was changed to 1 mL/min and pH of mobile phase was adjusted to pH 2.5 $\pm$ 0.05 units. This alternative method appeared to be optimal and was used for further analysis. As storage solvent for the column was used 65 % acetonitrile and 35 % water solution, because the column must not be stored with buffers.

4.6.2 HPLC Method Used

All samples were analysed at ambient temperature, a reversed phase HPLC analysis was employed. As the mobile phase was used phosphate buffer 20mM pH 2.5. 20 μl of the sample was injected via autosampler. The flow rate was set 1 ml/min with UV detection 220 nm.

4.6.3 Calibration Curves of AA and OA Making

Two solutions were prepared. 100 mg AA was resolved in ultrafiltered deionised water (UDW), put into 100ml volumetric flask and filled with UDW, 100 mg OA was prepared employing the same technique like AA solution in a 100ml volumetric flask. Both of these solutions were diluted 14.5 times and 30 times into a 10ml volumetric flask, and were analysed. 30 times dilution was suitable for making a calibration curve (see Figure 4.20), whereas 14.5 times dilution seemed to be much concentrated. The sample solution with 100 mg AA and 100 mg OA resolved with UDW in 100ml volumetric flask was prepared. Then the sample solution was diluted 30 times with UDW and used for calibration curve making (see figure 4.21) The calibration curve of AA and OA (see Figures 4.18 and 4.19) was determined by analysing five different concentrations at the concentration range between 50% - 125% of the sample solution in triplicate, namely 50, 75, 90, 100 and 125%. The calibration curves were constructed by plotting peak area versus concentration. The correlation coefficients (R^2) and relative standard deviations (RSD) were established.

Table 4.6: Correlation Coefficients (R^2) and Average Relative Standard Deviations (RSD) of AA and OA Samples

Vitamin	RSD	R^2
Ascorbic Acid	0.1531	0.9994
Oxalic Acid	0.4004	0.9990

Table 4.7: Average Peak Areas and Relative Standard Deviations (RSD) of AA and OA Sample Solutions

	OA		AA	
Concentration	Peak Area (%)	RSD	Peak Area (%)	RSD
50%	51.18	0.8547	50.51	0.2379
75%	75.61	0.0764	74.33	0.2005
90%	88.13	0.4607	88.49	0.1306
100%	100.00	0.3791	100.00	0.1484
125%	123.00	0.2311	123.33	0.0481

