

Investigations into the Pharmaceutical Issues
Associated with the Provision of Micronutrients
to Parenteral Nutrition (PN) Patients

Thomas Ian Ferguson

A thesis submitted in accordance with the conditions governing
candidates for the degree of

Philosophiæ Doctor in Cardiff University

Cardiff School of Pharmacy and Pharmaceutical Sciences
Cardiff University

September 2014

Acknowledgements

I would firstly like to thank my supervisors, Rebecca Price-Davies and Allan Cosslett, for their help, encouragement and guidance throughout this process. I would also like to thank Sophie Emery for her technical expertise and assistance.

I must thank Frensenius Kabi, for allowing me to use materials and equipment throughout this process.

Finally, I would like to thank all my family and friends for all their support, especially my father who proof read my thesis.

Summary

In recent years, there has been an increased emphasis to treat patients with parenteral nutrition (PN) at home in an attempt to reduce costs and improve clinical outcomes. This increased interest in home parenteral nutrition (HPN) has stimulated researchers to investigate potential sources of instability. One of the more unstable groups in PN is micronutrients, which can be divided into two groups: vitamins and trace elements. This thesis investigates the effects of artificial light sources (cool white, warm white and UVA) on the physico-chemical stability of vitamins. Vitamins were chemically analysed using a novel stability indicating HPLC assay that could quantify five water-soluble and three fat-soluble vitamins simultaneously in one run. Samples were physically analysed by visual analysis, microscope analysis, laser diffraction, pH and osmolality.

Initial experiments investigated the physico-chemical stability of vitamins exposed to artificial light sources over a period of 24 hours. In cool and warm white light there was approximately a 20% loss of riboflavin and 10% loss of retinol. In UVA light there was approximately a 20% loss of retinol. All other analysed vitamins were stable over the time period to these artificial light sources. Further experiments investigated these conditions following 6 days of storage between 2-8 °C. These experiments revealed similar results in the three types of artificial light source.

The protective effects of lipid emulsions on retinol were then investigated in containers and administration sets. Samples containing lipid emulsions in syringes and administration sets had a statistically significant increase in retinol stability. Nevertheless, degradation in excess of 10% still occurred in these groups. The protective mechanism of lipid emulsions was primarily thought to be a result of light obscuration. However, soybean oil (SBO), a clear liquid, provided unexpected obscuration of UVA light suggesting it may reflect or absorb damaging rays thereby improving retinol stability.

Table of Contents

Title Page.....	i
Declaration	ii
Acknowledgements	iii
Summary	iv
Table of Contents.....	vi
List of Abbreviations	xvi
List of Figures.....	xviii
List of Tables.....	xxiv
CHAPTER ONE - Introduction.....	1
1.1. Introduction to Parenteral Nutrition (PN).....	2
1.1.1. Introduction to stability Issues associated with PN	2
1.1.2. Indications of PN	3
1.1.3. Administration of PN.....	3
1.1.4. Home Parenteral Nutrition (HPN).....	4
1.2. Components of PN	5
1.2.1. Carbohydrates.....	5
1.2.2. Lipids	6
1.2.3. Proteins	7
1.2.4. Water and electrolytes.....	8

1.2.5. Micronutrients	9
1.3. Vitamins.....	9
1.3.1. Fat-soluble vitamins	10
1.3.1.1. Vitamin A.....	10
1.3.1.2. Vitamin D.....	11
1.3.1.3. Vitamin E	12
1.3.1.4. Vitamin K	13
1.3.2. Water soluble vitamins	14
1.3.2.1. Ascorbic acid	14
1.3.2.2. Thiamine.....	15
1.3.2.3. Riboflavin	16
1.3.2.4. Nicotinamide	17
1.3.2.5. Pantothenic acid.....	18
1.3.2.6. Pyridoxine	18
1.3.2.7. Biotin	19
1.3.2.8. Folic acid.....	20
1.3.2.9. Cyanocobalamin.....	21
1.4. Trace elements.....	22
1.4.1. Zinc	22
1.4.2. Copper	22
1.4.3. Selenium	23
1.4.4. Manganese	23
1.4.5. Molybdenum	24
1.4.6. Iron	24
1.4.7. Chromium	24

1.4.8. Iodine.....	25
1.5. Vitamin, Trace Element and Electrolyte Requirements	25
1.6. Stability and compatibility of vitamins and trace elements	27
1.6.1. Vitamins.....	27
1.6.1.1. Vitamin A.....	27
1.6.1.2. Vitamin D.....	29
1.6.1.3. Vitamin E	29
1.6.1.4. Vitamin K.....	31
1.6.1.5. Ascorbic acid	31
1.6.1.6. Thiamine.....	34
1.6.1.7. Riboflavin	34
1.6.1.8. Pyridoxine	35
1.6.1.9. Folic acid.....	36
1.6.1.10. Cyanocobalamin, nicotinamide, pantothenic acid and biotin.....	36
1.6.2. Trace elements.....	37
1.6.2.1. Zinc	37
1.6.2.2. Copper.....	37
1.6.2.3. Selenium.....	37
1.6.2.4. Manganese.....	38
1.6.2.5. Molybdenum	38
1.6.2.6. Iron	38
1.6.2.7. Chromium	39
1.6.2.8. Iodine	39
1.7. Methods developed to increase stability and compatibility of micronutrients in PN.....	40

1.8. Thesis Aim and Objectives.....	41
1.8.1. Thesis aim	41
1.8.2. Thesis objectives	42
 CHAPTER TWO - Designing a Novel Validated Stability-Indicating HPLC assay for Fat and Water-Soluble Vitamins	 43
2.1. Introduction	44
2.2. HPLC	44
2.2.1. HPLC system.....	46
2.2.2. Water-soluble vitamins	46
2.2.3. Fat-soluble vitamins	46
2.2.4. Reference standards	47
2.3. Development of a HPLC assay for the simultaneous analysis of fat and water-soluble vitamins	 48
2.3.1. Preparation of phosphate buffer	49
2.3.2. Preparation of water and fat-soluble vitamins	49
2.3.3. Identification, calibration and stability-indicating tests for water and fat- soluble vitamins.....	50
2.3.4. Results for HPLC assay identifying fat and water-soluble vitamins simultaneously	54
2.3.5. Discussion	58
 CHAPTER THREE - Stability of Vitamins in Artificial Light Sources Over 24 Hours	 62

