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HRADEC KRÁLOVÉ 2009 **LUCIE KHÝNOVÁ**

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FACULTY OF PHARMACY

DEPARTMENT OF ANALYTICAL CHEMISTRY

STABILITY OF VITAMINS IN PARENTERAL NUTRITION ADMIXTURES

HRADEC KRÁLOVÉ 2009 **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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Figure 4.25: Particle Size Distribution of Globules in Lipid Admixtures (Bag B-Day twenty-nine plus two)

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
mg	Milligram
ml	Milliliter
mm	Millimeter
mmol	Millimol
Mn	Manganese
Mo	Molybdenum
mosm/kg	Milliosmol per kilogram
MUFA	Monounsaturated fatty acids
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NICE	National Institute for Health and Clinical Excellence
nm	Nanometer
OA	Oxalic 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

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1.1 INTRODUCTION

Malnutrition is surprisingly common nowadays, especially in those who are unwell, and is both a cause and a consequence of ill-health. Many older people and those with any long term medical or psycho-social problems are chronically underweight and so are vulnerable to acute illness[1]. Parenteral nutrition 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, and is an important therapeutic requirement in many clinical situations[2].

1.2 AIMS OF PROJECT

The purpose of this diploma work was to obtain more punctual information on stability of the vitamins in parenteral nutrition admixtures depending on different storage conditions and different time. There is project which was done.

Stability of the fat-soluble vitamins (A, E) and the water-soluble (B_2, B_6) vitamins which were added to the Kabiven® Peripheral Bag, firstly together with trace elements and secondly without trace elements.

Assessing the chemical and 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.

CHAPTER TWO
THEORETICAL PART

CHAPTER TWO: THEORETICAL PART

2.1 PARENTERAL NUTRITION

Parenteral Nutrition (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. This intravenous route of administering artificial nutrition was pioneered by Dudrick et al (1968)[3], who demonstrated for the first time, the access of administering concentrated glucose, amino acids and other essential nutrients towards growth development in both animals and man[4]. With advances in the various aspects of clinical medicine the need and indication for parenteral nutrition are increasing progressively. This is due to availability of better medical and surgical facilities to treat conditions which were lethal few years ago; increasing awareness of the importance of providing adequate nutrition and of the fact that in potentially curable situations patients may actually die because of malnutrition[5].

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.

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[1]. 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). For adult patients in hospital it is suggested to use the Nutrition Risk Screening 2002. Assessment should incorporate the following principles:

- History and examination: to define all the factors leading to malnutrition and the likely natural history of the patient condition.
- Disease status: this includes history, examination, bed-side measurements such as temperature, pulse rate and blood pressure, laboratory tests of inflammation including full blood count, albumin and C- reactive protein.
- Laboratory tests: levels of vitamins, trace elements, changes in minerals.

- Fluid balance: examine for oedema or dehydration.
- Functional assessment: ask the patient about exercise tolerance and breathing. Measure peak flow and muscle strength[6].

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[6].

2.2 INDICATIONS FOR INTRAVENOUS NUTRITION

Patients selected for PN are malnourished patients or patients who have the potential to become malnourished[4]. Nutrition support should be considered in people, as defined by a BMI of less then 18.5; unintentional weight loss greater then 10% within the last 3 - 6 months; or a BMI of less then 20 and unintentional weight loss greater than 5 % within the last 3 - 6 months[1]. Conditions which need parenteral nutrition support are for example conditions characterized by loss of bowel, conditions characterized by functional bowel failure, burns, severe septicaemia and multiple trauma with or without surgery[5].

2.3 ROUTES OF ADMINISTRATION OF PN

PN means that nutrients are provided intravenously. Therefore venous access is necessary. PN can be administered via central route or via peripheral route[7].

2.3.1 Central Route

PN has to be given via a catheter with tip located in a central vein to infuse nutrients at high concentrations and smaller volume. Most often the catheter is inserted into the superior vena cava. Central PN (CPN) is indicated to patients who require long-term PN; those without adequate peripheral veins; those in whom peripheral PN

(PPN) has failed; who already have central venous access established; who have specific nutrition requirements and patients who are critically ill. PN for more than 7 days is usually provided via a central vein because of the risk of thrombophlebitis caused by hyperosmolarity of nutrient solutions[8].

2.3.1.1 Central Venous Catheters (CVC)

Nowadays CVCs are made of polyurethane or silicone. Some new CVCs are furthermore coated by antibiotics. Antibiotic-coated catheters have also been shown to result in significant reductions in infectious complication but have raised concern over the possibility of encouraging the growth of resistant organisms to the antibiotics employed.

2.3.1.2 Peripherally Inserted Central Catheters (PICCs)

These are usually inserted into either the cephalic or basilic vein in the cubital fossa. The catheter is advanced along the vein until the tip lies in the superior vena cava. Image post-insertion chest X-ray is mandatory for their safe use. These catheters are rapidly gaining in popularity. The use of PICCs carries the significant advantage that experience of direct puncture of central veins is not necessary. As with all CVCs these catheters must be inserted with strict attention to aseptic technique[8].

2.3.2 Peripheral Route

The concept of peripheral parenteral nutrition (PPN) was first described in 1945 by Brunschwig and colleagues. The term peripheral refers to superficial veins, most often of the upper extremities. Peripheral veins of the lower extremities, especially in adults, are not suitable for PN. This access should be avoided because of higher risks of thrombophlebitis and because of the need of confirming the patient to bed. PPN is provided when catheterization of a central vein is impossible or contraindicated, when bacteraemia or catheter sepsis is present. The patients suitable for PPN are those with available peripheral veins, who need short-term therapy with low osmolar solutions. Unsuitable patients are those without available

peripheral veins, requiring high energy and protein, electrolyte intake or those at risk of fluid overloading and prolonged nutritional support[7].

2.4 COMPLICATIONS OF PN

Complications can be categorized into two groups: Metabolic and Catheter related. Most of these complications can now be prevented or are easily treatable due to advances in modern medicine and clinical practice.

2.4.1 Catheter-related Complications (CRC)

CRC can be divided into three main categories: Mechanical Complications, Thrombosis and Catheter-related Sepsis.

2.4.1.1 Mechanical Complications

Mechanical complications include those that occur early, primarily related to subclavain catheter placement, and those occurring late with are primary related to the long-term presence of the central venous system. Pneumothorax is the most frequent complication associated with subclavain catheter placement.

2.4.1.2 Catheter-related Sepsis

Catheter-related sepsis still remains the most serious complication of total parenteral nutrition (TPN) in clinical practice today. The term ‘infection’, as related to the central vein catheter, is a universal term used to describe systematic or localized infections or the development of colonies along the length of the catheter. Systematic infections are frequently reported as catheter sepsis or bacteraemia. The material from which the catheter itself is made influences the risk of infection. Some materials favuor the collection of bacteria while others favour thrombogenesis. Materials which decrease thrombogenesis are PVC, polyethylene, polyurethane and silicone. The most important factor in prevention of this sepsis is aseptic technique.

2.4.1.3 Venous Thrombosis

Venous thrombosis is a late complication which may occur after extended use of central venous system. Subclinical venous thrombosis occurs with a much frequency, approaching 50 %. Clinical subclavain vein thrombosis has been reported to be in 3 – 7 %. The more rigid polyethylene catheters lead to thrombosis much more often and to a greater degree than much softer silicone and polyurethane catheters.

2.4.2 Metabolic Complications

The major deficiency states associated with prolonged use of TPN have been eliminated by recognizing requirements for the vitamins and trace elements. The most common metabolic complications are hypoglycaemia and hyperglycaemia.

Hypoglycaemia is caused by sudden slowing of the glucose infusion. Over-secretion of endogenous insulin at high infusion rates can lead to an extraordinary form of hypoglycaemia. The most common cause of hyperglycaemia is rapid initiation of the infusion. Other metabolic complications are abnormalities in plasma electrolytes. They are minimized by careful monitoring. At least 50 mEq of sodium, 40 mEq of potassium, 90 – 100 mEq of phosphorus and 28 – 32 mEq of magnesium and calcium should be administered daily to all patients receiving PN. Trace metal deficiencies belongs to metabolic complications too. Zinc deficiency can develop in patients who are very catabolic or have excessive diarrhoea. Cooper deficiency has been observed in patients receiving home PN. Selenium is an essential element for humans. Chromium deficiency is likely to occur only in patients on long-term TPN with minimal or not oral intake. Essential fatty acid deficiency may be prevented by administration of between 4 – 6 % of the daily calories as either soybean or safflower oil fat emulsion. The main symptom of fatty acid deficiency is dry skin with small reddish papules and alopecia[9].

2.5 COMPONENTS OF PN ADMIXTURES

PN admixture consists of three macronutrients: proteins as amino acid solutions, carbohydrates in the form of glucose, 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[4]. For mixtures which contain all described nutrients is used the term total parenteral nutrition (TPN).

PN regimens may be compounded as either 'two-in-one' mixtures, which do not contain lipid emulsion, or 'all-in-one' mixtures (AIO), in which the lipid emulsion is included[10]. TPN administration has developed from separate macronutrients in infusion bottles to administration of the AIO bags. The mixing of all, or most, of the TPN components into a larger, usually 1 or 3 litre capacity, flexible container or Big Bag is now accepted as the safest and most convenient method of TPN administration. The packing of the different components of TPN mixture has important consequences. Certain constraints apply specifically to these solutions. Hypertonic dextrose infusions are made in flexible plastic containers, amino acids injections are produced in glass bottles because of their instability in most plastic containers, especially PVC, such solutions. Amino acid solutions are filled under vacuum and often contain a reducing agent to avoid oxidation of particular amino acids. Fat emulsions are produced under vacuum in glass bottles, again to avoid oxidation of fatty acids during long-term storage. Additives such as vitamins and trace elements are packed in ampoules or vials[11].

2.5.1 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[12], 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[13]. Adult glucose requirements in healthy subjects can be set at 4 – 5 g/ kg/ day, equivalent to 50 % of the total energy requirement[14].

2.5.2 Proteins

Proteins are large organic compounds made of amino acids arranged in a linear chain and joined together by peptide bonds. Therefore proteins are a source of amino acids. Traditionally, amino acids are classified into essential and non-

essential. Essential amino acids in adults are 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[15].

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[16]. 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[17].

2.5.3 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[4]. Lipid requirement is about 1 – 1.5 g/ kg/ day in healthy adults[14].

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)[4]. 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[13].

2.5.4 Micronutrients

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

The micronutrients have two main functions. 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[18]. Both vitamins and trace elements are discussed under Vitamins (see 2.6) and Trace elements (see 2.7).

2.5.5 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. Sodium, potassium, magnesium, calcium and phosphate are some of the major electrolytes required[19]. Daily requirements of each ion can vary widely, depending on the disease state and may be very high[11].

2.6 VITAMINS

Vitamins are organic food components that promote the fast regular working of essential biochemical reactions within human body. These vitamins cannot generally

be synthesized in vivo, and must be obtained from food in trace amounts. They are required for growth, maintenance, lactation and reproduction[20].

Vitamins originally come from the term ‘vitamine’ as a generic descriptor for many such accessory factors associated with diets. This term was suggested by the Polish biochemist Casimir Funk.

The 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 or 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[21].

2.6.1 Water-soluble Vitamins

2.6.1.1 Thiamine (Vitamin B_1)

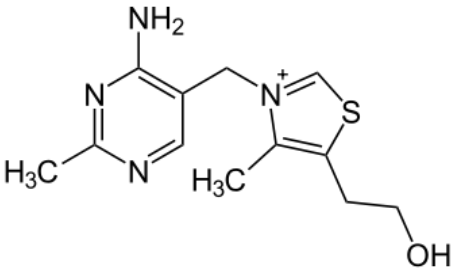


Figure 2.1: Chemical Structure of Thiamine

Thiamine was first crystallized by Jansen and Donath in 1926. They named it aneurine, the antineuritic vitamin, because of its function in curing polyneuritis.
Thiamine’s chemical structure contains a pyrimidine ring and a thiazole ring linked by a methylene bridge. Thiamine may be destroyed by several factors including neutral

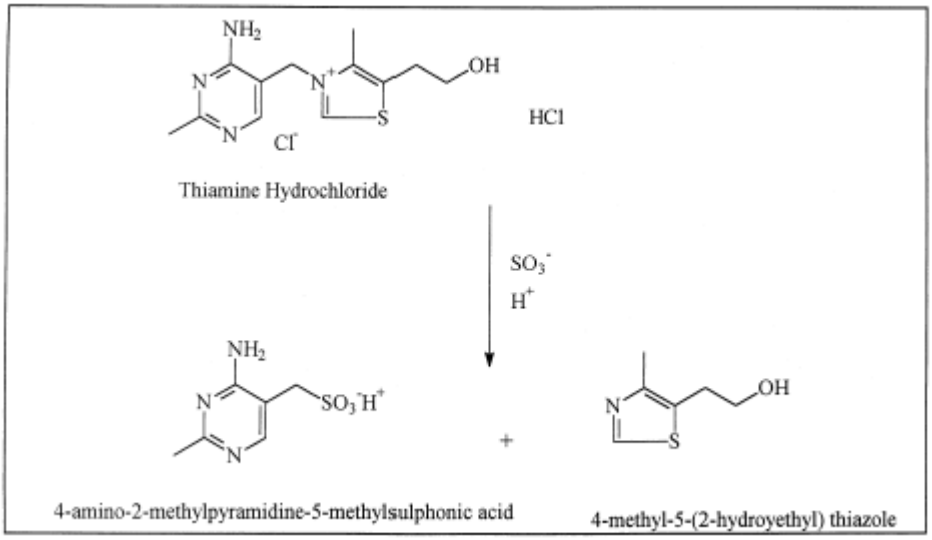
and alkaline conditions, heat, oxidation, and ionizing radiation. It is stable at low pH (pH <7). Thiamin is stable during frozen storage.
In foods derived from plants, thiamin occurs as free thiamin. In contrast, thiamin occurs in animal tissues almost entirely in phosphorylated forms. The predominant form being the coenzyme thiamin diphosphate, also called thiamin pyrophosphate (TPP). TPP is the coenzyme for the decarboxylases, the group of enzymes that remove carboxyl groups. One function of TPP is in the oxidative decarboxylation of pyruvate dehydrogenase. This is the key enzyme required for the conversion of pyruvate, the product of carbohydrate metabolism, to acetyl coenzyme A. Impaired entry of pyruvate into the citric acid cycle can lead to high concentrations of lactate and pyruvate, and to lactic acidodis[4].

Thiamin is widely distributed in foods, but in very low concentrations. The richest sources are yeasts and liver, especially pork liver. Cereal grains comprise the most important dietary sources of the vitamin in human diets[21].

Thiamine deficiency can result in beri-beri. This disease is prevalent in Southeast Asia, where polished rice is the dietary staple. Anorexia, cardiac enlargement, muscular weakness and lassitude are the general symptoms of beri-beri. This disease occurs in three clinical types. Dry (neuritic), wet (edematous), and infantile (acute) beri-beri[21].

Thiamine 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[22].