Figure 4.18: Calibration Curve of Ascorbic Acid

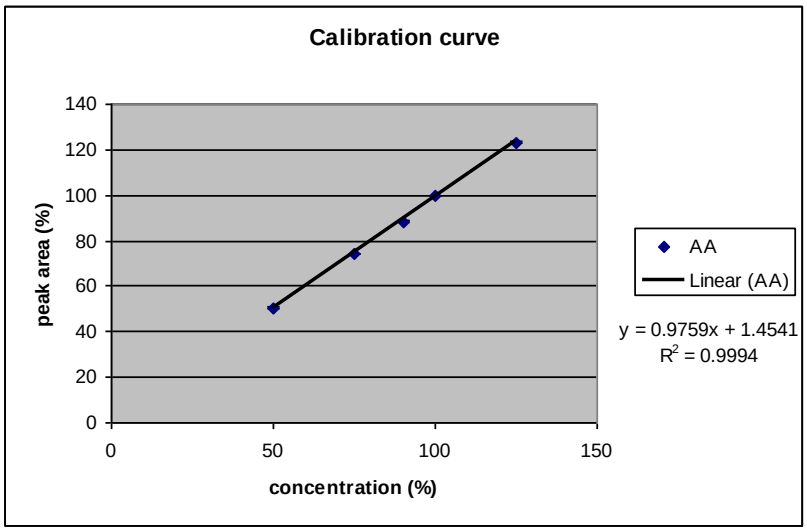


Figure 4.19: Calibration Curve of Oxalic Acid

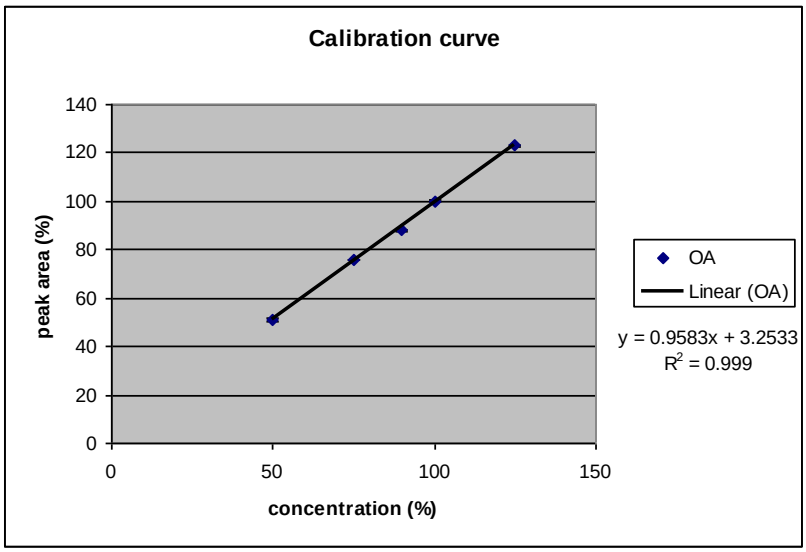


Figure 4.20: Chromatogram of OA Solution (rt. 2.64 minutes)

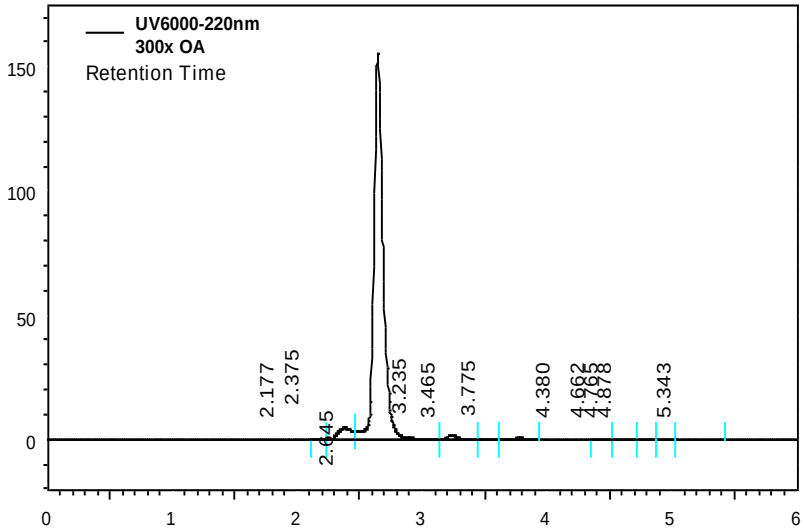
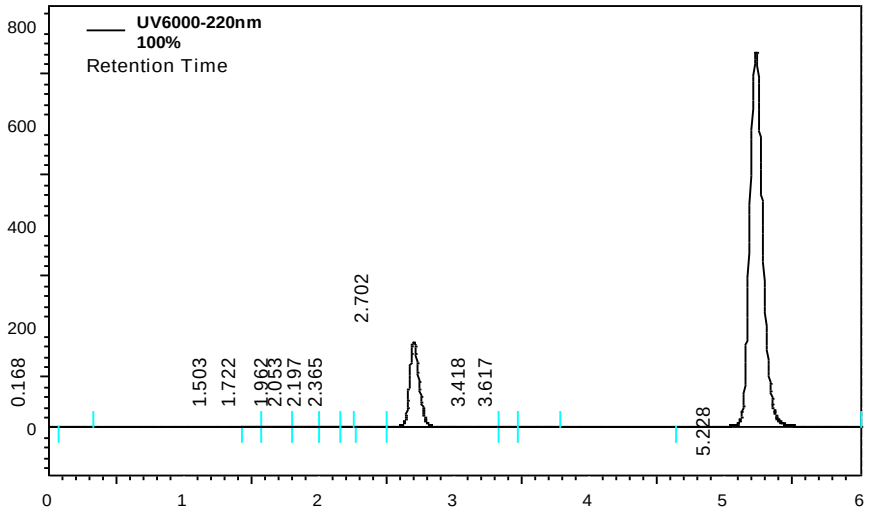