3.1. Introduction	63
3.2. Types of Light.....	63
3.3. Vitamins sensitive to light	64
3.4. Materials and methods	66
3.4.1. Physical analysis.....	67
3.4.1.1. Visual examination	67
3.4.1.2. Microscopy	68
3.4.1.3. Laser diffraction	69
3.4.1.5. Osmolality	71
3.4.1.6. pH	72
3.4.2. Experimental design.....	73
3.4.2.1. Sample preparation.....	74
3.4.2.2. Cool and warm white light experiments	74
3.4.2.3. UVA light experiment	76
3.4.2.4. Sampling of syringes.....	77
3.5. Results	78
3.5.1. Visual examination.....	78
3.5.2. Microscopy	78
3.5.3. Laser diffraction	80
3.5.4. Osmolality.....	81
3.5.5. pH	82
3.5.6. HPLC	83
3.5.6.1. Summary of HPLC results	83
3.5.6.2. Pyridoxine	84
3.5.6.3. Nicotinamide	85

3.5.6.4. Riboflavin	86
3.5.6.5. Tocopherol	87
3.5.6.6. Retinol	88
3.6. Discussion	89
3.7. Conclusion	91
 CHAPTER FOUR - Stability of Vitamins in Artificial Light Sources Over 6+1 Days	
.....	92
4.1. Introduction	93
4.2. Materials and Methods	93
4.2.1. Experimental Design	93
4.2.1.1. Sample preparation.....	93
4.2.1.2. Cool and warm white light	94
4.2.1.3. UVA Light.....	94
4.2.1.4. Physical and chemical testing.....	95
4.3. Results	96
4.3.1. Visual examination.....	96
4.3.2. Microscopy	96
4.3.3. Laser diffraction	98
4.3.4. Osmolality.....	100
4.3.5. pH	100
4.3.6. HPLC	101
4.3.6.1. Summary of HPLC results	101
4.3.6.2. Pyridoxine	103

4.3.6.3. Nicotinamide	104
4.3.6.4. Riboflavin	105
4.3.6.5. Tocopherol	106
4.3.6.6. Retinol	107
4.4. Discussion	108
4.5. Conclusion	110
 CHAPTER FIVE - Investigating the Photo-Protective Effects of Lipid Emulsions	
on Light-Sensitive Vitamins.....	111
5.1. Introduction.....	112
5.2. Materials and Methods.....	113
5.2.1. Stability-indicating HPLC assay used for the quantification of fat-soluble vitamins.....	113
5.2.1.1. Stability-indicating HPLC assay method	113
5.2.1.2. Calibration, identification and stability-indicating tests for fat-soluble vitamins.....	115
5.2.1.3. Types of lipid emulsion used.....	118
5.2.2. Sample Preparation.....	119
5.2.3. Experiment design and physico-chemical testing.....	120
5.2.4. Statistical analysis of vitamin degradation in ultra-filtered deionised water, IL10, IL20 and SMOF.....	120
5.3. Results	121
5.3.1. Visual examination.....	121
5.3.2. Microscopy	122

5.3.3. Laser diffraction	123
5.3.4. Osmolality.....	125
5.3.5. pH	126
5.3.6. HPLC analysis	128
5.3.6.1. Tocopherol	128
5.3.6.2. Retinol	130
5.3.7. Statistical analysis	133
5.4. Discussion	136
5.5. Conclusion	139

CHAPTER SIX - Investigating the Photo-Protective Effects of Lipid Emulsions on Light Sensitive Vitamins in Administration Sets During Simulated Delivery . 140

6.1. Introduction	141
6.2. Materials and Methods	142
6.2.1. Stability indicating HPLC assay used for quantification of retinol and tocopherol degradation	142
6.2.2. Sample preparation.....	142
6.2.3. Experimental design.....	143
6.3. Results	144
6.3.1. Visual examination.....	144
6.3.2. Microscopy	144
6.3.3. Laser diffraction	145
6.3.4. Osmolality.....	147
6.3.5. pH	149

6.3.6. HPLC	151
6.3.6.1. Tocopherol	152
6.3.6.2. Retinol	155
6.4. Discussion	158
6.5. Conclusion	159
 CHAPTER SEVEN - Investigating the Light Protective Effects of Lipid Emulsions	 161
7.1. Introduction	162
7.2. Materials and Methods	162
7.2.1. Experimental designs	162
7.2.1.1. Investigating light obscuration of containers and different types of lipid emulsion.....	163
7.2.1.2. Investigating the light protective effects of SBO on vitamins	164
7.3. Results	166
7.3.1. Light obscuration comparison of MDVs and Syringes.....	166
7.3.2. Light obscuration comparison between different lipid types in syringes ...	168
7.3.3. Light obscuration of SBO in cool white, warm white and UVA light	171
7.4. Discussion	173
7.5 Conclusion	176
 CHAPTER EIGHT - General Discussion	 177

8.1. General Discussion	178
8.2. Future Work.....	181
8.3. Limitations	182
8.4. Recommendations	183
8.5. Concluding Remarks.....	184
 References.....	 185
 Appendix I - Publications	 205

List of Abbreviations

ACP	Acyl-Carrier Protein
AIO	All-In-One
BANS	British Artificial Nutrition Survey
BD	Becton Dickinson®
CoA	Coenzyme A
DNA	Deoxyribonucleic Acid
EVA	Ethyl Vinyl Acetate
FAD	Flavine-Adenine Dinucleotide
FMN	Flavine Mononucleotide
HPLC	High-Pressure Liquid Chromatography
GI	Gastrointestinal
HPN	Home Parenteral Nutrition
IL10	Intralipid® 10
IL20	Intralipid® 20
IV	Intravenous
MCT	Medium-Chain Triglyceride
MDV	Multi-dose Vial
NHS	National Health Service
PN	Parenteral Nutrition
PICC	Peripherally-Inserted Central Catheter
PUFAs	Polyunsaturated fatty acids