Figure 2.2: Thiamine Degradation Pathway



2.6.1.2 Riboflavin

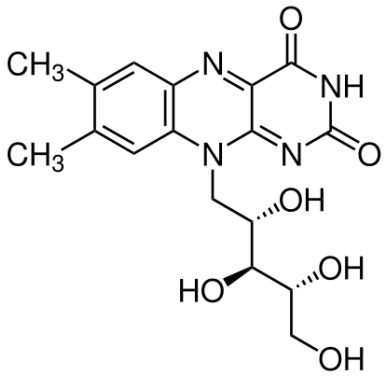


Figure 2.3: Chemical Structure of Riboflavin

Riboflavin, known as Vitamin B_2, was discovered as a 'yellow enzyme' in yeast by biochemists Warburg and Christian in 1932[23]. Riboflavin consists of an isoalloxazine derivate with a sugar alcohol ribityl side chain (Figure 2.3)[4]. Riboflavin is converted to its enzyme forms. The first step in this process is an ATP-dependent phosphorylation to yield riboflavin-5-phosphate, also called flavin mononucleotide (FMN). Most of FMN is then converted to the other coenzyme, flavin adenine dinucleotide (FAD), by FAD-pyrophosphorylase. FMN and FAD function as coenzymes for enzymes called flavoproteins or flavoenzymes, and acts as electron carriers in a variety of oxidation-

reduction reactions. They are essential for the metabolism of carbohydrates, amino acids, and lipids. Some are also essential for the activation of the vitamins pyridoxine and folate to their coenzyme forms[21].

Riboflavin is widely distributed in foods. Rapidly growing, leafy, green vegetables are rich in the vitamin.

Uncomplicated riboflavin deficiency becomes manifest in humans only after 3 - 4 months of deprivation of the vitamin. Signs include cheilosis (inflammation of the lips), angular stomatitis (cracks at the corners of the mouth), glossitis (inflammation of the tongue), edema and hyperemia of the oral mucosa. Although clinical signs of riboflavin deficiency are rarely seen, subclinical riboflavin deficiency is not uncommon. In children it may result in subnormal growth[21]. Riboflavin deficiency often coexists with nicotinic acid deficiency and can be complicated by pyridoxine deficiency[4].

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. 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[22].

2.6.1.3 Niacin (Vitamin B_3)

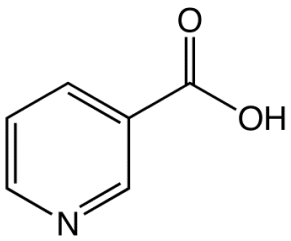


Figure 2.4: Chemical Structure of Nicotinic Acid

The term niacin is the generic descriptor for nicotinic acid (pyridine-3-carboxylic acid) (Figure 2.4) and nicotinamide (nicotinic acid amide). The active coenzyme forms of niacin are nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). In these both forms of the coenzymes is niacin essential[24]. NAD and NADP serve as coenzymes for a wide variety of reactions in the metabolism of carbohydrates, fatty acids and amino acids[4]. Niacin can be obtained from the diet or synthesised from amino acid tryptophan. This is an important route for meeting the body's niacin requirement. To estimate nutritional intake or niacin equivalents from tryptophan, an average conversion ratio of 60 mg tryptophan to 1 mg niacin was recommended by the Food and Nutrition Board of the National Research Council. The efficiency of conversion of dietary tryptophan to niacin is affected by a variety of nutritional and hormone factors. Deficiencies of vitamin B_6, riboflavin, or iron slow the conversion because these micronutrients are essential cofactors for enzymes involved in the pathway[24].

Niacin is widely distributed in plant and animal foods. Good sources are yeast, meats, cereals, liver, and seeds.

Pellagra is the classic dietary deficiency disease. This includes skin changes, nervous system and intestinal tract changes. The symptoms associates with the skin are most characteristic[24].

Both nicotinic acid and nicotinamide are very stable in dry form, but in solution nicotinamide is hydrolyzed by acids and bases[21].

2.6.1.4 Pyridoxine (Vitamin B_6)

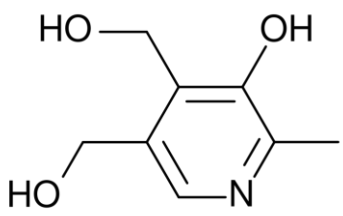


Figure 2.5: Chemical Structure of Pyridoxine

Vitamin B_6 is the generic descriptor for all 3-hydroxy-2-methylpyridine derivatives. This vitamin occurs naturally in three primary forms. Pyridoxine, which was called pyridoxol, its aldehyde pyridoxal and the amine pyridoxamine[21]. The bioavailability of vitamin B_6 is affected by food processing and the amount of pyridoxine glucoside in foods. After intestinal absorption, the three forms are taken up by the liver and converted to pyridoxal 5-phosphate in a metabolic process which also involves riboflavin-containing coenzymes. Pyridoxal 5-phosphate serves as cofactor for > 100 enzymes. These enzymes, which require pyridoxal 5-phosphate, influence several processes, including lipid metabolism, gluconeogenesis, niacin formation, nucleic acid metabolism and hormone modulation[25].

Vitamin B_6 is occurring in greatest amount in meats, whole-grain products (especially wheat), vegetables and nuts.

Although a frank deficiency of vitamin B_6 is uncommon, marginal deficiencies are more likely. This is probably associated with a deficiency of other water soluble vitamins. It may be more adverse if there is also deficiency of riboflavin because riboflavin is involved in the metabolism of vitamin B_6. The signs of deficiency are stomatitis, glossitis, cheilosis and depression[25].

Allwood M.C.[11] 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[22].

2.6.1.5 Folic Acid

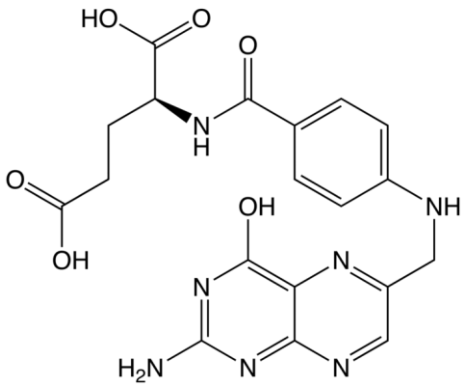


Figure 2.6: Chemical Structure of Folic Acid

Folate is the generic descriptor for 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. The pteridine nucleus is composed of two rings, pyrazine and pyrimidine[4].

Dietary folates are absorbed as folic acid, 5-methyl-tetrahydrofolate and 5-formyl-tetrahydrofolate. Tetrahydrofolic acid (FH_4), active coenzyme, is the product of reduction of folic acid.

Liver, mushrooms and green leafy vegetables are rich sources of folate.

Deficiencies of folate result in impaired biosynthesis of DNA and RNA, and thus in reduced cell division. This is manifested clinically as anemia, dermatologic lesions and poor growth. Anemic individuals show weakness, headache, palpitations and difficulty in concentrating. Folate deficiency also affects the intestinal epithelium. This is manifested clinically as malabsorption or diarrhea[21].

Most folates are easily oxidized and, therefore, are unstable to oxidation under aerobic conditions of storage. Under such conditions FH_4 derivatives can readily be oxidized to the corresponding derivatives of dihydrofolic acid (FH_2) or folic acid.

Folic acid 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[22].

2.6.1.6 Vitamin B_{12}

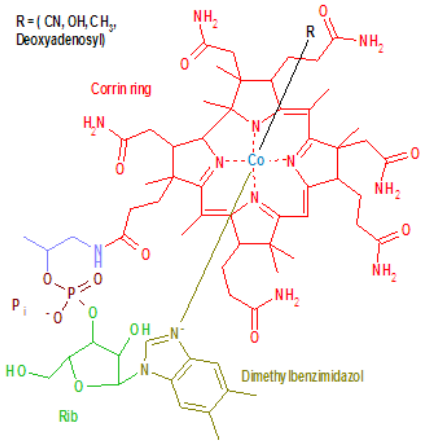


Figure 2.7: Chemical Structure of Cyanocobalamin

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)[21].

It is required by all DNA-synthesizing cells, including those of the hematopoietic and nervous system, and to facilitate the cyclic metabolism of folic acid. It transfers a methyl group from methylfolate, converting homocysteine to methionine[26]. There have been discovered three vitamin B_{12}–dependent enzymes in animals: methylmalonyl-CoA mutase, leucine mutase and methionine synthetase.

Vitamin B_{12} is synthesized by bacteria. It is found in the tissues of animals, which require the vitamin for growth. This vitamin is seldom found in foods derived form plants and is found only in foods that have been bacterially fermented and those

derived from the tissues of animals that have obtained it from their intestinal microflora.

Vitamin B_{12} deficiency in human is characterized by megaloblastic anemia and, after prolonged periods, neurological signs. Those signs include peripheral neuropathy. This is characterized by dementia, memory loss and numbness of the hands.

Vitamin B_{12} is very stable in both crystalline form and aqueous solution. However, the cobalamins are unstable to light[21].

2.6.1.7 Pantothenic Acid

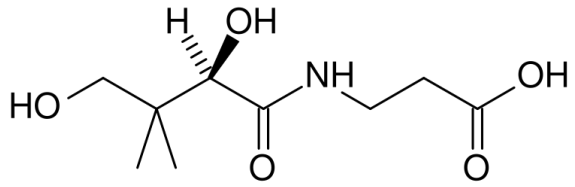


Figure 2.8: Chemical Structure of Pantothenic acid

Pantothenic acid is widely distributed in foods. Therefore the problems with its deficiency are rare. It is converted in the body to CoA, the form in which it accomplishes most of its biological functions. Pantothenic acid plays the essential roles in the synthesis and acetylation of small molecules and in respiratory metabolism. Pantothenate is also required for the modification of numerous proteins with acetyl and fatty acyl groups[27].

The most important food sources of pantothenic acid are meats, broccoli, mushrooms and yeast.

Pantothenic acid is yellow, viscous oil. Its calcium and other salts, however, are colorless crystalline substances. Each form is soluble in water and ethanol. Aqueous solutions of pantothenic acid are unstable to heating under acidic or alkaline conditions[21].

2.6.1.8 Biotin

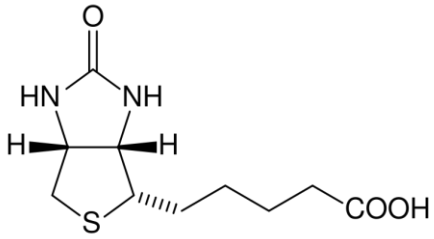


Figure 2.9: Chemical Structure of Biotin

Biotin is formerly known as vitamin H. This is widely distributed in food, but in very low concentrations. The most important natural sources of the vitamin are liver, milk and eggs. Because biotin is widespread among foods and is synthesized by the intestinal microflora, deficiencies are rare[21].

Biotin-dependent enzymes are involved in gluconeogenesis, biosynthesis of fatty acids, propionate metabolism and catabolism of leucine[4]. Biotin is unstable to oxidizing conditions and, therefore, is destroyed by heat, especially under conditions that support lipid peroxidation[21].

2.6.1.9 Ascorbic Acid (Vitamin C)

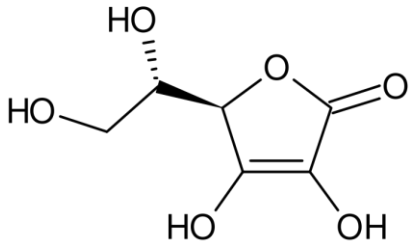


Figure 2.10: Chemical Structure of Ascorbic Acid

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

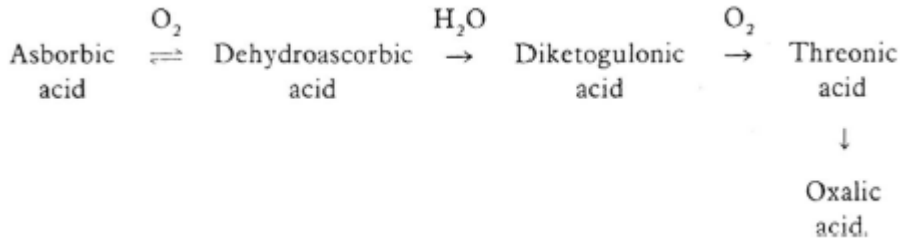
semidehydroascorbic acid. The three forms (AA, semidehydroascorbic acid, dehydroascorbic acid) compose a reversible redox system. It is therefore an effective quencher of free radicals such as singlet oxygen. It reduces ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron and the superoxide radical ($O_2^{\cdot -}$) to H_2O_2 and is oxidized to monodehydroascorbic acid in the process[21].

Vitamin C is required by only a few species which cannot synthesize it (human body). For most species, AA is a normal metabolite of glucose and is important for several physiological functions. Many of these functions involve redox characteristics that allow AA to play an important role in the antioxidant protection of cells.

Vitamin C is widely distributed in food. Fruits, vegetables, liver and kidney are generally the best sources. For practical reasons, citrus and others fruits are good daily sources of vitamin C. Its acute deficiency results in scurvy in individuals unable to synthesize it. Signs of the disease occur primarily in mesenchymal tissues. Scorbutic individuals may show swollen, bleeding gums with tooth loss, impaired wound healing, and weakening of collagenous structures in bone[21].

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[11]. Allwood M.C.[11] 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 and stage degradation product. The potential toxicity of this reaction may be significant[11]. 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[28].

2.6.2 Fat-soluble Vitamins

2.6.2.1 Vitamin A

Vitamin A is the generic descriptor for compounds with the qualitative biological activity of retinol (Figure 2.12). They are called retinoids. 1 international unit (IU) vitamin A activity is equivalent to 0.3 μg all-trans-retinol. Some compounds of the class of plant pigments called carotenoids and also have vitamin A activity. These are called provitamin A carotenoids and include β-carotene[21].

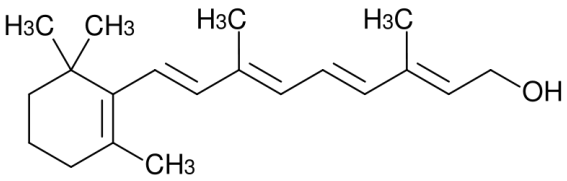


Figure 2.12: Chemical Structure of Retinol

Vitamin A is usually used in the form of esters such as the acetate, propionate and palmitate[4].

Vitamin A can support the maintenance of healthy epithelial cells differentiation, normal reproductive performance, and visual function. Vitamin A appears to have a role in maintaining the normal health of the skin. Its deficiency causes the skin to be thick, dry and scaly. Several carotenoids have been shown to have direct antioxidant activities. These include β-carotene, lycopene and lutein[21].

Deficiency of vitamin A can lead to night blindness and condition called xerophthalmia, or dryness of the eye[4].

Retinol is the most light-sensitive of the vitamins[22]. Retinol is known to be rapidly broken down by exposure to UV light. Therefore, daylight causes rapid degradation[11]. 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[22]. The presence of amino acids and fat emulsion in the bag affords considerable protection[11].

2.6.2.2 Vitamin D

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. Tissue cholecalciferol concentrations are dependent on the vitamin D content of the diet and the exposure to sunlight. Vitamin D is formed in animals by the action of UV light on 7-dehydrocholesterol in the skin. The activation reaction depends on the absorption of UV light by the steroid B ring of the sterol nucleus, and involves the opening of that ring in the formation of provitamin D_3 [21].

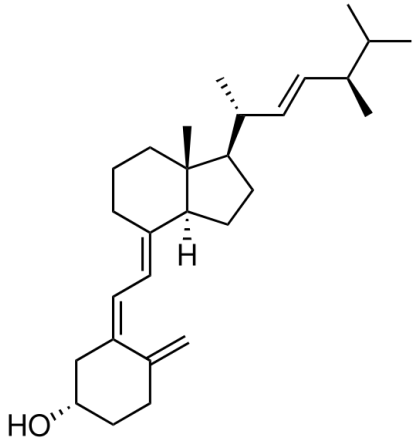


Figure 2.13: Chemical Structure of Ergocalciferol

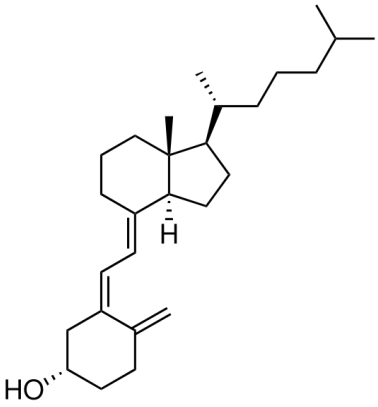


Figure 2.14: Chemical Structure of Cholecalciferol

The rich sources of vitamin D_3 are fish liver and oil.