Figure 4.21: Chromatogram of AA + OA Sample Solution (OA rt. 2.70 minutes, AA rt. 5.22 minutes)



4.6.4 Ascorbic Acid Degradation Results

It was not possible to finish the AA degradation investigation because of the conclusion of my research fellowship. Stability studies of AA and OA degradation were not complete by the time I have left, therefore it was not possible to publish them in this thesis.

CHAPTER FIVE

CONCLUSIONS

CHAPTER FIVE: CONCLUSIONS

Stability of vitamins is discussed in previous paragraph 2.4 Vitamins. Almost all vitamins are light-sensitive and are known to be rapidly broken down by exposure to UV light[2]. Intralipid® Syringes were daylight-protected throughout the investigation, therefore degradation was not rapid. According to the Laser diffraction results no lipid droplets in Intralipid® Syringes samples were enlarged. Emulsions in which > 0.4 % of the fat particles are above 5 μm, are likely to become unstable[40]. In discussed trials the volume of droplets bigger than 5 μm was 0 %. Concerning the lipid globules size this Intralipid® admixture could be used for intravenou delivery during whole storage period. Vitamin A in the mixture with fat emulsion was shown quite stable if its samples stored in the fridge. Gillis[19] reported that vitamin E may be strongly bound to plastics, especially to PVC. According to previous results vitamin E losses were not rapid in the mixture with fat emulsion. One of the reasons could be that Syringes used for storage of the mixtures are made from polypropylene/polyethylene and are PVC free. Allwood[2] reported that presence of a fat emulsion affords considerable protection. Previous HPLC results have shown that degradation of pyridoxine is negligible when the samples are light-protected. Pyridoxine was shown as a stable vitamin, because its losses were small during whole storage period. NA HPLC results have shown that its losses during storage in the fridge were small. More significant degradation of NA was noticed after two days storage at ambient temperature. Therefore samples containing NA should be stored in a refrigerator before administration to patients. Thiamine was shown to be stable in the mixture with fat emulsion. Almost no losses were detected when thiamine containing samples were stored in the fridge. Previous HPLC results have shown that FA was rapidly degraded when the samples were stored out of the fridge. Losses of FA in the mixture with fat emulsion were less if the samples stored in the fridge, around 17 % of its initial amount. FA degradation is rapid so mixtures containing FA should be administered to patients after preparation and not stored. Riboflavin losses were significant throughout investigation, especially when samples were stored at ambient temperature for two days. Riboflavin containing mixtures should be administered after preparation as well as mixtures with FA. Previous HPLC results have shown that PA degradation is small even after long time storage period.

Losses of this vitamin were negligible if samples stored in the fridge as well as at ambient temperature.

It was not possible to finish the AA degradation investigation because of the conclusion of my research fellowship. Stability studies of AA and OA degradation were not complete by the time I have left, therefore it was not possible to publish them in this thesis.

ABSTRACT

Malnutrition is a state in which a deficiency of energy, protein and other nutrients causes measurable adverse effects on tissue, body form, composition, function or clinical outcome[1]. Parenteral nutrition (PN) is an alternative method of providing nutritional support for patients via the intravenous route[3]. PN admixtures consist of fats, carbohydrates, proteins, vitamins, trace elements, electrolytes and fluids. Vitamins are believed to be among the least stable ingredients in PN mixtures and should be added immediately before commencing infusion. The purpose of this thesis was to obtain more punctual information on stability of the vitamins in Intralipid® emulsion depending on different storage conditions and different time.