PVC	Polyvinyl Chloride
PLP	Pyridoxal Phosphate
R^2	Correlation Coefficient
RDA	Recommended Daily Allowance
RNA	Ribonucleic Acid
RSD	Relative Standard Deviation
SBO	Soybean Oil
SD	Standard Deviation
SMOF	SMOFLipid® 20%
S/N	Signal-to-Noise Ratio
SVS	Small Volume Sampler
TPP	Thiamine pyrophosphate
UHPLC	Ultra High-Pressure Liquid Chromatography
UV	Ultraviolet
UVA	Ultraviolet A

List of Figures

Figure 1.1	Chemical structure of all-trans-retinol
Figure 1.2	Chemical structure of ergocalciferol
Figure 1.3	Chemical structure of α -tocopherol
Figure 1.4	Chemical structure of phylloquinone
Figure 1.5	Chemical structure of ascorbic acid
Figure 1.6	Chemical structure of thiamine
Figure 1.7	Chemical structure of riboflavin
Figure 1.8	Chemical structure of nicotinamide
Figure 1.9	Chemical structure of pantothenic acid
Figure 1.10	Chemical structure of pyridoxine
Figure 1.11	Chemical structure of biotin
Figure 1.12	Chemical structure of folic acid
Figure 1.13	Chemical structure of cyanocobalamin
Figure 1.14	The degradation cascade of ascorbic acid in PN adapted from Allwood and Kearney (1998)
Figure 2.1	Calibration curve for Ascorbic acid, n=3
Figure 2.2	Calibration curve for Pyridoxine, n=3
Figure 2.3	Calibration curve for Thiamine, n=3
Figure 2.4	Calibration curve for Folic acid, n=3
Figure 2.5	Calibration curve for Cyanocobalamin, n=3
Figure 2.6	Calibration curve for Riboflavin, n=3

- Figure 2.7 Calibration curve for Ergocalciferol, n=3
- Figure 2.8 Calibration curve for Tocopherol, n=3
- Figure 2.9 Calibration curve for Phylloquinone, n=3
- Figure 2.10 Calibration curve for Retinol, n=3
- Figure 2.11 HPLC chromatogram identifying 11 water and fat-soluble vitamins
- Figure 3.1 Sunlight spectra in the northern hemisphere recorded in North America (National Renewable Energy Laboratory 2003)
- Figure 3.2 Thoma hemocytometer. Dashed box indicates the overall area to be considered. Grey shading shows one of the counting areas within the dashed box
- Figure 3.3 Scattering process adapted from the Malvern reference manual
- Figure 3.4 The experimental set up for the 50 ml BD syringes exposed to either cool white or warm white light
- Figure 3.5 Spectra of cool white light using a multi-ray lamp
- Figure 3.6 Spectra of warm white light using a multi-ray lamp
- Figure 3.7 Spectra of UVA light in SANYO® stability chamber
- Figure 3.8 Degradation of pyridoxine when exposed to (a) cool white light, (b) warm white light and (c) UVA light
- Figure 3.9 Degradation of nicotinamide when exposed to (a) cool white light, (b) warm white light and (c) UVA light
- Figure 3.10 Degradation of riboflavin when exposed to (a) cool white light, (b) warm white light and (c) UVA light

- Figure 3.11 Degradation of tocopherol when exposed to (a) cool white light, (b) warm white light and (c) UVA light
- Figure 3.12 Degradation of retinol when exposed to (a) cool white light, (b) warm white light and (c) UVA light
- Figure 4.1 Degradation of pyridoxine when exposed to (a) cool white light, (b) warm white light and (c) UVA light following up to 7 days of storage
- Figure 4.2 Degradation of nicotinamide when exposed to (a) cool white light, (b) warm white light and (c) UVA light following up to 7 days of storage
- Figure 4.3 Degradation of riboflavin when exposed to (a) cool white light, (b) warm white light and (c) UVA light following up to 7 days of storage
- Figure 4.4 Degradation of tocopherol when exposed to (a) cool white light, (b) warm white light and (c) UVA light following up to 7 days of storage
- Figure 4.5 Degradation of retinol when exposed to (a) cool white light, (b) warm white light and (c) UVA light following up to 7 days of storage
- Figure 5.1 Chromatogram of Vitlipid® N Adult in water. Peaks include: ergocalciferol, tocopherol and retinol
- Figure 5.2 Calibration curves for (a) ergocalciferol, (b) tocopherol and (c) retinol with trendlines and R^2 values

- Figure 5.3 Tocopherol degradation in 50 ml Becton Dickinson® syringes when exposed to cool white light
- Figure 5.4 Tocopherol degradation in 50 ml Becton Dickinson® syringes when exposed to warm white light
- Figure 5.5 Tocopherol degradation in 50 ml Becton Dickinson® syringes when exposed to UVA light
- Figure 5.6 Retinol degradation in 50 ml Becton Dickinson® syringes exposed to cool white light
- Figure 5.7 Retinol degradation in 50 ml Becton Dickinson® syringes exposed to warm white light
- Figure 5.8 Retinol degradation in 50 ml Becton Dickinson® syringes exposed to UVA light
- Figure 5.9 Retinol degradation following 24 hours of exposure to cool white light
- Figure 5.10 Retinol degradation following 24 hours of exposure to warm white light
- Figure 5.11 Retinol degradation following 24 hours of exposure to UVA light.
- Figure 6.1 Experimental set-up of simulated delivery of Vitlipid® N Adult and either ultra-filtered deionised water, IL10, IL20 or SMOF
- Figure 6.2 Tocopherol degradation following simulated delivery when exposed to cool white light
- Figure 6.3 Combined tocopherol degradation following simulated delivery in cool white light