Vitamin D functions as a steroid hormone. Its physiological role is the maintenance of calcium and phosphate homeostasis, impairment of which produces the lesions in bone called rickets and osteomalacia[21].

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

2.6.2.3 Vitamin E

Vitamin E is the generic descriptor for all tocoretinol derivates that exhibit qualitatively the biological activity of α-tocopherol (Figure 2.15). Synthetic preparations of vitamin E presently available are mixtures of all eight possible stereoisomers. The acetate esters are used in medicine and animal feeding, whereas the unesterified forms are used as antioxidants in foods and pharmaceuticals. Other forms are also used in multivitamin preparations.

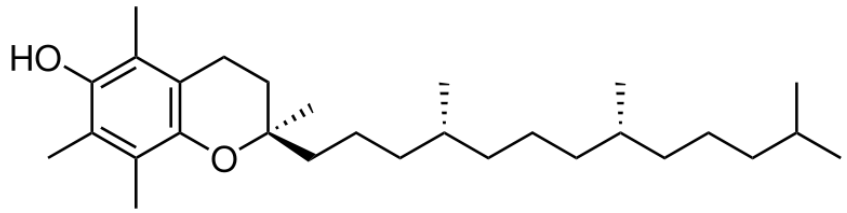


Figure 2.15: Chemical Structure of α-tocopherol

Vitamin E has a role in the normal metabolism of all cells. It serves as an antioxidant and its function involves the reduction of free radicals[21]. Because of its antioxidative properties, vitamin E is believed to prevent diseases that are associated with oxidative stress such as cardiovascular disease, cancer and neurologic disorders[4].

Tocopherol appears to be relatively stable in PN mixtures[22]. But Gillis[29] 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[30].

2.6.2.4 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 (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)[21].

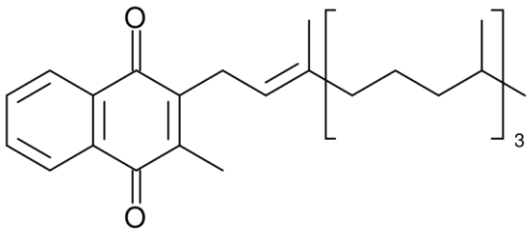


Figure 2.16: Chemical Structure of Phylloquinone

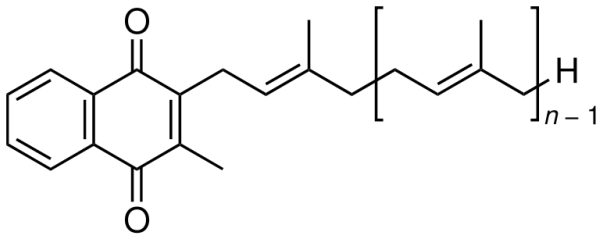


Figure 2.17: Chemical Structure of Menaquinone

Vitamin K is used by bacteria and plants, which can synthesize it, for electron transport and energy production. Animals cannot synthesize the vitamin, but require it for blood clotting and bone formation. Vitamin K reduces risks to postsurgical thrombosis and cardiac patients. Warfarin and dicumarol and other inhibitors of the vitamin K oxidation/carboxylation/reduction cycle are valuable in this purpose. Vitamin K has clear roles in the metabolism of calcified tissues.

There are two natural sources of vitamin K. Green plants synthesize the phylloquinones and bacteria (include those of the normal intestinal microflora) synthesize the manaquinones. Green leafy vegetables tend to be rich in vitamin K, whereas fruits and grains are poor sources[21].

Vitamin K deficiency is rare among humans. It is because of the significant microbial synthesis of the vitamin that occurs in the intestines. But neonates are at special risk of vitamin K deficiency for several reasons:

- Placental transport of the vitamin is poor
- The neonatal intestine is sterile for the first few days of life
- Human milk is an inadequate source of vitamin K
- Hepatic biosynthesis of the clotting factors is inadequate in the infant[21].

2.7 TRACE ELEMENTS (TE)

TE are an essential additive to PN mixtures. Currently considered necessary TE include copper, zinc, manganese, iron, iodine, fluorine, chromium, selenium and molybdenum. It is also recognized, that TE are present in variable quantities as contaminants[31]. For example zinc can be extracted from rubber stoppers used in glass bottles and from PVC additives[11]. Aluminium (AL) is a contaminant of several substances, such as calcium and phosphate salts and albumin[32].

Chromium, copper, iron, manganese, zinc and selenium are routinely added to TPN solutions to avoid the development of deficiency manifestations[32].

In healthy subjects, TE are present in the organism at very low levels. However, they are involved in a number of key metabolic functions. Some of the best known TE are considered as essential because of their role in maintaining important enzymatic and metabolic functions. Among them are copper, iron and zinc. Some of TE are enzymatic cofactors, vitamin cofactors, or hormone cofactors. Many act as antioxidants[2].

2.7.1 Zinc

Zinc (Zn) is a small ion (0.065nm) and has a concentrated 2+ charge (Zn^{2+}). It is a strong Lewis acid (electron acceptor).

Zinc has catalytic, structural and regulatory function in biology. Catalytic roles are found in enzymes from all six classes of enzymes. Examples are the RNA nucleotide transferases (RNA polymerases I, II, III), alkaline phosphatase and the carbonic anhydrases[33]. Approximately 100 enzymes require zinc for their catalytic activity, especially the enzymes of protein and nucleic acid synthesis. Zinc also permits the folding of other proteins by binding to cysteine and histidine residues, forming zinc fingers. These have roles in controlling gene transcription. Zinc is absorbed after digestion of macronutrients in the gut. Absorption may be markedly affected by ingestion of large amounts of other elements such as iron or cooper. Zinc deficiency is well characterized. Reduced growth in children is early sign. Severe zinc deficiency gives rise to alopecia, diarrhoea, skin rash especially on the face and impaired appetite[34].

2.7.2 Copper

Copper (Cu) is a transition metal widely distributed in nature. In solution, as well as in living organisms, cooper is found almost exclusively in the +2 and +1 valence states. At neutral pH in aqueous media copper ions form hydroxides that precipitate out of solution unless chelated by organic molecules[35].

Copper has primarily a catalytic role for certain metalloenzymes, which act as oxidases. For example, cytochrome C oxidase is especially important in energy metabolism. Copper is transported in plasma bound to caeruloplasmin, which participates in tissue iron release. Deficiency of cooper is rare, but it has been observed in TPN and causes normocytic hypochromic anaemia, neutropenia and skeletal disturbances[34].

2.7.3 Iron

Iron (Fe) is the fourth most common element on earth after oxygen, silicon and aluminium. In aqueous solution iron exists in two oxidation states, Fe^{2+}, the ferrous form, and Fe^{3+}, the ferric form[36].

In the adult human body, over half the iron is present in haemoglobin and in iron-binding proteins, such as myoglobin, transferrin, lactoferrin, ferritin, and hemosiderin. The remainder occurs in various enzymes. The main function of Fe in the body is oxygen transport within the blood and muscle[32].

Iron is stored in the liver and bone marrow in the form of ferritin[34]. Fe deficiency develops quite slowly, because the Fe stores in the body can suffice for up to one year. Those who develop true Fe deficiency anaemia due to blood losses from an ulcer, a malignant tumor, or during excessive menstrual flow, require Fe supplementation. In contrast, hospitalized patients who develop anaemia due to chronic inflammation or infections have low serum Fe, but adequate stores[32].

2.7.4 Selenium

Selenium (Se) plays a structural role as a constituent of selenoproteins, and enzymic role as part of many key antioxidant enzymes such as glutathione peroxidase, as selenocysteine[4].

Se deficiency may result in different clinical outcomes. A fatal cardiomyopathy due to Se deficiency, mainly in children, was described in the Keshan district of China, where Se levels are very low in the soil and in foods. Supplementation with sodium selenite corrected this problem. Similar cases of fatal cardiomyopathy have been reported in adult patients receiving long-term TPN without Se supplementation[32].

Absorption of Se from the diet is very efficient – much is in the form of selenomethionine or selenocysteine. Commercial selenium supplements in the form of selenite or selenate are well absorbed. It seems likely that selenium deficiency alone does not cause obvious illness[34].

2.7.5 Chromium

Trivalent chromium (Cr) is an essential TE for improved glucose tolerance, but hexavalent Cr has been shown to be a carcinogen. Normally, all circulating Cr is

trivalent[32]. Cr increases the action of insulin, possibly through amplifying insulin receptor tyrosine kinase activity. Cr is absorbed largely in the form of Cr^{3+} [34].

A very small number of cases of chromium deficiency have been observed during TPN. Subclinical deficiency may lead to impaired glucose tolerance in type 2 diabetes. Assessment of Cr status is extremely difficult due to very low plasma concentrations[34].

2.7.6 Molybdenum

Molybdenum (Mo) is a co-factor for several oxidiser enzymes, especially sulphite oxidase and xanthine oxidase[34].

A case of Mo deficiency was reported in an adult receiving long-term TPN, who developed intolerance to infused amino acids, elevated serum methionine, decreased serum uric acid, and low urinary excretion of sulphate. Supplementation of ammonium molybdate corrected these biochemical abnormalities. Mo has a low level of toxicity[32].

Assessment is rarely done. Plasma concentration is very low and difficult to measure [34].

2.7.7 Manganese

Manganese (Mn) is associated with many enzymes, such as Mn-superoxide dismutase (antioxidant protection) and pyruvate carboxylase (energy metabolism). Deficiency of Mn can impair the production of hyaluronic acid, chondroitin sulphate and other mucopolysaccharides needed for growth and maintenance of connective tissue, cartilage and bone. Mn deficiency may not be evident in some patients on TPN because Mn can be derived from the contaminants present in the parenteral fluids.

Mn is excreted through the bile. Patients with cholestatic liver disease should not be given supplements of this metal[32].

2.7.8 Other TE

Cobalt (Co) is required as a component of vitamin B_{12} and is provided as cyanocobalamin at a dosage of 1 μg/day for children in TPN preparations. Iodine is an integral part of the thyroid hormones, thyroxine and triiodothyronine. Deficiency of iodine leading to thyroid enlargement and goiter development are prevented in adults by a daily oral intake of approximately 150 μg iodine.

2.8. MICRONUTRIENT REQUIREMENTS

There is the difficulty in establishing recommendations for intakes of micronutrients in health and disease. The problem is not too difficult for those micronutrients (water-soluble vitamins) where there is a wide safety margin between effectiveness and toxicity. However, fat-soluble vitamins and for many TE (such as Se) there is a relatively narrow margin of safety and caution is required to prevent over-dosage. This is also true for nutrients where homeostasis achieved by controlling absorption from the gut[18].

Parenteral vitamins requirements differ from enteral nutrition requirements, where enteral are designed for healthy individuals to prevent deficiencies and to minimise the risk from nutrition-related chronic disease. Dosing guidelines for parenteral vitamins are approximates to the needs of patients with increased requirements in acute and chronic disease[4].

Table 2.1: Daily Parenteral Vitamin Requirements in Adults[18]

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

Table 2.2: Daily Parenteral TE Requirements in Adults[18]

Trace elements	**Adults parenteral requirements**
Zn	3.2 – 6.5 mg
Cu	0.3 – 1.3 mg
Se	30 – 60 µg
Mn	0.2 – 0.3 mg
Cr	10 – 20 µg
Mo	19 µg
Fe	1.2 mg
I	131 µg
F	0.95 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[4].

2.9 POTENTIAL INTERACTIONS OF COMBINED TPN MIXTURES IN BIG BAGS

Mixing TPN components, which include glucose, lipids, amino acids, electrolytes, vitamins and TE, provides a chemically unstable mixture with many possible reactions occurring[37]. These reactions include precipitation and degradation of particular compounds. Exposure to light may cause degradation while temperature changes can result in precipitation. pH changes can lead to significant loss of stability or to precipitation. 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[11].

2.9.1 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[11].

2.9.2 Electrolytes

The principal 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. It may be very high. 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[11].

2.9.3 Trace Elements (TE)

The addition of TE 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[2]. TE, in particular copper and iron, may interact with complete PN mixtures leading to precipitate formation. According to Allwood M.C.[22] 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 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[2]. Harraki[2] reported that the absorption of Se was much decreased when sodium selenite and ascorbic acid were provided orally together.

2.9.4 Vitamin Stability

Vitamins are generally 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[22]. Stability of particular vitamins was discussed in detail under Vitamins (see 2.6).

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 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
Kabiven® Peripheral Bag 900 kcal/5gN, Emulsion for infusion, 1440 ml	Fresenius Kabi Limited, Runcorn, Cheshire, U.K.	11/2008
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
Additrace®, Concentrate for solution for infusion, 10 ml	Fresenius Kabi Limited, Runcorn, Cheshire, U.K.	01/2009

Kabiven® Peripheral 900 kcal/5gN

Emulsion for infusion, 1440 ml

1. Glucose 11% 885 ml contain: Glucose anhydrous 97 g, Water for injections to 885 ml

2. Vamin® 18 300ml contain: Alanine 4.8 g, Arginine 3.4 g, Aspartic acid 1.0 g, Glutamic acid 1.7 g, Glycine 2.4 g, Histidine 2.0 g, Isoleucine 1.7 g, Leucine 2.4 g, Lysine 2.7 g, Methionine 1.7 g, Phenylalanine 2.4 g, Proline 2.0 g, Serine 1.4 g, Threonine 1.7 g, Tryptophan 0.57 g, Tyrosine 0.07 g, Valine 2.2 g, Calcium chloride 0.22 g, Sodium glycerophosphate anhydrous 1.5 g, Magnesium sulphate 0.48 g, Potassium chloride 1.8 g, Sodium acetate 1.5 g, Water for injections to 300ml. Glacial acetic acid q.s. to pH approx. 5.6

3. Intralipid® 20% 255 ml contain: Purified soybean oil 51 g, Purified egg phospholipids 3.1 g, Glycerol 5.6 g, Water for injections to 255 ml, Sodiun hydroxide q.s. to pH approx. 8

Electrolytes in total: Sodium 32 mmol, Potassium 24 mmol, Magnesium 4.0 mmol, Calcium 2.0 mmol, Phosphate 11 mmol, Sulphate 4.0 mmol, Chloride 47 mmol, Acetate 39 mmol

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

Additrace®

Concentrate for Solution for Infusion

10.0 ml contains:

Active ingredients: Ferric chloride 20 micromol, Zinc chloride 100 micromol, Manganese chloride 5 micromol, Copper chloride 20 micromol, Chromic chloride 0.2 micromol, Sodium selenite 0.4 micromol, Sodium molybdate 0.2 micromol, Sodium fluoride 50 micromol, Potassium iodide 1 micromol.

Inactive ingredients: Xylitol 3 g, Hydrochloric acid q.s., Water for injections to 10 ml.

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

Table 3.3: Sterile Equipment

Sterile equipment	Supplier
BD Microlance™ 3 Needles 19G, 21G and 23G	Becton Dickinson
BD Plastipak™ Syringes 1-20 ml	Becton Dictinson

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 Preparation of the Kabiven® Peripheral Bag Samples

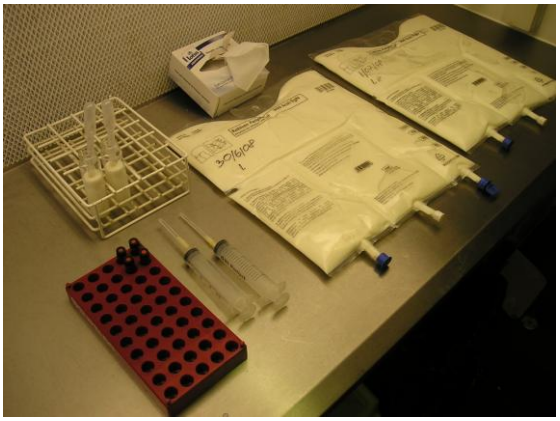
Two different mixtures were prepared by mixing a reconstituted Kabiven® Peripheral Bag 900 kcal/5gN firstly with Additrace®, Vitlipid® N and Solvitio® N, and secondly with Vitlipid® N and Solvitio® N without Additrace®. Manipulation with each bag was carried out in the laminar flow workstation, which was cleaned by 70% isopropyl alcohol. It provides a protected work area without microbial contamination.