Water-soluble vitamins and fat-soluble vitamins were investigated in the mixture of Intralipid® emulsion (Fresenius Kabi) with Solvitio® N (water-soluble vitamins) and Vitlipid® N (fat-soluble vitamins) Adult Injections. There were prepared six 50ml Luer-Lock Syringes in total. Each of them was filled with 47 ml of the Intralipid® mixture and closed by Multi-Ad Luer-Lock Syringe cap. Assessing the chemical and physical stability was carried out after: zero time, 7, 14 and 29 days in a refrigerator followed by 24 and 48 hours storage at ambient temperature and day-light protected. Physical tests included pH, osmolality, microscopy and particle size determination by laser diffraction. Chemical tests used validated stability indicating reversed phase HPLC methods. Eight vitamins were suitable for investigation via HPLC method, namely vitamin A and E, thiamine, pyridoxine, riboflavin, pantothenic acid, folic acid and nicotinamide. Ascorbic acid (AA) was eluted very early in gradient run and was not suitable for analysing by HPLC method used.

AA is rapidly oxidised, the oxidation of AA clearly proceeds to inactive products. There is potential appearance of oxalic acid (OA) in the mixture as an end stage degradation product[2]. The purpose of my investigation was to prove the presence of OA as a degradation product of AA dissolved in water after couple-day storage at ambient temperature via HPLC analysis.

According to the Laser diffraction results no lipid droplets in Intralipid® Syringes samples were enlarged. In discussed trials the volume of droplets bigger than 5 μm was 0 %. Concerning the lipid globules size this Intralipid® admixture could be used for intravenou delivery during whole storage period. Vitamin A in the mixture with fat emulsion was shown quite stable if its samples stored in the fridge. Losses of the vitamin E were not rapid in the mixture with fat emulsion. One of the reasons could be that Syringes used for storage of the mixtures are made from polypropylene/polyethylene and are PVC free. HPLC results have shown that degradation of pyridoxine is negligible when the samples are light-protected. Pyridoxine was shown as a stable vitamin, because its losses were small during whole storage period. NA HPLC results have shown that its losses during storage in the fridge were small. More significant degradation of NA was noticed after two days storage at ambient temperature. Thiamine was shown to be stable in the mixture with fat emulsion. Almost no losses were detected when thiamine containing samples were stored in the fridge.

HPLC results have shown that FA was rapidly degraded when the samples were stored out of the fridge. Losses of FA in the mixture with fat emulsion were less if the samples stored in the fridge. Riboflavin losses were significant throughout investigation, especially when samples were stored at ambient temperature for two days. Degradation of PA was small even after long time storage period. Losses of this vitamin were negligible if samples stored in the fridge as well as at ambient temperature.

It was not possible to finish the AA degradation investigation because of the conclusion of my research fellowship. Stability studies of AA and OA degradation were not complete by the time I have left, therefore it was not possible to publish them in this thesis.

ABSTRAKT

Podvýživa je stav, ve kterém nedostatek energie, proteinů a ostatních živin má znatelně nepříznivý dopad na tkáně, jejich funkci a složení[1]. Parenterální výživa je alternativní metoda poskytování nutriční podpory pro pacienty prostřednictvím žilního systému[3]. Směsi parenterální výživy se skládají z tuků, sacharidů, proteinů, vitamínů, stopových prvků, elektrolytů a tekutin. Vitamíny jsou považovány za nejméně stabilní složky v parenterálních směsích, a tudíž by měly být přidány bezprostředně před aplikací infuze. Cílem mé práce bylo získat informace o stabilitě vitamínů ve směsi Intralipid® v závislosti na různých skladovacích podmínkách a na době skladování.