- Figure 6.4 Tocopherol degradation following simulated delivery when exposed to warm white light
- Figure 6.5 Combined tocopherol degradation following simulated delivery in warm white light
- Figure 6.6 Tocopherol degradation following simulated delivery when exposed to UVA light
- Figure 6.7 Combined tocopherol degradation following simulated delivery in UVA light
- Figure 6.8 Retinol degradation following simulated delivery when exposed to cool white light
- Figure 6.9 Combined retinol degradation following simulated delivery in cool white light
- Figure 6.10 Retinol degradation following simulated delivery when exposed to warm white light
- Figure 6.11 Combined retinol degradation following simulated delivery in warm white light
- Figure 6.12 Retinol degradation following simulated delivery when exposed to UVA light
- Figure 6.13 Combined retinol degradation following simulated delivery in UVA light
- Figure 7.1 Set-up for an experiment investigating the obscuration of cool white, warm white and UVA light
- Figure 7.2 Comparison of container material on obscuration of (a) cool white, (b) warm white and (c) UVA light

Figure 7.3 Comparison of contents on light obscuration in (a) cool white, (b) warm white and (c) UVA light

Figure 7.4 Comparing light obscuration of syringes containing SBO with those containing different types of lipid in (a) cool white and (b) warm white light and (c) UVA light

List of Tables

Table 1.1	The influence of hormones on glucose metabolism and storage
Table 1.2	Classes of amino acids adapted from Koletzko et al. (2005a)
Table 1.3	Reference nutrient intakes of vitamins for male and females between 19-50 years (Department of Health 2009)
Table 1.4	Reference nutrient intake for trace element and electrolytes in males and females aged between 19-50 years (Department of Health 2009)
Table 2.1	Method of stability-indicating HPLC assay
Table 2.2	The peaks and approximate retention times identified on the HPLC chromatogram
Table 2.3	Relative standard deviation and R^2 values of calibration curves for water and fat-soluble vitamins
Table 3.1	Microscope analysis of control and test syringes exposed to cool white light
Table 3.2	Microscope analysis of control and test syringes exposed to warm white light
Table 3.3	Microscope analysis of control and test syringes exposed to UVA light
Table 3.4	Means and Standard Deviation (SD) of D[4,3] (μm) and 'Size Under' (μm) in control and test samples when exposed to cool white, warm white and UVA light over 24 hours

Table 3.5	Osmolality values for control and test syringes
Table 3.6	pH values for control syringes and test syringes exposed to cool white, warm white and UVA light
Table 3.7	Percentage of vitamins remaining \pm SD in control syringes that were protected from light and test syringes (mean) that were exposed to light for 24 hours
Table 4.1	Microscope analysis of control and test syringes exposed to cool white light.
Table 4.2	Microscope analysis of control and test syringes exposed to warm white light.
Table 4.3	Microscope analysis of control and test syringes exposed to UVA light.
Table 4.4	Mean and SD of D[4,3] and 'size under' results in control (C) and test (T) samples at 0, 6 and 6+1 days
Table 4.5	Summary of osmolality values recorded on day 6+1
Table 4.6	pH of syringes exposed to cool white, warm white and UVA light at 0 and 6+1 days
Table 4.7	Mean percentage \pm SD of vitamins remaining after 6+1 days in control syringes and test syringes
Table 5.1	Stability-indicating HPLC assay method
Table 5.2	Retention times of fat-soluble vitamins in a chromatogram generated by the validated stability-indicating HPLC assay described

Table 5.3	R ² values and mean RSD of ergocalciferol, tocopherol and retinol analysed by a stability-indicating HPLC assay
Table 5.4	Contents of IL10, IL20 and SMOF in 1000 ml
Table 5.5	Control and test sample means and SD of D[4,3] (µm) and 'Size under' (µm) when exposed to cool white light over 24 hours
Table 5.6	Control and test sample means and SD of D[4,3] (µm) and 'Size under' (µm) when exposed to warm white light over 24 hours
Table 5.7	Control and test sample means and SD of D[4,3] (µm) and 'Size under' (µm) when exposed to UVA light over 24 hours
Table 5.8	Mean osmolality values for syringes exposed to cool white light
Table 5.9	Mean osmolality values for syringes exposed to warm white light
Table 5.10	Mean osmolality values for syringes exposed to UVA light
Table 5.11	pH of syringes at 0 and 24 hours exposed to cool white light
Table 5.12	pH of syringes at 0 and 24 hours exposed to warm white light
Table 5.13	pH of syringes at 0 and 24 hours exposed to UVA light
Table 6.1	Mean and SD of D[4,3] (µm) and 'Size under' (µm) of control and test samples before (0 Hours) and after (2 Hours) exposure to cool white light
Table 6.2	Mean and SD of D[4,3] (µm) and 'Size under' (µm) of control and test samples before (0 Hours) and after (2 Hours) exposure to warm white light
Table 6.3	Mean and SD of D[4,3] (µm) and 'Size under' (µm) of control and test samples before (0 Hours) and after (2 Hours) exposure to UVA light

Table 6.4	Average osmolality values for samples exposed to cool white light
Table 6.5	Average osmolality values for samples exposed to warm white light
Table 6.6	Average osmolality values for samples exposed to UVA light
Table 6.7	pH of samples before (0 hours) and after (2 hours) exposure to cool white light
Table 6.8	pH of samples before (0 hours) and after (2 hours) exposure to warm white light
Table 6.9	pH of samples before (0 hours) and after (2 hours) exposure to UVA light
Table 7.1	Summary table of mean \pm SD light obscuration of glass MDVs and BD plastic syringes containing ultra-filtered deionised water
Table 7.2	Summary of mean \pm SD light obscuration of syringes containing ultra-filtered deionised water, ultra-filtered deionised water and Vitlipid® N adult, IL10, IL20 and SMOF
Table 7.3	Summary of mean \pm SD light obscuration in syringes containing ultra-filtered deionised water, ultra-filtered deionised water and Vitlipid® N Adult, IL10, IL20, SMOF and SBO

CHAPTER ONE

Introduction

1.1. Introduction to Parenteral Nutrition (PN)

1.1.1. Introduction to stability issues associated with PN

In the past, PN was prepared on wards and administered to patients via a multi-bottle system (Barnett et al. 2009), which was labour intensive and liable to contamination. The shortcomings of this system called for the introduction of an all-in-one (AIO) bag. This was a cost-effective and convenient alternative aimed at decreasing the incidence of infection experienced with the multi-bottle system previously used (Allwood 2000; Barnett et al. 2009; Menne et al. 2008). However, using an AIO bag meant that micronutrients, macronutrients and electrolytes were mixed in the same bag prior to administration causing numerous stability and formulation issues.