3.3.1.1 Bags with Additrace® Concentrate (Bags A)

10 ml of Additrace® concentrate was syringed into a reconstituted bag via a 10ml syringe. Then another 10ml syringe was filled with 10 ml of Vitlipid® N emulsion, and one vial of Solvitio® N was reconstituted by this emulsion. This mixture was altogether put into the same syringe and added to the bag. The volume of the bag was carefully mixed up. In all, three bags were prepared this way. From each bag, 20 ml of the mixture was taken away into the Hach tubes (25 ml) for chemical and physical assays at time zero. A 1ml sample was taken from this tube via a plastic pipette into autosampler vial for HPLC assay. Two vials were prepared this way. The rest of the sample in Hach tube served for physical tests. Bags were stored in the refrigerator until other stability tests were carried out at a different time.

Figure 3.1: Hach Tube

Figure 3.2: Kabiven® Peripheral Bags A and B



3.3.1.2 Bags without Additrace® Concentrate (Bags B)

A mixture of the Vitlipid® N and Solvitio® N was syringed into a reconstituted bag via a 10ml syringe. Preparation of this mixture was the same as in previous paragraph. Additrace® concentrate was not added. There were three bags prepared. From each bag 20 ml of the mixture was taken away and put into the Hach tubes (25 ml) for chemical and physical assays at time zero. A 1ml sample was taken from this tube via a plastic pipette into autosampler vial for HPLC assay. Two vials were prepared this way. The rest of the sample in Hach tube served for physical tests. Bags were stored in the refrigerator until another stability tests were carried out at a different time.

Figure 3.3: Additrace®, Vitlipid® N, Solvitio® N

3.3.1.3 The Sampling Technique of the Kabiven® Peripheral Bags

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

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

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.

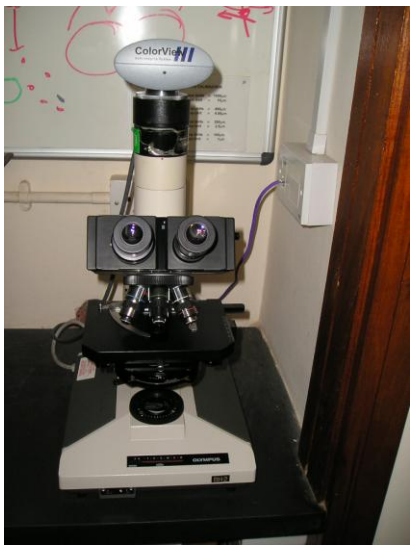
3.5 INSTRUMENTS USED FOR PHYSICAL TESTS

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 3.4: Olympus® BH-2



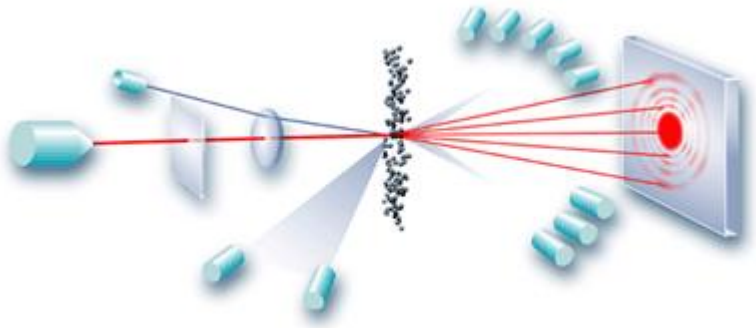
One drop of the emulsion was placed in the centre of a Thoma-welled counting chamber slide of a height of 0.2 mm, and gridlines of a total of 256 squares. A 1ml syringe was used and the sample was then covered with a glass cover slip. It is important to avoid creating air bubbles, which may cause a problem,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

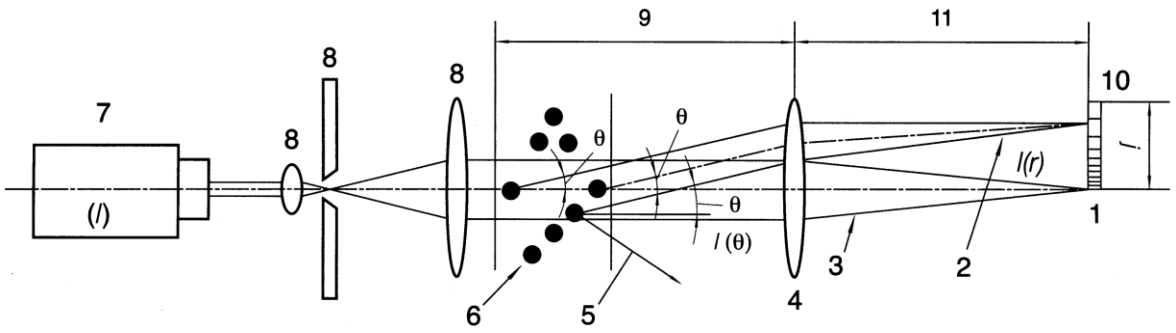
The Malvern Mastersizer® particle size analyzer uses the laser diffraction technique which is based on the analysis of the diffraction pattern produced when particles are exposed to a beam of monochromatic light.

Figure 3.5: 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 3.6: 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)

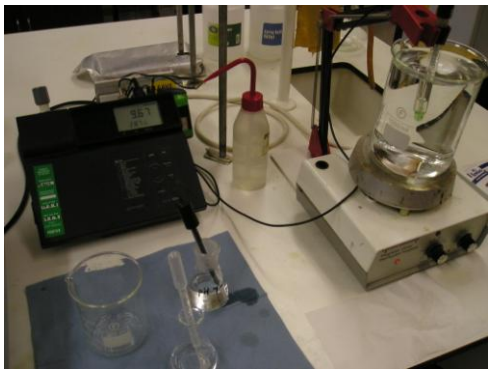
The particles can enter the laser beam in 2 positions. In the conventional case the particles enter the parallel beam before the collecting lens and within its working distance. In so-called reversed Fourier optics the particles enter behind the collecting lens and thus, in a converging beam. The advantage 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[38].

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 Kabiven® Peripheral Bag emulsions required to add about 15 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[39]. 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 3.7: 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. 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[40].

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.

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[41]:

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

Figure 3.8: 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 ultrafiltered air and allows a clean air work environment[42].

Throughout this project, all manipulation with the Kabiven® Bags and preparation of the samples was performed in the laminar flow workstation. It provides aseptic work and it is necessary for avoidance of bacterial contamination.

Figure 3.9: Laminar Flow Workstation

3.6 CHEMICAL ASSAYS BY HPLC

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[43]. HPLC is becoming a standard method in vitamin assay, especially for routine work[44].

3.6.1 HPLC Instrument System Used for Kabiven® Bags 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 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 Preparation of Phosphate Buffer Used for Kabiven® Bags 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. This solution was put into a 1000ml volumetric flask and filled with ultrafiltered deionised water. Amount of weighed $K_2HPO_4.3H_2O$ (formula mass FM= 228.22) was counted via the following figure: 0.05M × 228.22 = 11.41 g (± 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 ± 0.05 units by using orthophosphoric acid. 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 KABIVEN® PERIPHERAL BAGS

Firstly it was necessary to develop an optimal method for vitamin assay by HPLC. The initial step was reading the PhD. thesis of Said[42] paragraph 2.3: Chemical Assays by HPLC, whose project was similar to the one discussed in this diploma thesis. Previously published literature about vitamin assays by HPLC was consulted as well.

A similar column and HPLC system, like Said[42] used, was employed. Ultraviolet (UV) detection for the vitamins was recommended. The maximum absorbance for the water-soluble vitamins was found to be in ranges of 200-220 nm and 250-280 nm. The wavelength between 250-280 nm was preferred because most interference will absorb much less above 240 nm. Therefore, the methods that used UV detection between 250 - 280 nm were carried out[42].

The separation method from Said[42] thesis was adapted for the water-soluble vitamins, which used a C18 column on gradient run, UV detection 265 nm and using phosphate buffer 0.05M pH 7 and methanol as the mobile phase.

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[42]. According to Said[42] 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.

Following sections describe optimization of method for determination of vitamins in mixture Kabiven® Peripheral Bag with Solvitio® N solution, using given method for stability tests of tested samples, and HPLC instrument system.

4.1.1 Determination of Optimal Method for Water-soluble Vitamins

For finding the optimal separating method, it was essential to change retention time of vitamins, because they were covered mono-acid peaks in the mixture of Bag with Solvitio®-Vitlipid® emulsion. Four samples were prepared. First was the clear reconstituted Kabiven® Peripheral Bag sample, according to which we identified mono-acid peaks. Second sample was Solvitio® N vial reconstituted with 10 ml of distilled water, and syringed into Kabiven® Bag. Another sample analysed was Solvitio® N solution, prepared from Solvitio® N vial reconstituted with 10 ml of distilled water and diluted 10 times with distilled water into 100ml volumetric flask. This sample served as standard for determination of vitamin peaks and evaluation of their retention times. The last sample was prepared from 10 time diluted Solvitio® N solution. 330 µl of this solution was put into 10ml volumetric flask and filled with distilled water to 10ml volume. Several different methods with various gradient runs were investigated. Different types of columns were employed. For a couple of samples acetonitrile with phosphate buffer pH 7 was used as a mobile phase. However this mobile phase seemed to be strong and moving the peaks was not optimal. When the optimal method was developed, Kabiven® Peripheral Bag samples could be analysed.

4.1.2 HPLC Analysis for Water-soluble Vitamins Assay

Solvitio® N Adult is an injectable multivitamin, in the form of a yellow powder for reconstitution[42].

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 4.1: 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

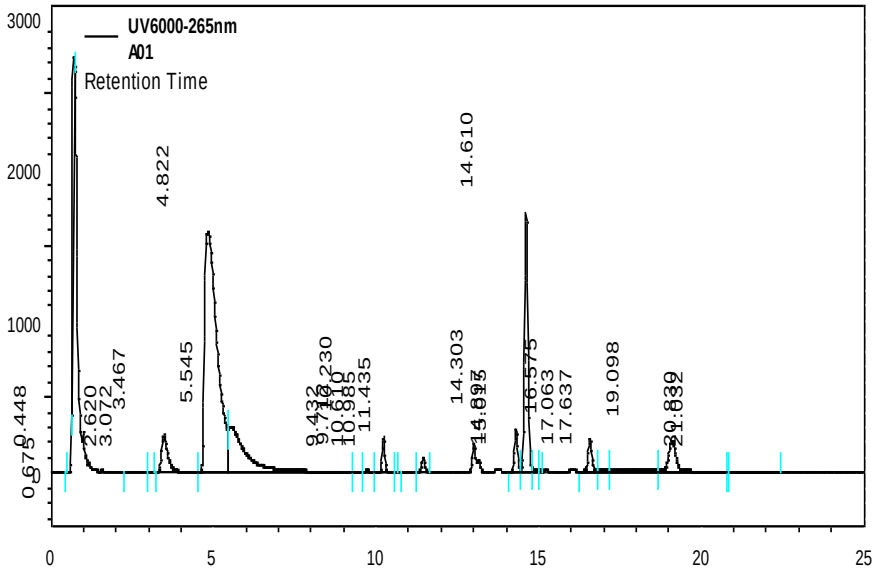
20 μl sample was injected via autosampler. The flow rate was set 1 ml/min with UV detection 265 nm.

4.1.3 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 Said's[42] results and compared to chromatograms of Solvitio® N solution in her thesis. Resulting retention times of Said's[42] chromatogram peaks slightly differ from the ones recorded in this research. This is because a different gradient run was used. The chromatogram peaks with the approximate retention times identified in Said [42] assays were:

Ascorbic acid rt. 0.76 minutes
Pyridoxine hydrochloride rt. 3.70 minutes
Nicotinamide rt. 4.80 minutes
Thiamine hydrochloride rt. 6.30 minutes
Folic acid rt. 11.10 minutes
Riboflavin sodium phosphate rt. 12.40; 14.90; 16.90; 17.30; 20.50 minutes
Pantothenic acid rt. 22.47 minutes

Figure 4.1: Chromatogram of Solvitio® N Standard Solution



The chromatogram peaks with retention times, identified using my method were:

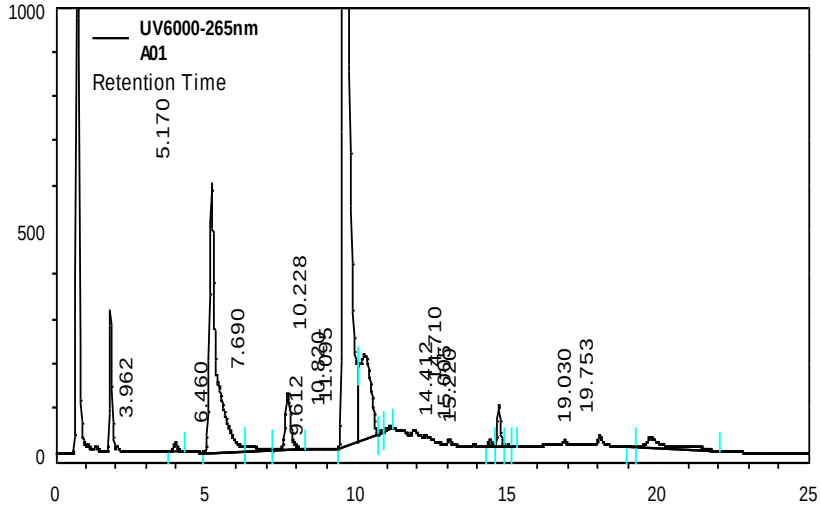
Ascorbic acid	rt. 0.67 minutes
Pyridoxine hydrochloride	rt. 3.46 minutes
Nicotinamide	rt. 4.82 minutes
Thiamine hydrochloride	rt. 5.54 minutes
Folic acid	rt. 10.23 minutes
Riboflavin sodium phosphate	rt. 14.61 minutes
Pantothenic acid	rt. 19.09 minutes

Five peaks for riboflavin sodium phosphate were identified according to Said's[42] 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[42] 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. Another degraded product peaks were detected after 20 minutes, therefore making this assay unsuitable for the analysis of pantothenic acid.

Five vitamins were found to be suitable for assay by this method, which are pyridoxine, nicotinamide, thiamine, folic acid and riboflavin sodium phosphate[42]. On the other hand, only two water-soluble vitamins were possible to analyse at different times in the Kabiven® Bags, which are pyridoxine and riboflavin sodium phosphate (Figure 4.2). It was not possible to analyse other water-soluble vitamins, because they were covered by mono-acids peaks. It was not possible to shift every vitamin peak from mono-acid peaks.

Figure 4.2: Chromatogram of Kabiven® Bag with Vitamins - Detection of Water-soluble Vitamins. Separation of Pyridoxine (rt. 3.96 minutes), and Riboflavin Sodium Phosphate (rt. 15.22 minutes)



4.1.3.1 Results for Water-soluble Vitamins in Kabiven® Bags A

Amount of vitamin B6 was not less than 90 % during the storage period. Around 91 % of the vitamin left after 7 days storage in the refrigerator followed two days storage at ambient temperature. After 14 days storage almost 95 % of the vitamin left and at the day 29+1 there was over 97 % of the vitamin (see Table 4.2). Amount of vitamin B2 was not less than 92 % of it's initial amount. There were detected also values over 100 % of the initial amount of the vitamins. It might be caused by measure error. Degradation of these vitamins is not rapid and their losses are insignificant under assayed storage conditions. All results are expressed via graphs (see Figures 4.3, 4.4 and 4.5) and Table 4.2.