Vitamíny rozpustné ve vodě a v tucích byly testovány ve směsi Intralipid® emulze (Fresenius Kabi) se substancí Solvitio® N obsahující vitamíny rozpustné ve vodě a s emulzí obsahující vitamíny rozpustné v tucích-Vitlipid® N. Celkem bylo připraveno šest 50ml injekčních stříkaček. Každá z nich byla naplněna 47 ml Intralipidové směsi a uzavřena. Testování chemické a fyzikální stability bylo provedeno v čase nula, po 7, 14 a 29 dnech skladování v lednici následované 24 a 48 hodinovým skladováním těchto směsí při okolní teplotě. Provedené fyzikální testy zahrnovaly měření hodnot pH, osmolality, mikroskopii, a měření velikosti částic laserovou difrakcí. K chemickým testům byly použity validované a stabilitu určující metody HPLC s užitím reverzní fáze. Pomocí HPLC bylo možno testovat osm vitamínů, konkrétně vitamíny A a E, thiamin, riboflavin, pyridoxin, kyselinu listovou, kyselinu pantothenovou a nicotinamid. Jelikož vitamín C byl eluován brzy v gradientové eluci, nebylo možné provést jeho analýzu pomocí popsané HPLC metody.

Kyselina askorbová je rychle oxidována na neaktivní produkty. Existuje zde potencionální možnost přítomnosti oxalové kyseliny ve směsi jako konečného degradačního produktu[2]. Cílem analýzy bylo dokázat přítomnost oxalové kyseliny jako degradačního produktu askorbové kyseliny rozpuštěné ve vodě po několika dnech skladování při okolní teplotě prostřednictvím HPLC analýzy.

Podle výsledků laserové difrakce, ve vzorku s Intralipidovou směsí, nebyla žádná z lipidových částic zvětšená. V provedených testech byl objem lipidových částic větších než 5 μm roven 0%. Co se týká velikosti lipidových globulí, Intralipidová směs by mohla být použita pro intravenozní aplikaci ve všech zkoušených časových jednotkách. Vitamín A byl ve směsi s tukovou emulzí zhodnocen jako stabilní, pokud byly analyzované vzorky uchovávány po dobu skladování v lednici. Ztráty vitamínu E ve směsi s tukovou emulzí nebyly rapidní. Jedním z důvodů by mohlo být, že stříkačky, použité pro skladování analyzovaných směsí, byly utvořeny z polypropylenu/polyethylenu, bez obsahu PVC. Výsledky HPLC analýzy ukázaly, že degradace pyridoxinu byla zanedbatelná, když vzorky byly chráněny před světlem a ztráty vitamínu byly nepatrné během celé doby skladování. Výsedky HPLC analýzy NA ukázaly, že jeho ztráty během skladování v lednici byly malé. Rozsáhlejší degradace NA byla zaznamenána po dvou dnech skladování směsi při pokojové teplotě. Thiamin byl ve směsi s tukovou emulzí zhodnocen jako stabilní. Téměř žádné ztráty tohoto vitamínu nebyly zaznamenány, pokud směsi byly uchovávány v lednici.

HPLC analýza kyseliny listové ukázala rychlou degradaci vitamínu, pokud směsi byly uchovávány při pokojové teplotě. Ztráty kyseliny listové nebyly tak výrazné, pokud vzorky směsi byly uchovávány v lednici. Degradace riboflavinu byla zaznamenána po celou dobu skladování směsí, zejména pokud byly vzorky uchovávány po dva dny při pokojové teplotě. Degradace kyseliny pantothenové byla zanedbatelná dokonce i po dlouhé skladovací době. Vliv skladovací teploty na degradaci vitamínu nebyl prokázán.

Z důvodu nedostatku času nebylo možné dokončit analýzu degradace kyseliny askorbové. Studijní stáž byla ukončena před dokončením stabilitních studií degradace kyseliny askorbové a kyseliny oxalové, tudíž nekompletní výsledky nebylo možné v této práci publikovat.

REFERENCES

1. NICE guideline of Nutrition support in adults, oral nutrition support, enteral tube feeding and parenteral nutrition, published by the National Collaborating Centre for Acute Care at The Royal College of Surgeons of England in 2006
Available at:
URL:http://www.nice.org.uk/nicemedia/live/10978/29981/29981.pdf

2. ALLWOOD, M.C. 1984. Compatibility and Stability of TPN Mixtures in Big Bags. *Journal of Clinical and Hospital Pharmacy* 9 (3), pp. 181-198.