In recent years, there has been an increased emphasis in the National Health Service (NHS) to treat patients at home thereby reducing costs and improving clinical outcomes (NHS 2010). An increased interest in home parenteral nutrition (HPN) has prompted researchers to investigate the instabilities of PN components, in order to validate shelf lives and enable more convenient administration in a domiciliary setting.

An area that requires further investigation is the addition of micronutrients (vitamins and trace elements) to PN admixtures. Micronutrients are very reactive and their addition to PN can cause numerous pharmaceutical issues.

Reactions involving micronutrients are dependent on: pH, temperature, container material, relative concentration of reactants and the presence of any other components that are catalytically active (Ribeiro et al. 2011). Due to the reactive nature of micronutrients, they should be introduced close to the time of administration in order to ensure that the integrity of the admixture is retained (Wormleighton & Catling 1998). A better understanding of how vitamins and trace elements interact with their storage environments and other PN components will help to ensure the safe use of them in the future.

1.1.2. Indications of PN

PN should be considered for patients who are malnourished or at risk of malnourishment with inadequate or unsafe oral or enteral intakes. PN should also be considered for patients who are malnourished or at risk of malnourishment with a non-functional or inaccessible GI tracts (NICE 2006).

1.1.3. Administration of PN

PN can be given by central or peripheral routes. In hospital, PN may be given via a dedicated peripherally-inserted central catheter (PICC) (Pikwer et al. 2012), however, the use of these lines has some limitations. Firstly, insertion may be difficult, as many patients who receive PN may have damaged peripheral veins. In addition, some patients, such as those with short bowels, will require large

volumes of PN with a high osmolality, which will exceed the capacity of PICC lines (Staun et al. 2009). Therefore, for short-term treatment (i.e. less than 2 weeks), patients may be fed via a peripheral venous catheter. In long term use (i.e. more than 30 days) the central route is preferred and tunnelled subclavian lines are recommended to reduce infection risk (NICE 2006). Central catheterisation via the subclavian vein is preferred to the jugular vein as it is less likely to result in catheter-related infections (McGee & Gould 2003).

PN can either be administered continuously or cyclically. Continuous PN is given for 24 hours without a break and is the preferred method for severely ill patients. Cyclical PN is administered for less than 24 hours with a break between administrations and should be considered for patients requiring PN for longer than 2 weeks or those requiring routine catheter changes (NICE 2006; Friel & Bistran 1997).

1.1.4. Home Parenteral Nutrition (HPN)

As previously mentioned, the NHS is trying to treat more patients at home to improve patient care and lower costs (NHS 2010). HPN should be considered with those patients who cannot get sufficient nutrition from enteral intake and who are able to receive therapy in a domiciliary setting (Staun et al. 2009). The most recent annual British Artificial Nutrition Survey (BANS) recorded 624 patients receiving HPN and this number is rising each year (Smith et al. 2011).

Patients receiving this service require a large amount of support and teaching before they are treated at home. To ensure patients' regimens are appropriate, HPN patients should have regular anthropometry and biochemistry measurements taken (NICE 2006) with micronutrient status measurements taken every 6 months (Staun et al. 2009).

To allow administration of PN at home the stability of the admixture is a major consideration, to ensure patient safety and that the correct amount of each constituent is given. Expiries of 14 days are preferred to allow convenient delivery of treatments at home and to guarantee the adequate care and safety of patients (Wormleighton & Catling 1998).

1.2. Components of PN

1.2.1. Carbohydrates

Glucose is the carbohydrate form used in PN. It is the major circulating carbohydrate fuel and can be used by many cells in the body (Koletzko et al. 2005b). When circulating glucose levels are high, glucose is converted to glycogen and stored in skeletal muscle and the liver. When circulating glucose levels decrease, glycogen is converted to glucose in a process called glycolysis (Sherwood 2001). When glycogen stores have been exhausted, glucose is synthesised through gluconeogenesis from lactate, glycerol and amino acids.

Hormones control glucose metabolism and their effects can be seen in Table 1.1. Glucagon, cortisol, adrenaline and growth hormone oppose many of the effects stimulated by insulin. In PN, the maximal infusion rates of glucose tend to be around $5\text{mg.kg}^{-1}.\text{min}^{-1}$ (Sobotka 2004).

Table 1.1. The influence of hormones on glucose metabolism and storage.

	Insulin	Glucagon	Cortisol	Adrenaline	Growth Hormone
Glycogenolysis	↓↓	↑↑		↑↑	
Gluconeogenesis	↓	↑	↑↑	↑	↑
Muscle/adipose glucose uptake	↑↑		↓	↓	↓
Glycogen storage	↑	↓	↑	↓	↓
Glucose oxidation	↑		↓	↓	↓

1.2.2. Lipids

Lipids are vital to the body as they provide energy and essential fatty acids, alongside transporting fat-soluble vitamins (Milla 2001). In PN, lipid emulsions have been made to model intestinal chylomicrons with a triglyceride core containing some lipid soluble vitamins and a surface comprised of free cholesterol, phospholipids and other lipid-soluble vitamins (Carpentier 2009). Although these exogenous globules do not share the exact chemical makeup of

an endogenous chylomicron, they still follow the same metabolic pathway. Lipid may account for up to 50% of all non-protein calories in the diet (Sobotka 2004).

1.2.3. Proteins

In PN, protein is delivered to patients as amino acids. There are eight essential amino acids as outlined in Table 1.2. These amino acids are known as essential amino acids as they can only be obtained from the diet (Koletzko et al. 2005a). Other amino acids are essential in particular conditions, for example histidine is required for the growth of infants and cysteine is required in liver disease, as it cannot be synthesised in the body (Fürst et al. 2001). Proteins are required for many bodily functions such as, growth and maintenance, energy, maintaining fluid and electrolyte balance, maintaining acid-base balance, transportation and production of enzymes, hormones and antibodies. In a healthy young adult a daily protein intake of around 0.75g.kg^{-1} is desirable. During illness, convalescence or in the elderly quantities of $1\text{-}1.5\text{g.kg}^{-1}$ are recommended (Sobotka 2004).