Table 4.2: Peak Area and RSD of WSV in Bags A throughout the Investigation

vitamin	**B6**		**B2**	
day	**peak area (%)**	**RSD**	**peak area (%)**	**RSD**
0	100	0,54	100	0,60
7	91,3	1,48	102,9	0,60
7+1	90,5	0,23	96,5	2,20
7+2	91,5	2,02	103,4	2,58
	B6		**B2**	
day	**peak area (%)**	**RSD**	**peak area (%)**	**RSD**
0	100	1,29	100	0,68
14	94,5	0,71	94,8	11,3
14+1	101,8	0,07	134,7	5,32
14+2	106,4	1,05	99,8	5,45
	B6		**B2**	
day	**peak area (%)**	**RSD**	**peak area (%)**	**RSD**
0	100	6,71	100	13,5
29	100,9	3,79	92,7	4,16
29+1	97,8	1,59	95,9	5,82
29+2	111,2	0,59	93,5	5,19

Figure 4.3: Peak Area for WSV in Kabiven® Bag A after 7 Days Storage in the Fridge

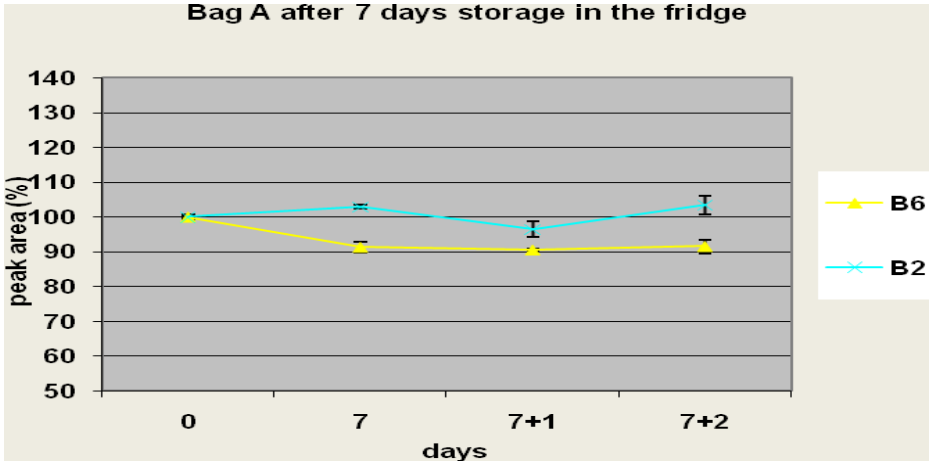


Figure 4.4: Peak Area for WSV in Kabiven® Bag A after 14 Days Storage in the Fridge

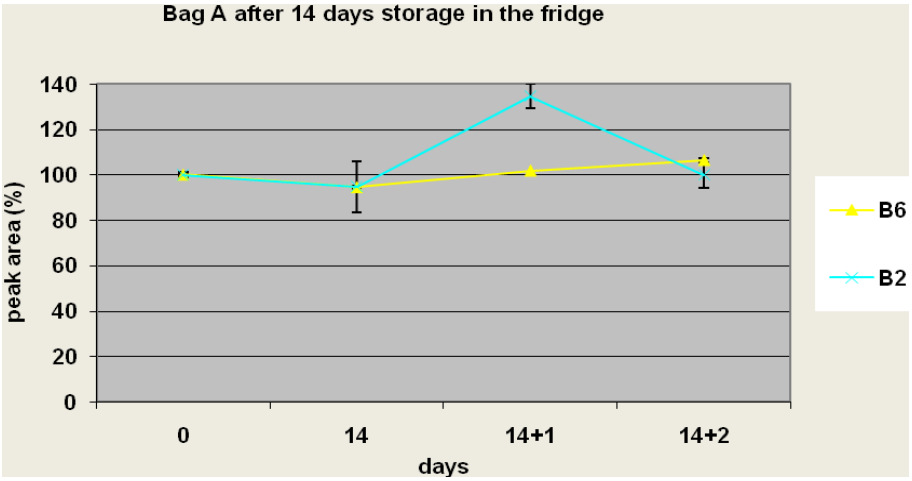
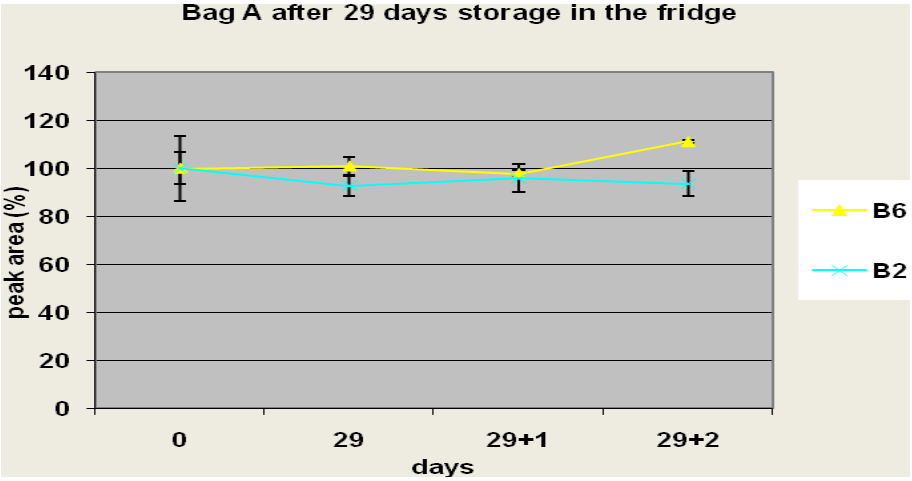


Figure 4.5: Peak Area for WSV in Kabiven® Bag A after 29 Days Storage in the Fridge



4.1.3.2 Results for Water-soluble Vitamins in Kabiven® Bags B

Amount of vitamin B6 was not less than 93 % of its initial amount during storage period. Losses of this vitamin are insignificant when the bags are light-protected. On the other hand losses of vitamin B2 were more significant than vitamin B6 losses. Only 81 % of the vitamin left after 14 days storage in the refrigerator (see table 4.3). The results having values over 100 % of their initial amount are probably influenced by measure error. Some results may be influenced by changing ambient temperature during stability studies, exactly the sensitivity of the detector may be influenced by big temperature difference between single assays. All results are expressed in the graphs (see Figures 4.6, 4.7 and 4.8) and in the Table 4.3.

Table 4.3: Peak Area and RSD of WSV in Bags B throughout the Investigation

	B6		B2	
day	**peak area (%)**	**RSD**	**peak area (%)**	**RSD**
0	100	0,36	100	1,64
7	93,4	0,11	97,9	1,38
7+1	93,3	0,43	93,8	0,93
7+2				
	B6		**B2**	
day	**peak area (%)**	**RSD**	**peak area (%)**	**RSD**
0	100	2,97	100	2,43
14	96,5	0,27	81,9	2,05
14+1	95,6	1,64	145,3	2,03
14+2	97,8	1,28	88,4	1,24
	B6		**B2**	
day	**peak area (%)**	**RSD**	**peak area (%)**	**RSD**
0	100	3,90	100	2,88
29	93,2	0,88	98,7	1,09
29+1	98,2	0,08	102,7	2,56
29+2	98,2	0,29	103,0	4,26

Figure 4.6: Peak Area for WSV in Kabiven® Bag B after 7 Days Storage in the Fridge

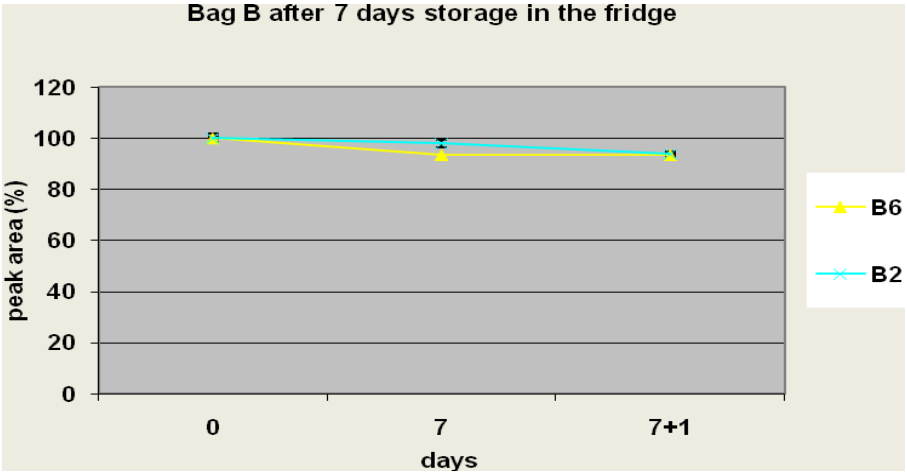


Figure 4.7: Peak Area for WSV in Kabiven® Bag B after 14 Days Storage in the Fridge

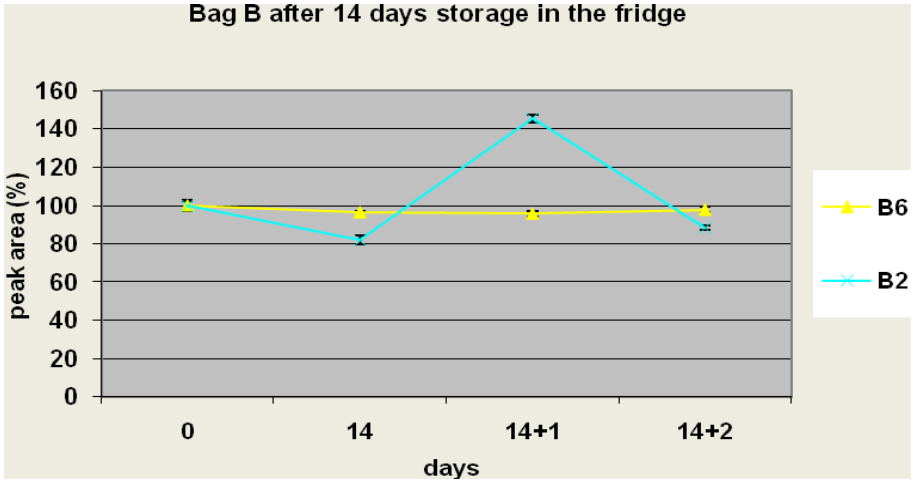
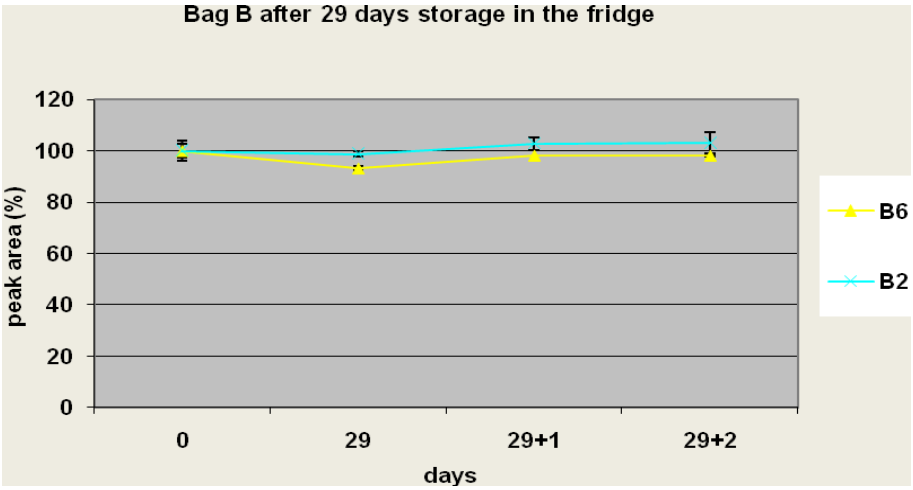


Figure 4.8: Peak Area for WSV in Kabiven® Bag B after 29 Days Storage in the Fridge



4.1.3.3 Discussion for Water-soluble Vitamins in Kabiven® Bags

Stability of vitamins is discussed in previous paragraph 2.6 Vitamins. Riboflavin is degraded rapidly when exposed to daylight[11]. Previous HPLC results have shown that degradation of riboflavin is not rapid when the bags are light protected. Commencing infusion in the evening makes sure us that the patient receives greater amounts of this vitamin. The results also have shown that degradation of riboflavin was not influenced by presence of trace elements, because there was not any bigger

difference between values in Kabiven® Bags A and B. Pyridoxine is also light-sensitive also degradation is far less than is observed with riboflavin[11]. Almost no losses of pyridoxine were detected in light-protected bags throughout the investigation. There was not any influence of TE to pyridoxine stability in Kabiven® Bags as the results showed. Therefore both discussed vitamins should be deliver and stored light-protected and then they should be stable and their losses should be only very small.

4.1.4 HPLC Analysis for Fat-soluble Vitamins Assay

A reversed phase HPLC analysis using two different UV detections in a single run was employed. The mobile phase was methanol HPLC grade, set at a flow rate of 1.5 ml/min. 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[42].

4.1.5 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[42] 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 4.9) and at 8.53 minutes for vitamin A (Figure 4.10). Peaks for vitamin K and vitamin D were observed too, but according to Said[42] results these two vitamins are unsuitable for analyses 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[42].