3. DUDRICK, S.J., WILMORE, D.W., VARS, H.M. and RHOADS, J.E. 1968. Long-term total parenteral nutrition with growth, development, and positive nitrogen balance. *Surgery* 64(1), pp. 134 – 142.

4. NICE 2006a New NICE guideline will help tackle the problem of malnutrition in the NHS – Press Release. [online] NICE 2006/006(Issued: 21 February 2006). Available at:
URL:http://www.nice.org.uk/niceMedia/pdf/2006_006_nutrition_guideline_launch.pdf

5. BARENDREGT, K., SOETERS, P.B., ALLISON, S.P., KONDRUP, J. 2004. Chapter 1.3: Diagnosis of malnutrition- Screening and Assessment. In: Sobotka, L. *Basics in Clinical Nutrition*. Third ed. Prague: Galen, pp.11 – 18.

6. KHÝNOVÁ, L. 2009. Chapter 2: Theoretical part. In: KHÝNOVÁ L. 2009. *Stability of Vitamins in Parenteral Nutrition Admixtures*. Diploma Thesis, Hradec Králové, CZ, pp. 18-50.

7. SAID, S.N. 2006a. Chapter 1: Introduction. In: Said S.N. 2006. *Problems associated with the provision of micronutrients to parenteral nutrition patients.* Thesis.Cardiff, UK. pp. 1 – 38.

8. SAUERWEIN, H.S. and ROMIJN, J. A. 2001. Chapter 10: Adult macronutrient requrements. In: Payne-James, J. et al. *Artificial Nutrition Support in Clinical Practice*. Second ed. London: Greenwich Medical Media Limited, pp. 177 – 191.

9. FURST, P., KUHN, K. and STEHLE, P. 2001. Chapter 23: *Parenteral nutrition substances*. In: Payne-James, J. et al. *Artificial Nutrition Support in Clinical Practice*. Second ed. London: Greenwich Medical Media Limited, pp. 401 – 434.

10. CUTHBERTSON, D.J.R. and RENNIE, M.J. 2001. Protein and amino acid metabolism in the whole body and in the tissues. In: Payne-James, J. et al. *Artificial Nutrition Support in Clinical Practice.* Second ed. London: Greenwich Medical Media Limited, pp. 25 – 29.

11. WOOLFSON, A.M.J. 1985. Intravenous Feeding – A Review of Aspects of Current Practice. *Clinical Nutrition* 4, pp. 187 – 194.

12. VAN DER HULST, R.R.W.J., VAN KREEL, B.K., VON MEYENFELDT, M.F., BRUMMER, R.J.M., ARENDS, J.W., DEUTZ, N.E.P. and SOETERS, P.B. 1993. Glutamine and the preservation of gut integrity. *The Lancet* 341, pp. 1363 – 1365.

13. TAPPY, L. 2004. Chapter 2.3: *Carbohydrate metabolism*. In: Sobotka, L. *Basics in Clinical Nutrition*. Third ed. Prague: Galen, pp.66 – 71

14. SOBOTKA, L., ALLISON, S. P. and STANGA, Z. 2004. Water and electrolytes during nutritional support. In: Sobotka, L. *Basics in Clinical Nutrition*. Third ed. Prague: Galen, pp. 165 – 168.

15. SHENKIN, A. 2001. Adult micronutrient requirements. In: Payne-James, J. et al. *Artificial Nutrition Support in Clinical Practice.* Second ed. London: Greenwich Medical Media Limited, pp. 193 – 212.

16. COMBS Jr., G.F. 1998 *The Vitamins: Fundamental Aspects in Nutrition and Health*. Second ed. San Diego: Academic Press.

17. ALLWOOD, M.C. and KEARNEY, M.C.J. 1998. Compatibility and Stability of Additives in Parenteral Nutrition Admixtures. *Nutrition* 14(9), pp. 697 – 706.