Table 1.2. Classes of amino acids adapted from Koletzko et al. (2005a)

Essential	Conditionally Essential	Non Essential
Histidine	Arginine	Alanine
Isoleucine	Cysteine	Aspartic Acid
Leucine	Glycine	Asparagine
Lysine	Proline	Glutamic Acid
Methionine	Tyrosine	Glutamine
Phenylalanine		Serine
Threonine		
Tryptophan		
Valine		

1.2.4. Water and electrolytes

Water and electrolytes are essential components in the provision of PN care to patients. Water is a carrier for nutrients and is a large proportion of human body mass (Koletzko et al. 2005c). Some of the major electrolytes required in the body are calcium, magnesium, chloride, sodium, potassium and phosphorus. Regular monitoring of fluid and electrolyte levels are essential for maintaining physiological function (Sobotka 2004).

1.2.5. Micronutrients

Micronutrients are an essential part of the human diet and their roles were discovered through the emergence of deficiency states across the world (Shenkin 2006b). Deficiency states gave rise to a variety of conditions, such as scurvy in vitamin C deficiency (Koletzko et al. 2005d). Micronutrients can be split into two groups, vitamins and trace elements. Their biological functions include: co-factors in metabolism, co-enzymes in metabolism, genetic control and antioxidant activity (Shenkin 2006a).

1.3. Vitamins

Casimir Funk published a paper in 1912 presenting vitamin theory. Initially he called them vital amines, which was subsequently changed to vitamins and over time was shortened to vitamins following discoveries that the group did not always have an amine component. Vitamins are essential for survival and can be divided into two groups: fat-soluble and water-soluble vitamins (Combs 1998).

1.3.1. Fat-soluble vitamins

1.3.1.1. Vitamin A

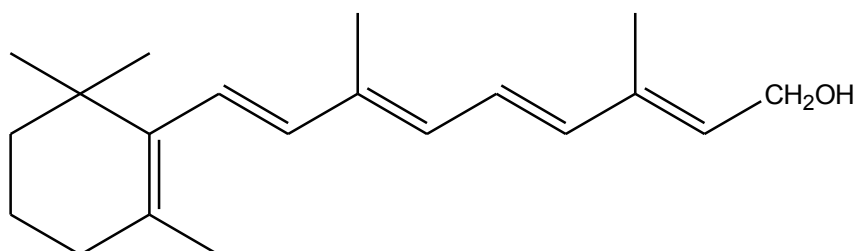


Figure 1.1. Chemical structure of all-trans-retinol

Vitamin A refers to a family of complex molecules including retinol (Figure 1.1), retinal and retinoic acid. Vitamin A has a number of roles within the body. Most notably, it maintains the structure of the cornea, converts light into neural signals within the eye, regulates structural protein gene expression and is involved with enhancing the immune system (Sobotka 2004). Deficiency of vitamin A leads to conditions such as night blindness (xerophthalmia), corneal scarring and eventual blindness (Tanumihardjo 2011). In PN, retinol palmitate is the member of the vitamin A family that is most commonly used.

1.3.1.2. Vitamin D

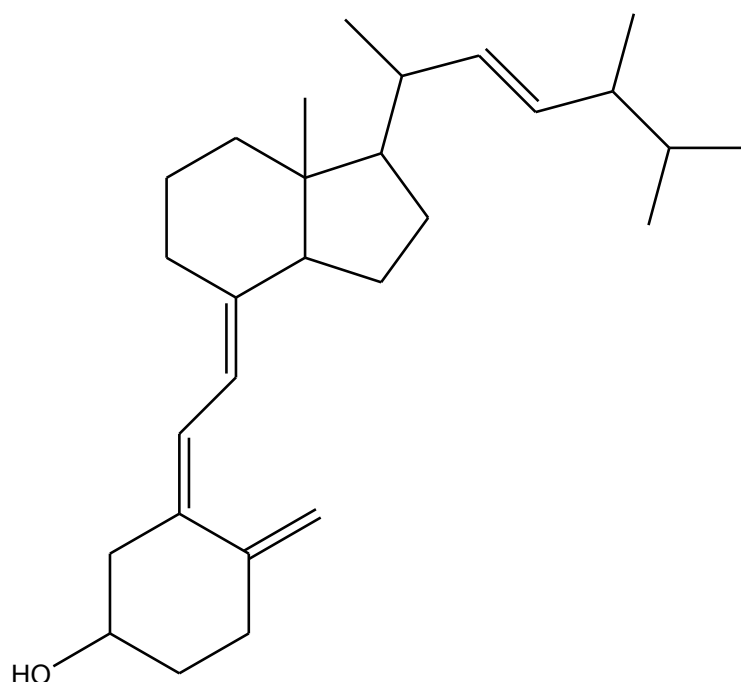


Figure 1.2. Chemical structure of ergocalciferol

Vitamin D can be obtained through the diet or uniquely it can be synthesised through a photolytic reaction in the skin, where 7-dehydrocholesterol is converted to pre-vitamin D₃ in the presence of light (Holick 2012).

In the body, vitamin D has to be converted to its active form. Firstly, it is 25-hydroxylated in the liver where it is converted to 25-OH-D₃ by a cytochrome P450 enzyme yet to be positively identified. 25-OH-D₃ is biologically inactive and so it is metabolised to the hormone 1,25-(OH)₂D₃ in the kidneys. This activated form of vitamin D has an essential function in regulating calcium and phosphorus. It is thought to have a number of other effects in the body

including reducing the incidence of autoimmune disease, colorectal cancer and heart attacks (DeLuca 2009).

Vitamin D deficiency can result from insufficient irradiation of the skin, inadequate intake in diets or from problems associated with disease states. Deficiency in this vitamin can lead to skeletal diseases such as rickets in children and osteomalacia in adults (Pittas et al. 2010). In PN, ergocalciferol (Figure 1.2) is the form of Vitamin D that is used.