Figure 4.9: Chromatogram of Vitlipid® N Injection - Detection of Vitamin E (rt. 2.65 minutes)

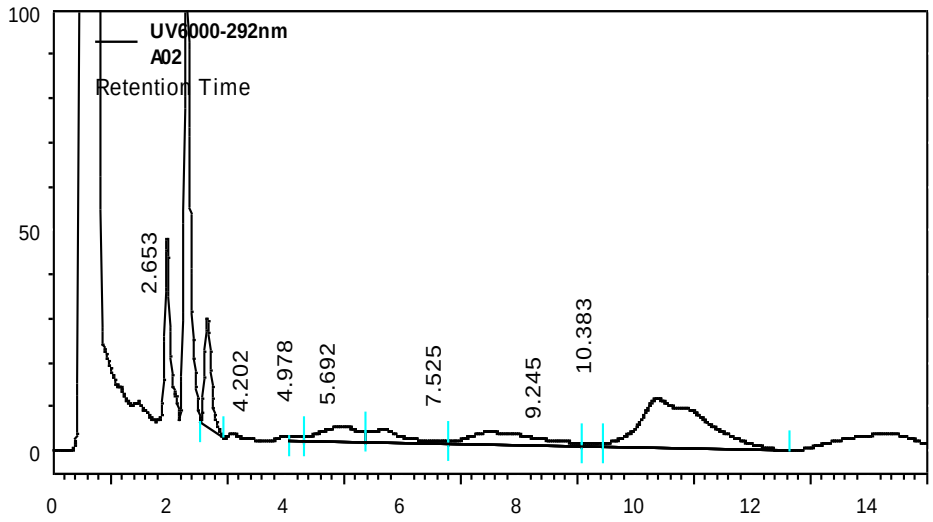


Figure 4.10: Chromatogram of Vitlipid® N Injection- Detection of Vitamin A (rt. 8.53 minutes)

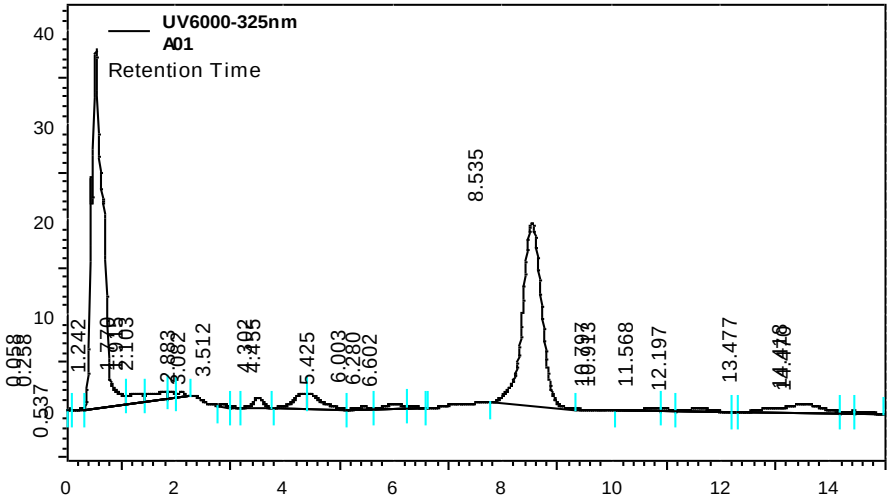


Figure 4.11: Chromatogram of Kabiven® Bag with Vitamins- Detection of Vitamin E (rt. 2.35 minutes)

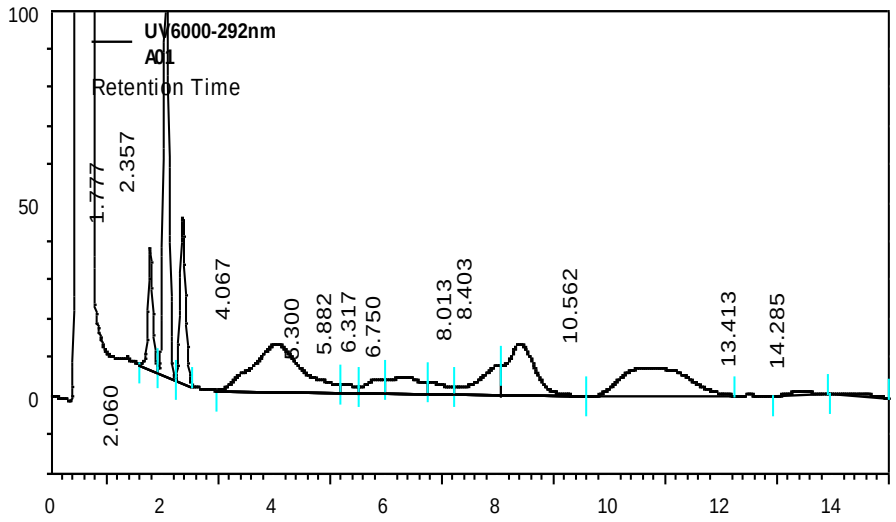
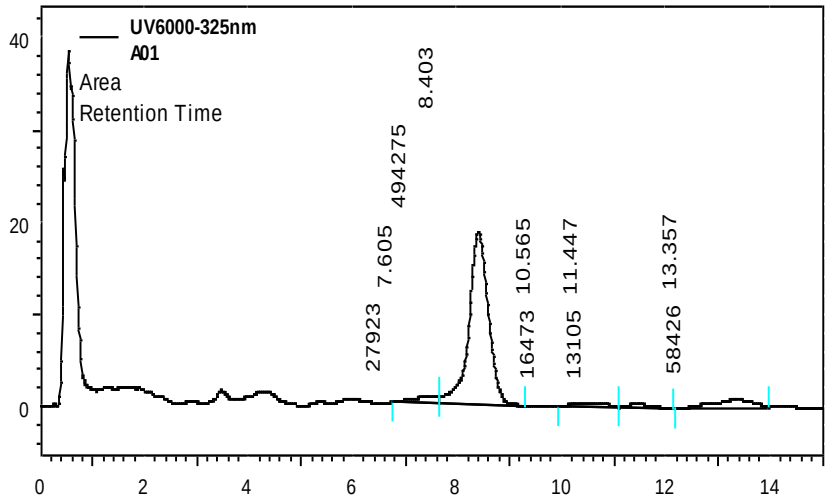


Figure 4.12: Chromatogram of Kabiven® Bag with Vitamins- Detection of Vitamin A (rt. 8.40 minutes)



4.1.5.1 Results of HPLC for Fat-soluble Vitamins in Kabiven® Bags A

Amount of vitamin A was found about 65% of its initial amount throughout storage period. Only 65 % left after 29 days storage in the refrigerator followed two days storage at ambient temperature. Losses after storage in the refrigerator were smaller than losses after storage at ambient temperature (see Table 4.4). Some values were over 100 %, it was probably caused by measure error. Degradation of vitamin A was biggest after 29 days storage in the fridge followed two days storage at ambient temperature. Degradation of Vitamin E was massive, especially when the bags were stored at ambient temperature. If they were stored in the refrigerator, the losses were not so significant (see table 4.4). All results are expressed in the following graphs (see Figures 4.13, 4.14 and 4.15) and in the Table 4.4.

Table 4.4: Peak Area and RSD of FSV in Bags A throughout the Investigation

vitamin	**A**		**E**	
day	**peak area (%)**	**RSD**	**peak area (%)**	**RSD**
0	100	7,82	100	1,94
7	116,18	0,77	108,18	10,7
7+1	98,18	13,6	94,45	3,13
7+2	96,10	6,99	46,84	22,6
	A		**E**	
day	**peak area (%)**	**RSD**	**peak area (%)**	**RSD**
0	100	7,74	100	0,43
14	109,86	6,46	101,29	3,24
14+1	94,76	1,82	84,90	6,80
14+2	91,30	15,9	72,17	2,85
	A		**E**	
day	**peak area (%)**	**RSD**	**peak area (%)**	**RSD**
0	100	4,63	100	2,86
29	84,49	1,56	81,75	4,16
29+1	81,19	3,14	56,78	7,58
29+2	65,45	1,90	27,22	4,93

Figure 4.13: Peak Area for FSV in Kabiven® Bag A after 7 Days Storage in the Fridge

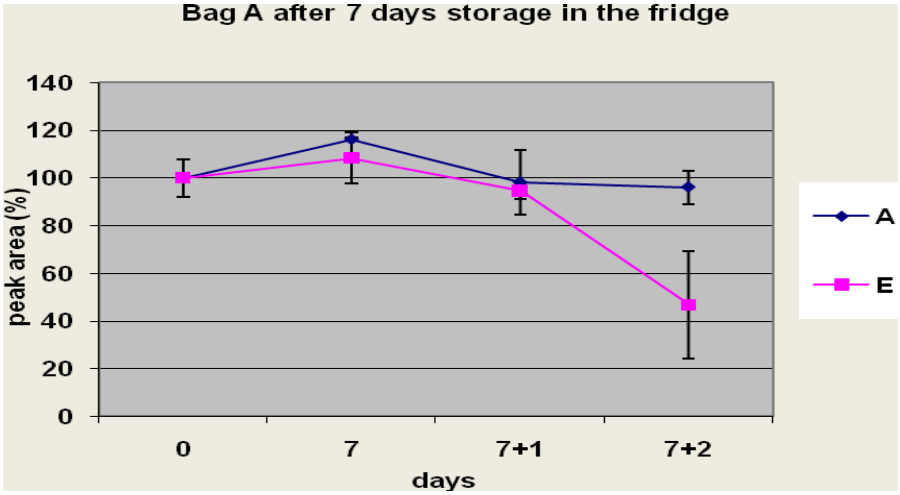


Figure 4.14: Peak Area for FSV in Kabiven® Bag A after 14 Days Storage in the Fridge

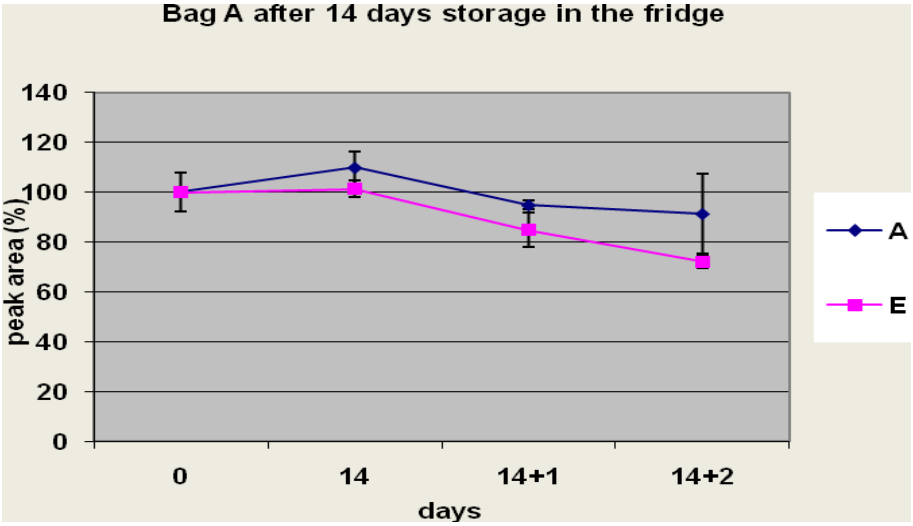
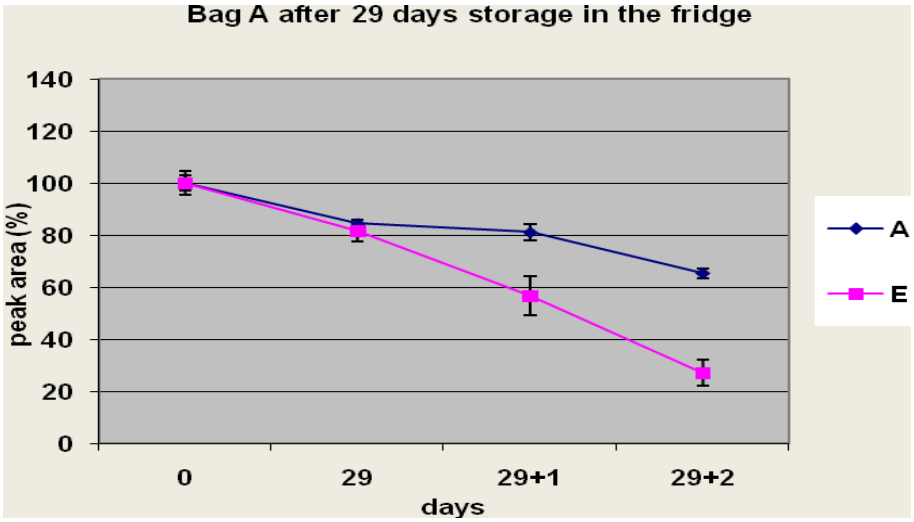


Figure 4.15: Peak Area for FSV in Kabiven® Bag A after 29 Days Storage in the Fridge



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4.1.5.2 Results of HPLC for Fat-soluble Vitamins in Kabiven® Bags B

Amount of vitamin A was found about 83 % of its initial amount throughout storage period. Some values over 100 % were probably influenced by measure error. Especially the results at days 14, 14+1 and 14+2 were influenced by detector sensitivity, because in these days the ambient temperature was much higher than during other single assays (see Table 4.5). Degradation of vitamin A was bigger if the bags were stored at ambient temperature. Vitamin E losses were not as rapid as losses in the bags A. Amount of vitamin E was not less than 66 % at day 7+1. Other values showed that amount of degraded vitamin E is less 20 %. All results are showed in the graphs (see Figures 4.16, 4.17 and 4.18) and in the Table 4.5.

Table 4.5: Peak Area and RSD of FSV in Bags B throughout the Investigation

	A		E	
day	**peak area (%)**	**RSD**	**peak area (%)**	**RSD**
0	100	1,08	100	1,52
7	101,18	0,31	81,18	11,9
7+1	87,73	2,76	66,51	10,1
7+2	83,13	5,12	95,60	7,03
	A		**E**	
day	**peak area (%)**	**RSD**	**peak area (%)**	**RSD**
0	100	4,11	100	0,70
14	116,99	1,05	106,82	1,50
14+1	108,96	1,29	93,65	0,40
14+2	115,42	2,72	102,72	1,04
	A		**E**	
day	**peak area (%)**	**RSD**	**peak area (%)**	**RSD**
0	100	3,61	100	10,1
29	100,57	3,73	97,02	5,16
29+1	94,60	1,88	92,53	1,25
29+2	97,41	2,66	92,77	1,47

Figure 4.16: Peak Area for FSV in Kabiven® Bag B after 7 Days Storage in the Fridge

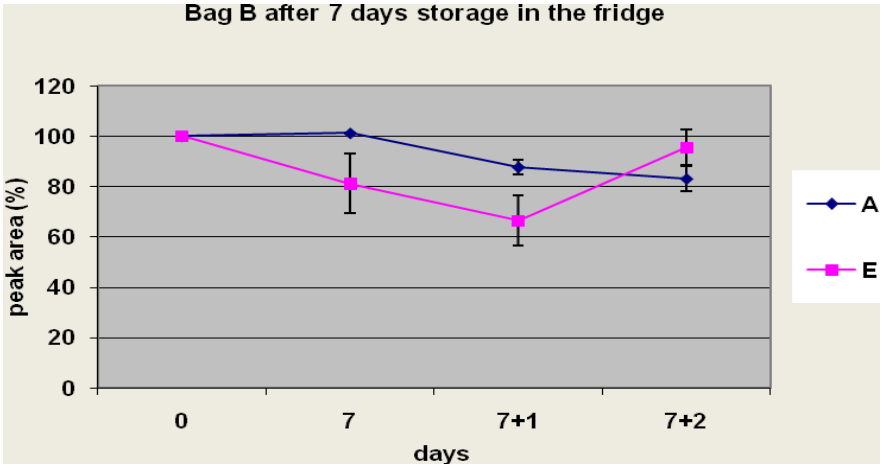


Figure 4.17: Peak Area for FSV in Kabiven® Bag B after 14 Days Storage in the Fridge

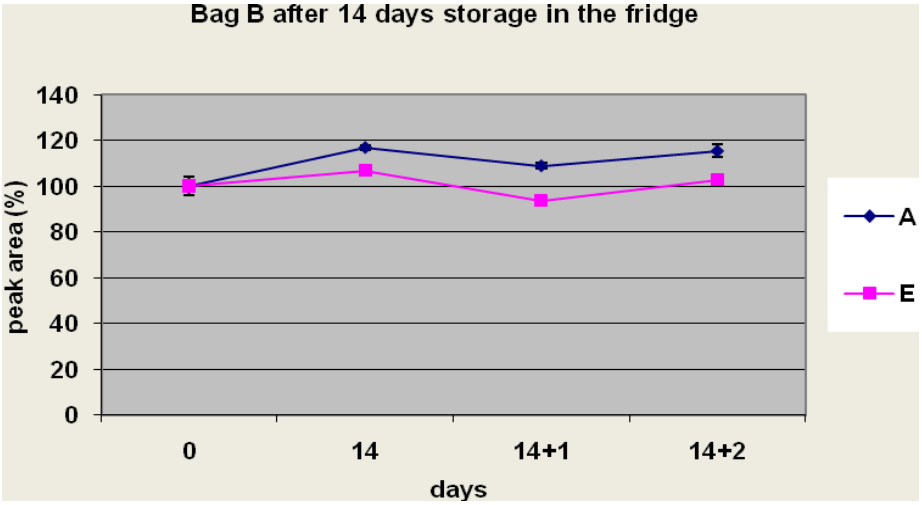
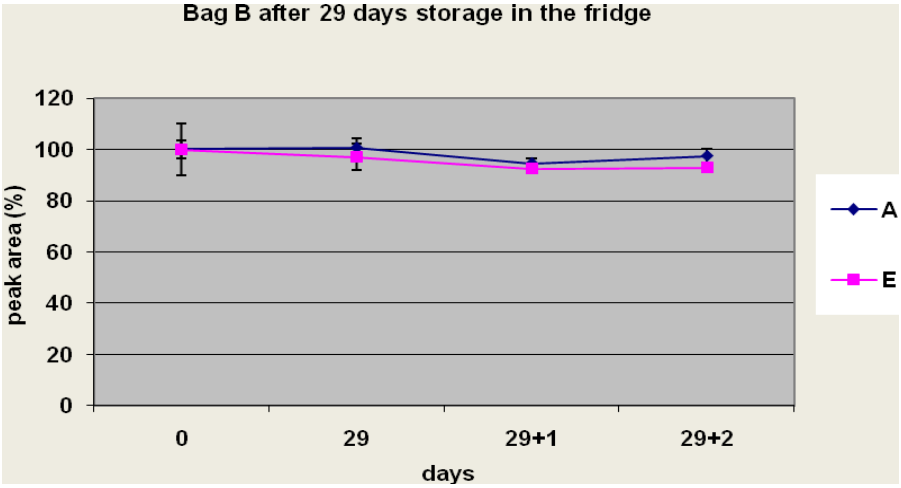


Figure 4.18: Peak Area for FSV in Kabiven® Bag B after 29 Days Storage in the Fridge



4.1.5.3 Discussion for Fat-soluble Vitamins in Kabiven® Bags

Stability of vitamins A and E was discussed in previous paragraph 2.6 Vitamins. Retinol is the most light-sensitive of the vitamins and is known to be rapidly broken down by exposure to UV light[11]. Kabiven® Bags were light-protected throughout the project, therefore degradation was not rapid. Losses of vitamin A were less when the

bags were stored in the refrigerator. The presence of amino acids and fat emulsion in the bag affords considerable protection of retinol[11]. This might be the reason why losses of retinol were reduced. Stability of retinol was not influenced by presence of TE as obtained results showed. On the other hand, degradation of vitamin E was not as rapid in the Bags B as in the Bags A. It was probably caused by presence of TE. Harraki[2] reported that there is an important interaction between Se and vitamin C and E. Vitamin E degrades very fast if the Bags are stored out of refrigerator and if the TE are presented. There is also possibility of sorption vitamin E to the bag as Allwood[11] reported.