18. ROCKWELL, G.F., CAMPFIELD, T., NELSON, B.C. and UDEN, P.C. 1998. Oxalogenesis in parenteral nutrition solution components. *Nutrition* 14 (11-12), pp. 836-839.

19. GILLIS, J., JONES, G. and PNECHARZ, P. 1983. Delivery of vitamin A, D and E in total parenteral nutrition solutions. *Journal of Parenteral and Enteral Nutrition* 7, pp. 11 – 14

20. ALLWOOD, M.C. and MARTIN, H.J. 2000. The photodegradation of vitamins A and E in parenteral nutrition mixtures during infusion. *Clinical Nutrition* 19 (5), pp. 339 – 342

21. GIBBONS, E., ALLWOOD, M.C., NEAL, T. and HARDY, G. 2001. Degradation of dehydroascorbic acid in parenteral nutrition mixtures. *Journal of Pharmaceutical and Biomedical Analysis* 25, pp. 605 – 611.

22. HARRAKI, B., GUIRAUD, P., ROCHAT, M.H., ALARY, J., FAVIER, A. 1995. Interactions related to trace elements in parenteral nutrition. *Pharmaceutica Acta Helvetiae* 70(4), pp. 269-278.

23. British Pharmacopoeia 2008. Appendix XVII P. Particle Size Analysis by Laser Light Diffraction. http://www.pharmacopoeia.co.uk/ixbin/bp.cgi?r=cGyEz3JucdN&id=825&n=2&a=display

24. British Pharmacopoeia 2008. Appendix V L. Determination of pH Values. http://www.pharmacopoeia.co.uk/ixbin/bp.cgi?id=940&a=display

25. MEIER, P., LOHRUM, A. and GAREISS, J. 1989. Practice and Theory of pH Measurement. An outline of pH measurement. Information and practical hints. Ingold

26. British Pharmacopoeia 2008. Appendix V N. Osmolality. http://www.pharmacopoeia.co.uk/ixbin/bp.cgi?r=sJzOR9eUVki&id=938&n=34&a=display

27. SAID, S.N. 2006b. Chapter 2: General Methods. In: Said S.N. 2006. *Problems associated with the provision of micronutrients to parenteral nutrition patients*. Thesis. Cardiff, UK, pp. 39 – 67.

28. RIZZOLO, A. and POLESELLO, S. 1992. Chromatographic determination of vitamins in foods. *Journal of Chromatography* 624, pp. 103-152.

29. British Pharmacopoeia 2008. Appendix III D. Liquid Chromatography. http://www.pharmacopoeia.co.uk/ixbin/bp.cgi?id=960&a=display#WIXLINKp2p985

30. KHÝNOVÁ, L. 2009. Chapter 4: Results and Discussion. In: KHÝNOVÁ L. 2009. *Stability of Vitamins in Parenteral Nutrition Admixtures*. Diploma Thesis, Hradec Králové, CZ, pp. 66-93.

31. KOSTER, V.S., KUKS, P.F.M., LANGE, R., TALSMA, H. 1996. *Particle size in parenteral fat emulsions, what are the truelimitations? International Journal of* Pharmaceutics 134, pp 235-238.

32. TORMO, M. and IZCO, J.M. 2004. Alternative reversed-phase high-performance liquid chromatography method to analyse organic acids in diary products. *Journal of Chromatography A* 1033, pp. 305 – 310.

33. NOVÁKOVÁ, L., SOLICHOVÁ, D., SOLICH, P. and PAVLOVICOVÁ, S 2008. Hydrophilic interaction liquid chromatography method for the determination of ascorbic acid. *Journal of Separation Science* 31, pp. 1634 – 1644.

34. BURINI, G. 2007. Development of a quantitative method for the analysis of total L-ascorbic acid in foods by high-performance liquid chromatography. *Joural of Chromatography A* 1154, pp. 97 – 102.

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