1.3.1.3. Vitamin E

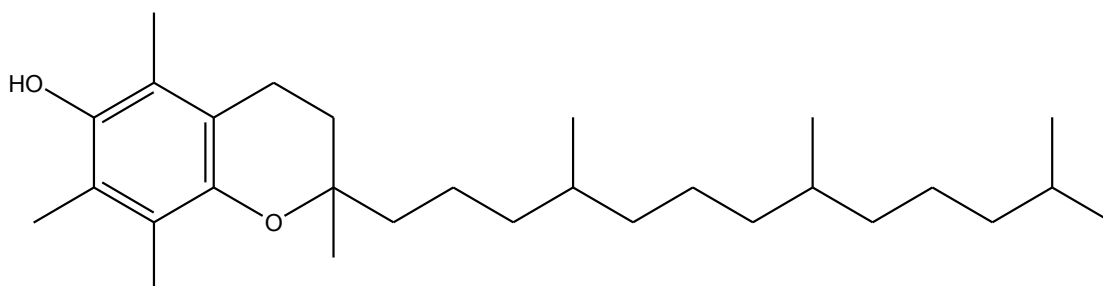


Figure 1.3. Chemical structure of α -tocopherol

Vitamin E is a naturally occurring antioxidant that quenches free radicals within the body. Maintaining adequate vitamin E levels is thought to help prevent conditions associated with oxidative stress. Vitamin E's most important known function is to protect polyunsaturated fatty acids (PUFAs) in cell membranes from peroxidation by free radicals (Biesalski 2009). This protective function has

been utilised in PN containing fish oils. Higher concentrations of vitamin E are added to these admixtures to prevent the oxidation of fish oils (Waitzberg et al. 2006).

The main form of vitamin E used in PN is α -tocopherol (Figure 1.3). α -tocopherol commonly presents as a mixture of eight isomers, some of which have higher activity than others (Biesalski 2009; Allwood & Kearney 1998).

1.3.1.4. Vitamin K

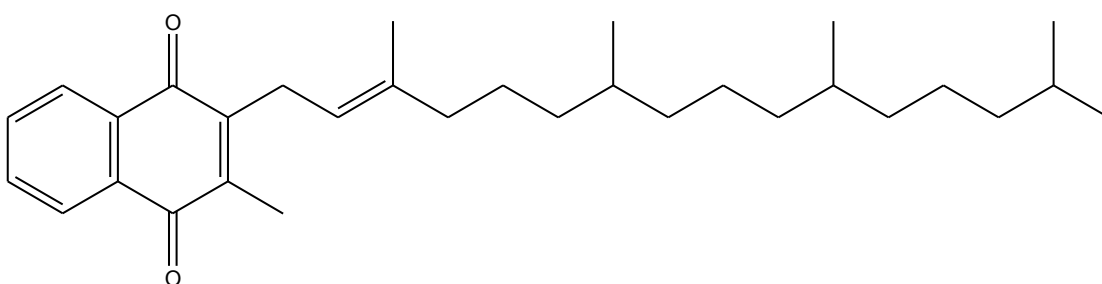


Figure 1.4. Chemical structure of phylloquinone

Vitamin K is a cofactor essential for the production of γ -carboxy glutamic acid residues. Deficiency of vitamin K prevents the synthesis of coagulation factors, resulting in bleeding syndromes. Vitamin K also has some role in vascular and bone health (Shearer 2009). In PN, phylloquinone (vitamin K₁), a naturally occurring extract synthesised in plants, is most commonly used (Billion-Rey et al. 1993). Phylloquinone (Figure 1.4) is found naturally in lipid emulsions, with some emulsions having higher concentrations than others (Shearer 2009).

1.3.2. Water soluble vitamins

1.3.2.1. Ascorbic acid

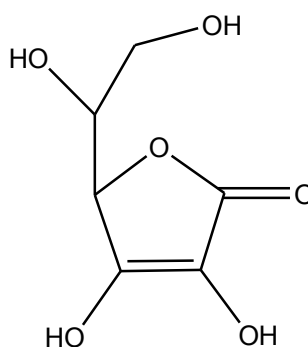


Figure 1.5. Chemical structure of ascorbic acid

In the body, ascorbic acid (Figure 1.5) is a strong antioxidant that quenches reactive nitrogen and oxygen species. In addition, it is an important co-factor for a number of enzymes and is involved with the synthesis of compounds such as dopamine and serotonin (Berger 2009). Alongside its antioxidant role, ascorbic acid is thought to support redox recycling of α -tocopherol (Rümelin 2009). Mammals are part of a small group of animals that are unable to synthesise ascorbic acid from glucose. This is owing to an absence of L-gulonolactone oxidase, which converts L-gulonolactone into L-ascorbic acid towards the end of the synthesis reaction (Combs 1998).

1.3.2.2. Thiamine

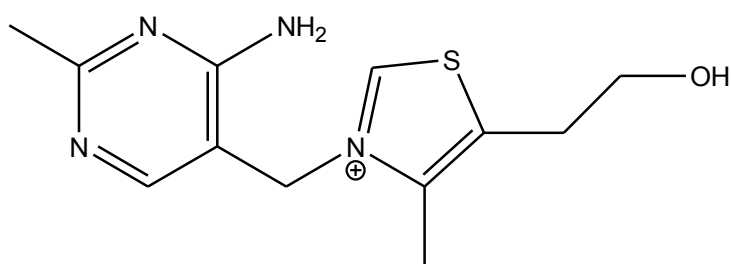


Figure 1.6. Chemical structure of thiamine

Thiamine (Figure 1.6) has an important role in the metabolism of carbohydrates and neural function (Sobotka 2004). In the body, it is converted to thiamine diphosphate (also known as thiamine pyrophosphate (TPP)) and is used by several enzymes as an important co-factor in the decarboxylation of α -keto acids and keto sugars (Baumgartner et al. 1997; Francini-Pesenti et al. 2009). Signs of deficiency include muscle weakness, anorexia, weight loss and mental alteration. These symptoms are better known under the umbrella term beriberi (Sobotka 2004).