4.2 RESULTS AND DISCUSSION OF pH FOR KABIVEN® BAGS

4.2.1 pH Results of Kabiven® Bags A and B

a) The measured pH in the Bags A was in range between 5.44 at day seven plus one and 6.00 at day twenty nine. Only one value differs from the initial value of pH in more than 0.5 units. This value 6.00 was measured after 29 days storage in the fridge (see Table 4.6). The changes of pH after 24 and 48 hours storage at ambient temperature are quite small. The biggest differece is 0.36 units and it is between day seven and day seven plus two. The other changes are insignificant because they are not to enormous. The graphical results are expressed in Figures 4.19, 4.20 and 4.21 in differen time.

b) The measured pH in the Bags B was in range between 5.38 at day seven plus two and 5.97 at day twenty nine plus one (see Table 4.6). Except of the difference 0.43 units between pH at day seven and pH at day seven plus one, all changes of pH are quite small and all values of pH are less than 0.5 units. The graphical results are expressed in Figures 4.19, 4.20 and 4.21.

Table 4.6: pH of Kabiven® Bags throughout the Investigation

pH/day	0	7	7+1	7+2	14	14+1	14+2	29	29+1	29+2
Bag A	5.49	5.45	5.44	5.85	5.88	5.86	5.77	6.00	5.88	5.84
Bag B	5.55	5.53	5.96	5.38	5.92	5.92	5.88	5.97	5.94	5.91

Figure 4.19: pH of Kabiven® Bags A and B after 7 Days Storage in the Fridge

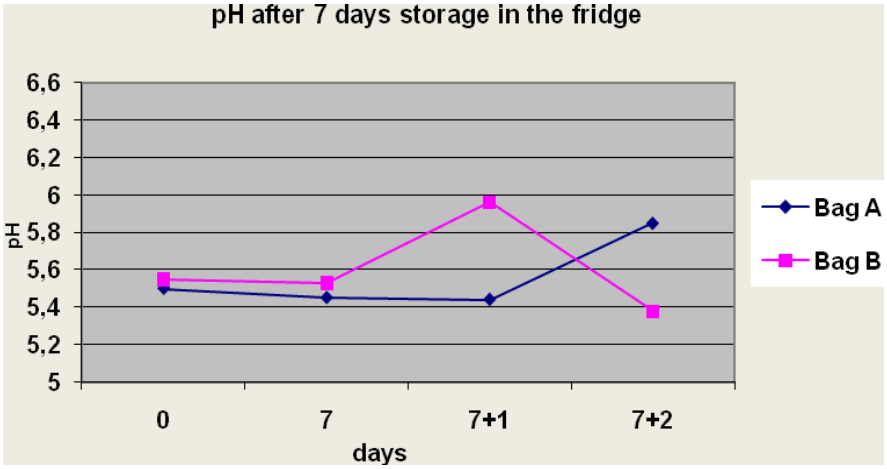


Figure 4.20: pH of Kabiven® Bags A and B after 14 Days Storage in the Fridge

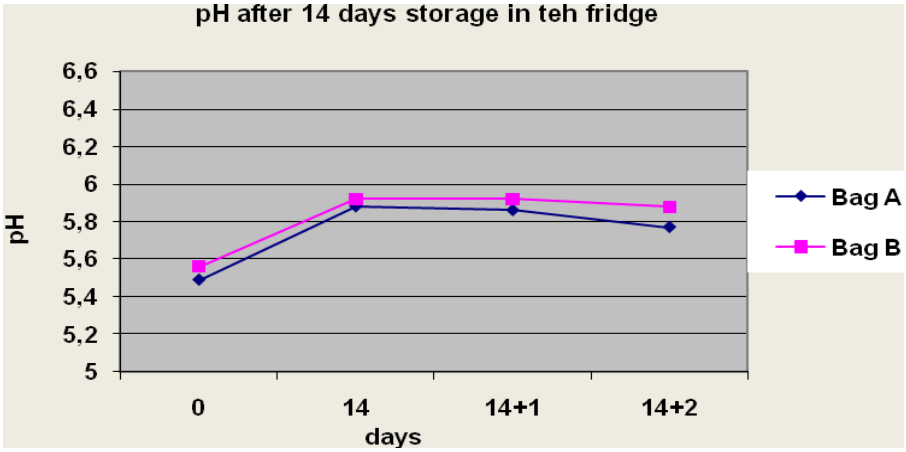
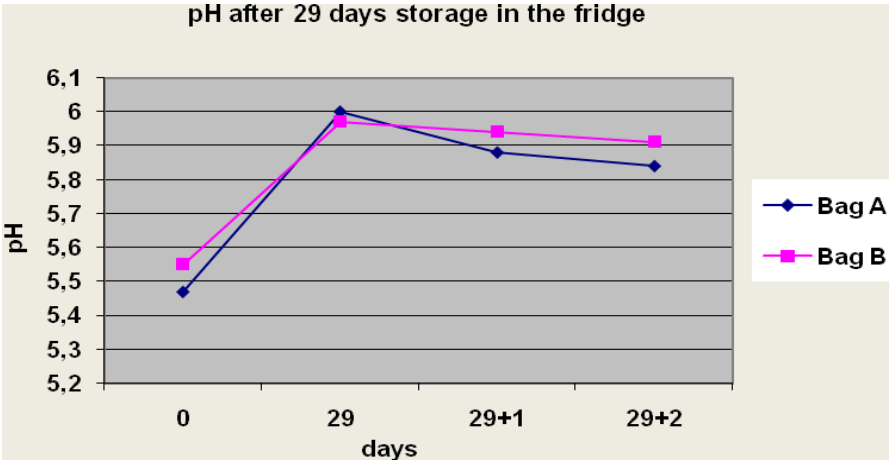


Figure 4.21: pH of Kabiven® Bags A and B after 29 Days Storage in the Fridge



4.2.2 Discussion

Allwood[11] reported that pH changes can lead to significant loss of stability or to precipitation. That is the reason why observation of pH is 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 pH changes were in norm, except one value which differed from initial pH value in more than 0.5 units. This change was observed after 29 days storage in refrigerator in Bag A. Addition TE into the Bag showed, that probably has not any influence to pH changes in time. Therefore, investigated admixtures are suitable for intravenous delivery. The most stable are admixtures stored for 7 days in refrigerator and then followed one day storage at ambient temperature. After longer storage period (14 or 29 days) stability of admixtures is decreasing.

4.3 Results and Discussion of Kabiven® Bags Microscopy

4.3.1 Microscopy Results of Kabiven® Bag A

Many enlarged particles were seen in Kabiven® admixtures A. The biggest lipid globule of 25 µm was observed at day twenty-nine plus two (see Table 4.11). At all time points there were globules bigger than 10 µm except day zero. Particles in range 7.5 and 10 µm are showed in Table 4.7. All other lipid globules were 5 µm or smaller at all time points.

Table 4.7: Results of Microscopy of Kabiven® Bags A

Bag A			
day	**biggest particle size (µm)**	**amount of particles >10µm**	**particle size 7.5-10 µm**
0	10	0	4
7	10		5
7+1	12,5	1	5
7+2	12,5	1	4
14	10		3
14+1	20	4	4
14+2	20	2	5
29	20	1	6
29+1	20	3	6
29+2	25	4	6

4.3.2 Microscopy Results of Kabiven® Bag B

Many enlarged particles were seen in Kabiven® admixtures B. The biggest lipid globules of 25 µm were observed at days fourteen plus two and twenty-nine plus two (see Table 4.8). One particle bigger than 10 µm was observed at day seven plus two, two particles at day fourteen plus two, three particles were observed at day seven

plus one and four particles at day twenty-nine plus two. Partcles in range 7.5 and 10 µm are showed in Table 4.8. All other lipid globules were 5 µm or smaller at all time points.

Table 4.8: Results of Microscopy of Kabiven® Bags B

Bag B			
day	**biggest particle size (µm)**	**amount of particles >10µm**	**particle size 7.5-10 µm**
0	10	0	5
7	10	0	4
7+1	12,5	3	4
7+2	12,5	1	4
14	10	0	3
14+1	10	0	5
14+2	25	2	4
29	10	0	5
29+1	10	0	6
29+2	25	4	7

4.3.3 Discussion

Potential interactions of combined TPN mixtures were discussed in paragraph 2.9. Allwood[11] reported that precipitation may be a result of temperature changes, pH changes or poor mixing. The results showed that most stable admixtures are those stored for seven days in refrigerator. If they are stored for more than 7 days they become unstable because of higher amount of enlarged lipid globules. Bags without TE contain less enlarged particles, especially after longer storage period (14 and 29 days), it means that stability of admixtures is decreasing when TE are presented and storage period is prolonged.

4.4 Results and Discussion of Laser Diffraction of Kabiven® Bags

4.4.1 Kabiven® Bag A Laser Diffraction Results

Very few enlarged lipid droplets were detected in the Kabiven® Bag A containing samples. Many samples presented a maximum particle size of just 4.3 µm. Particles of 8.04 µm were observed at day seven plus two, exactly 0.12 volume percentage. Enlarged lipid particles were observed at day twenty-nine plus one and twenty-nine plus two as well, it means particles of 12.21 µm were contained in 0.12 volume percentage at both time points (see Figure 4.23). All other lipid globules were 4.3 µm or smaller at all time points.

Figure 4.22: Particle Size Distribution of Globules in Lipid Admixtures (Bag A-Day Zero)

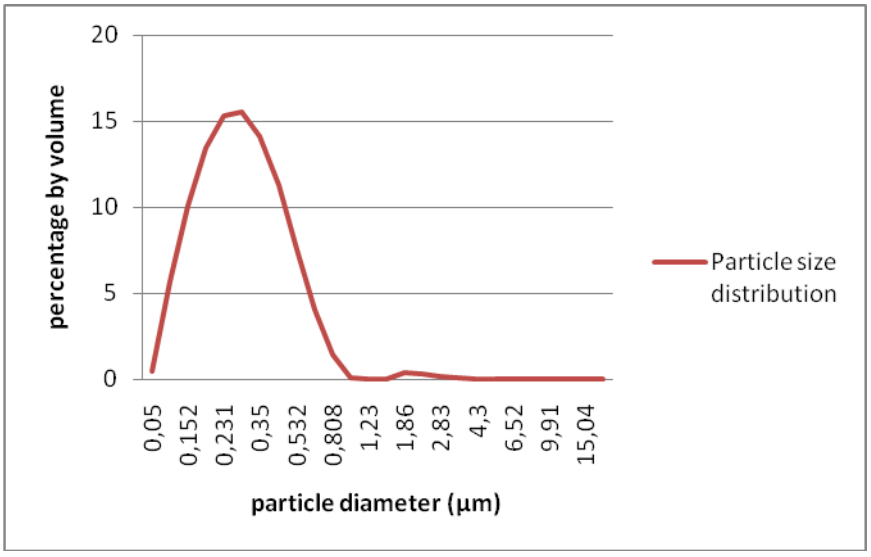
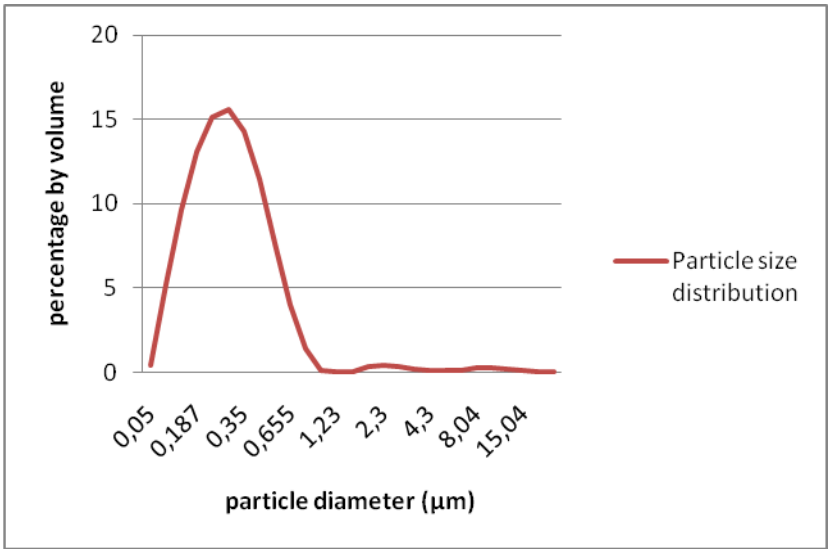


Figure 4.23: Particle Size Distribution of Globules in Lipid Admixtures (Bag A-Day twenty-nine plus one)



4.4.2 Kabiven® Bag B Laser Diffraction Results

Very few lipid droplets greater than 4.3 µm were detected in the Kabiven® Bag B containing samples. Many samples presented a maximum particle size of 3.49 µm. There were detected couple enlareged lipid particles of 6.52 µm in 0.178 volume percentage and 12.21 µm in 0.148 volume percentage at day twenty-nine plus two (see Figure 4.25). All other lipid globules were 4.3 µm or smaller at all time ponits (see Figure 4.24).

Figure 4.24: Particle Size Distribution of Globules in Lipid Admixtures (Bag B-Day Zero)

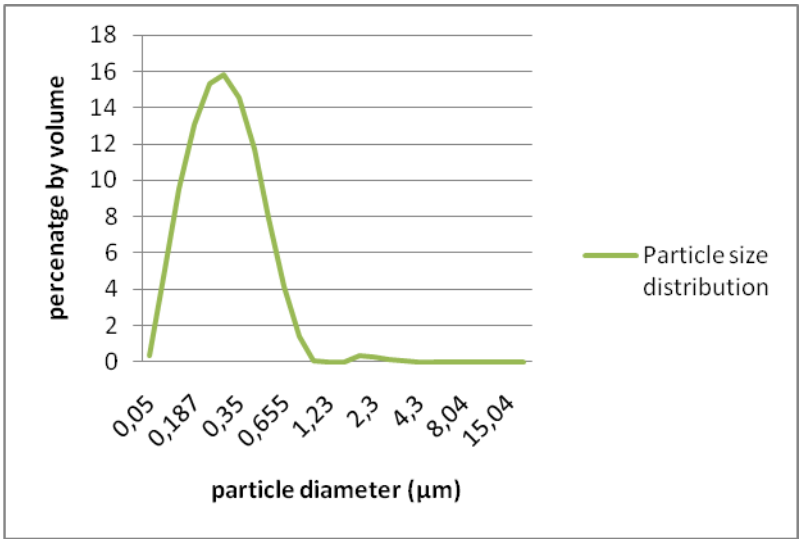
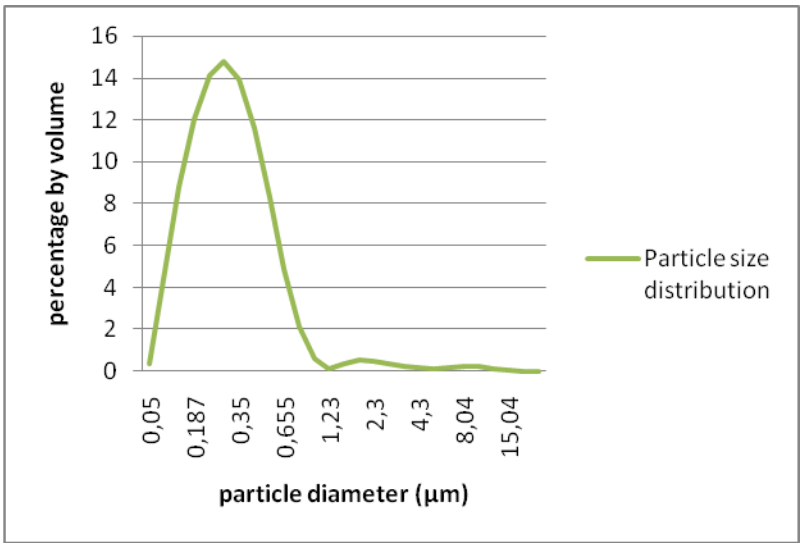


Figure 4.25: Particle Size Distribution of Globules in Lipid Admixtures (Bag B-Day twenty-nine plus two)



4.4.3 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 authors take 5 μm as the upper limit, since larger particles pose a risck of lung embolism[45].

Very few lipid globules in Kabiven® Bag A samples were enlarged. Emulsions in which >0.4 % of the fat particles are above 5 μm, are likely to become unstable[45]. In my trials the volume of droplets bigger than 5 μm was not so big, exactly was less than 0.4 %. Larger lipid droplets were presented after 29 days storage in the refrigerator and then after two days storage at ambient temperature. Concerning the lipid globules size this admixture could be used for intravenous delivery after seven days and fourteen days storage in the refrigerator. There is bigger probability adverse reaction after delivery admixture after twenty-nine days storage in the fridge followed two days storage at ambient temperature. On the other hand lipid particles with diameter even greater than 7.5 μm can deform and pass through the pulmonary vasculature without difficulty[45].

4.5 Results of Osmolality of Kabiven® Bags

Osmolality was measured at day zero, day twenty-nine, day twenty-nine plus one and day twenty-nine plus two. The osmolality values are expressed in Table 4.14.

Table 4.9: Osmolality of Kabiven® Bags

	Bag A	**Bag B**
day	**Osmolality (mOsm/kg)**	**Osmolality (mOsm/kg)**
0	**818**	**795**
29	**799**	**800**
29+1	**809**	**798**
29+2	**817**	**794**

CHAPTER FIVE
CONCLUSIONS

CHAPTER FIVE: CONCLUSIONS

All discussed vitamins are light-sensitive, especially vitamin A and riboflavin. All analysed bags were light protected throughout the thesis therefore degradation was not as rapid as in case if they were not light-protected. Loses of all vitamins were also less when the bags were stored in a refrigerator. Degradation was faster when the bags were stored at ambient temperature. Presence of TE caused rapid degradation of vitamin E, on the other hand stability of riboflavin, vitamin A and pyridoxine was not influenced by TE. Prolonged storage period caused less stability of all assayed bags, especially when TE were presented, because of enlarged lipid globules. It means that the most stable are these admixtures which are stored for seven days in a refrigerator. Pyridoxine was found the most stable among all analysed vitamins. Its losses were negligible even after 29 days storage or if trace elements were presented. The least stable vitamin was found vitamin E, especially in a mixture with trace elements.

All results could be summarize by following points:

- All discussed vitamins are light-sensitive and day-light protection caused that losses of these vitamins were multiple less.
- It was found that vitamin E is rapidly degraded when is in the mixture with trace elements. Degradation of vitamin E is not so significant when the mixture is trace elements free.
- All vitamins are stable when they are stored in a refrigerator for first seven days. If they are stored at ambient temperature, stability has decreasing tendency.
- Vitamin A should not be stored more than 14 days in a refrigerator if trace elements are presented. Vitamin E should not be stored more than 7 days in a fridge if trace elements are presented. In these cases degradation of vitamins is rapid.
- Pyridoxine is the most stable vitamin and its losses were negligible.
- The most stable admixtures are those stored for seven days in refrigerator. If they are stored for more than 7 days they become unstable because of higher amount of enlarged lipid globules. Bags without TE contain less enlarged particles, especially after longer storage period (14 and 29 days), it means that

stability of admixtures is decreasing when TE are presented and storage period is prolonged.

SOUHRN

Parenterální výživa se stala akceptovanou metodou pro prevenci a nápravu podvyživenosti u pacientů, u kterých nemůže být dostatečná výživa poskytnuta gastrointestinální cestou[2]. 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 této diplomové práce bylo získat informace o stabilitě vitamínů ve směsích parenterální výživy v závislosti na odlišných skladovacích podmínkách a na době skladování. Konkrétně byla sledována stabilita vitamínů rozpustných ve vodě (B_2, B_6) i vitamínů rozpustných v tucích (A, E), které byly přidány do Kabiven® Peripheral vaku (viz. Tabulka 3.1) nejdříve se stopovými prvky a poté bez stopových prvků. Fyzikální a chemická analýza směsí byla provedena v následujících intervalech:

- V čase přípravy směsi
- Po sedmi dnech skladování v lednici následována 24 a 48 hodinami skladování při pokojové teplotě.
- Po čtrnácti dnech skladování v lednici následována 24 a 48 hodinami skladování při pokojové teplotě.
- Po dvaceti devíti dnech skladování v lednici následována 24 a 48 hodinami skladování při pokojové teplotě.

Všechny infuzní vaky byly po celou dobu testování chráněny před denním světlem.

Infuzní vaky byly připraveny dvěma různými způsoby:

- První sada vaků (A) byla připravena smísením rekonstituovaného vaku Kabiven® Peripheral s Additrace® koncentrátem (viz. Kapitola 3.2) pomocí 10ml injekční stříkačky. Solvitio® N prášek (viz. Kapitola 3.2) byl rekonstituován pomocí Vitlipid® N emulze (viz. Kapitola 3.2) 10ml stříkačkou a vzniklá emulze byla stejnou stříkačkou přidána do infuzního vaku A, který byl následně dobře promíchán. Tímto způsobem byly připraveny celkem tři infuzní vaky A. Z každého vaku bylo odebráno injekční stříkačkou 20 ml směsi,

přemístěno do Hach zkumavky (viz. Obr. 3.1) a připraveno k fyzikální analýze v čase přípravy směsi. 1 ml této směsi z každé zkumavky byl požit pro analýzu HPLC. Po odebrání vzorku byly vaky uskladněny v lednici, dokud nebyla provedena další analýza.

- Druhá sada vaků (B) byla připravena obdobným způsobem jako sada A, ale bez přídavku Additrace® koncentrátu. Stopové prvky tudíž v těchto vacích chyběly. Bylo připraveno stejné množství vaků jako v předešlém odstavci, které byly následně analyzovány stejným způsobem jako vaky A.

Manipulace se všemi vaky byla provedena v boxu s laminárním prouděním, aby se zabránilo mikrobiální kontaminaci.

Fyzikální analýza zahrnovala následující metody: emulze byly zkontrolovány prostým okem a pomocí světelného mikroskopu. Velikost lipidových částic byla určena laserovou difrakcí prostřednictvím Malvern Mastersizer® X a osmolalita byla změřena pomocí The Advanced™ Osmometer. pH emulzí bylo změřeno pomocí Orion® pH metru (viz. Tabulka 3.4). Chemická analýza byla provedena pomocí HPLC analýzy. Směs metanolu a 0.05M fosfátového pufru pH 7 jako mobilní fáze byla použita pro analýzu vitamínů B_2 a B_6 (viz. Kapitola 3.6). Jako mobilní fáze pro analýzu vitamínů A a E byl použit metanol (viz. Kapitola 4.1.4).

Všechny analyzované vitamíny jsou citlivé na světlo, speciálně vitamín A a riboflavin. Proto byly všechny vaky, použité k analýze, chráněny před denním světlem a tudíž degradace vitamínů nebyla tak rozsáhlá jak by tomu bylo v opačném případě. Vitamínové ztráty byly menší, pokud infuzní vaky byly uloženy po dobu trvání stabilitních studií v lednici. Degradace byla rychlejší v případě uchovávání vaků při pokojové teplotě. Přítomnost stopových prvků ve směsi způsobila významnou degradaci vitamínu E, ale stabilita ostatních vitamínů jimi nebyla nijak významně ovlivněna (viz. Tabulka 4.4 a 4.5). Stabilita všech analyzovaných vaků klesala s prodlužováním doby skladování, obzvlášť v přítomnosti stopových prvků, kvůli přítomnosti zvětšených lipidových částic. Nejstabilnější byly tudíž ty směsi, které byly uchovávány maximálně po dobu sedmi dní za snížené teploty. Ze všech analyzovaných vitamínů byl pyridoxin shledán jako nejstabilnější. Jeho ztráty byly zanedbatelné dokonce i po 29 denním skladování nebo ve směsi se stopovými

prvky. Nejméně stabilním vitamínem byl vitamín E, zvlášť ve směsi se stopovými prvky.

Všechny výsledky můžeme shrnout do několika bodů:

- Všechny výše zmíněné vitamíny jsou citlivé na světlo, tudíž ochrana infuzí před denním světlem má za následek, že ztráty vitamínů jsou několikanásobně nižší.
- Bylo zjištěno, že vitamín E rychle degraduje ve směsi se stopovými prvky. Jestliže je vitamín E ve směsi prosté stopových prvků, degradace není tak výrazná.
- Všechny vitamíny byly stabilní po dobu prvních sedmi dní uskladnění v lednici. Pokud jsou uskladněny při pokojové teplotě, stabilita rychle klesá.
- Vitamín A by neměl být skladován v lednici po dobu delší než 14 dní, pokud je ve směsi se stopovými prvky. Vitamín E by neměl být skladován v lednici více než 7 dní, pokud jsou přítomny stopové prvky. V těchto případech stabilita obou vitamínů rapidně klesá.
- Pyridoxin je nejvíce stabilní vitamín a jeho ztráty byly zanedbatelné.
- Nejvíce stabilní směsi se ukázaly ty, které byly uskladněny v lednici po dobu 7 dnů. V případě delšího skladování se stávají méně stabilními, díky většímu množství zvětšených lipidových částic. Infuzní vaky bez přídavku stopových prvků obsahují mnohem méně zvětšených lipidových částic, obzvlášť po delší době skladování (14 a 29 dní). To znamená, že stabilita směsí klesá s přítomností stopových prvků a s prodlužující se dobou skladování.

ABSTRACT

Parenteral Nutrition (PN) is an alternative method of providing nutritional support for patients via the intravenous route. Patients selected for PN are malnourished patients or patients who have the potential to become malnourished. 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 is to obtain information on stability of the vitamins in PN admixtures depending on different storage conditions and different time.

Water-soluble vitamins and fat-soluble vitamins were investigated in the mixture of Kabiven® Bag (Fresenius Kabi) with Vitlipid® N (fat-soluble vitamins) and Solvitio® N water-soluble vitamins) Adult Injections. There were prepared 6 Bags altogether, three with addition of trace elements contained in Additrace® concentrate and three Bags without trace elements. Assessing the chemical and physical stability was carried out after: zero time, 7, 14 and 29 days in a refrigerator followed by 24 hours and 48 hours storage at ambient temperature and day-light protected. Physical tests include pH, osmolality, microscopy and particle size determination by laser diffraction. Chemical tests used validated stability indicating reversed phase HPLC methods. Only four vitamins were suitable for investigation via HPLC: vitamin A, vitamin E, riboflavin and pyridoxine.

It was found that pyridoxine is the most stabile vitamin, because its losses were negligible. All discussed vitamins are light-sensitive and day-light protection caused that losses of these vitamins were multiple less. All vitamins are stable when they are stored in a refrigerator for first seven days. If they are stored at ambient temperature, stability has decreasing tendency. It was found that vitamin E is rapidly degraded when is in the mixture with trace elements. Degradation of vitamin E is not so significant when the mixture is trace elements free. The most stable admixtures are those stored for seven days in a refrigerator. If they are stored for more than 7 days they become potentially unstable because of higher amount of enlarged lipid globules.

ABSTRAKT

Parenterální výživa je alternativní metoda poskytování nutriční podpory pro pacienty prostřednictvím žilního systému. Pacienti vybraní pro parenterální výživu jsou podvyživení nebo mají potenciál k podvyživenosti. 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 směsích s parenterální výživou v závislosti na různých skladovacích podmínkách a na době skladování.

Vitamíny rozpustné ve vodě a v tucích byly testovány ve směsi Kabiven® Bag (Fresenius Kabi) s Vitlipid® N emulzí obsahující vitamíny rozpustné v tucích a s roztokem obsahujícím vitamíny rozpustné ve vodě - Solvitio® N. Celkem bylo připraveno šest směsí, tři s přídavkem stopových prvků obsažených v Additrace® koncentrátu a tři bez přídavku stopových prvků. Testování fyzikální a chemické stability bylo provedeno v čase přípravy směsí, po 7, 14 a 29 dnech skladování v lednici následované 24 a 48 hodinovým skladováním těchto směsí při okolní teplotě. Směsi byly chráněny před světlem po celou dobu skladování. Provedené fyzikální testy zahrnovaly měření hodnot pH, osmolarity, 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 pouze čtyři vitamíny, konkrétně vitamíny A a E, dále riboflavin a pyridoxin.

Bylo zjištěno, že pyridoxin je nejstabilnější vitamín, protože jeho ztráty v průběhu stabilitních studií byly zanedbatelné. Všechny zmiňované vitamíny jsou citlivé na světlo. Ochrana před denním světlem způsobila, že ztráty vitamínů jsou tak mnohonásobně nižší. Všechny vitamíny se ukázaly být stabilní po dobu prvních sedmi dní skladování v lednici. Jestliže jsou směsi uchovávány při okolní teplotě, stabilita vitamínů klesá. Bylo zjištěno, že vitamín E rychle degraduje, jestliže je ve směsi se stopovými prvky. Degradace vitamínu E není tak zřejmá, jestliže je ve směsi prosté stopových prvků. Nejvíce stabilní jsou ty směsi, které se uchovávají v lednici po dobu sedmi dní od připravení. Jestliže jsou skladovány déle, stávají se

potencionálně nestabilními, kvůli většímu množství zvětšených nežádoucích lipidových globulí.

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