1.3.2.3. Riboflavin

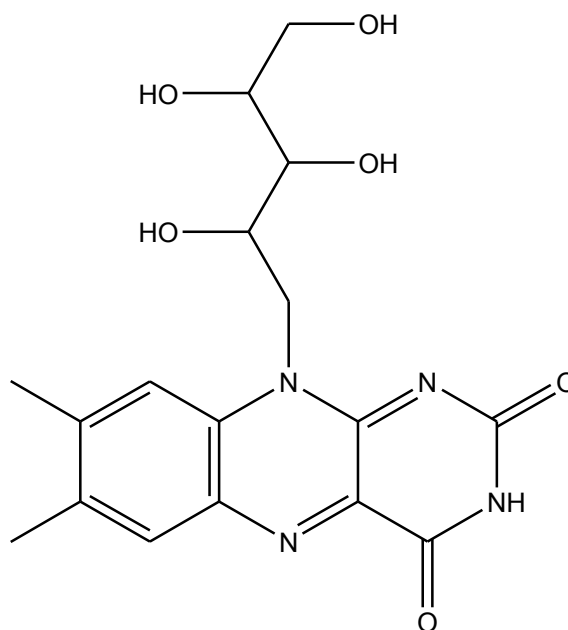


Figure 1.7. Chemical structure of riboflavin

Riboflavin (Figure 1.7) is a vital component of two co-enzymes, flavine mononucleotide (FMN) and flavine-adenine dinucleotide (FAD). These co-enzymes are important for redox-reactions occurring in a number of metabolic pathways (Sobotka 2004). Deficiency of riboflavin results in conditions such as dermatitis, stomatitis and anaemia (Koletzko et al. 2005d). Subclinical riboflavin deficiency is not uncommon and some children may experience subnormal growth as a result (Combs 1998).

1.3.2.4. Nicotinamide

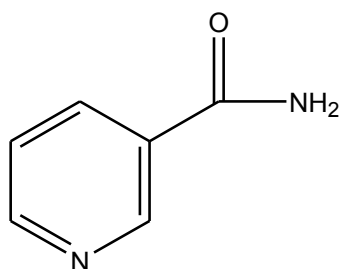


Figure 1.8. Chemical structure of nicotinamide

Nicotinamide (Figure 1.8) is used in the body to synthesise NAD(H) and NADP(H), which have key roles in numerous metabolic processes. A large amount of this vitamin is synthesised from one of its precursors, an amino acid called tryptophan (Sriram et al. 1996). Deficiency states usually result from an unbalanced diet with respect to nicotinamide, tryptophan and frequently pyridoxine, which is involved in the synthesis of nicotinamide from its amino acid precursor (Combs 1998). Deficient individuals usually suffer from pellagra, which is a condition that is characterised by symptoms such as dermatitis, dementia and diarrhoea (Austin & Stroud 2007).

1.3.2.5. Pantothenic acid

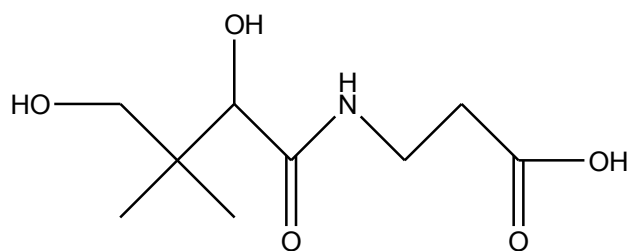


Figure 1.9. Chemical structure of pantothenic acid

Pantothenic acid (Figure 1.9) has a vital role in metabolism as it is an integral part of acyl-carrier protein (ACP) and coenzyme A (CoA). The deficiency of this vitamin results in impairments of metabolism, for example, reduced lipid synthesis and energy production. Due to the wide distribution of pantothenic acid in foods, deficiency is relatively rare (Combs 1998).

1.3.2.6. Pyridoxine

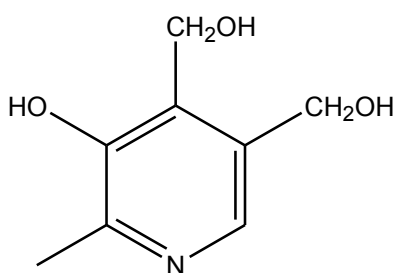


Figure 1.10. Chemical structure of pyridoxine

Pyridoxine (Figure 1.10) is a pre-cursor for pyridoxal phosphate (PLP), which is used as a co-enzyme for a wide range of enzymes and is primarily involved with amino acid metabolism (Sobotka 2004; Renwick & Walker 2008). The broad nature of its function means that it is important in diverse areas such as cognitive development, immune function and steroid hormone activity. In humans, symptoms exhibited in pyridoxine deficiency states can be quickly corrected through vitamin administration (Combs 1998). Symptoms that may be presented in deficiency states include: hypochromic anaemia, convulsions, and glossitis (Koletzko et al. 2005d; Austin & Stroud 2007).

1.3.2.7. Biotin

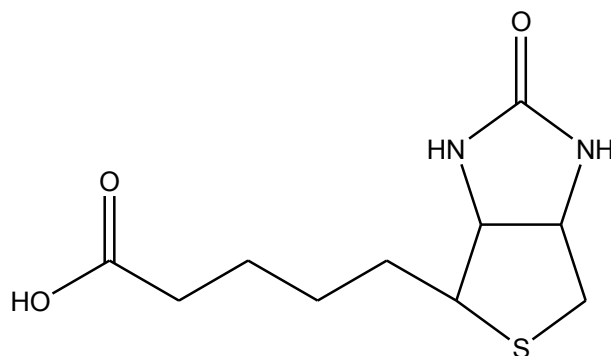


Figure 1.11. Chemical structure of biotin

Biotin (Figure 1.11) deficiency was first identified with the discovery that supplementation of biotin could prevent egg white injury, a deficiency state arising from eating raw egg whites. Later it was recognised that avidin, an

enzyme found in egg whites, strongly binds to biotin preventing its utilisation in the body. Following this, biotin was categorised as a vitamin (Combs 1998).

In the body, biotin-containing carboxylases are involved in the metabolism of glucose, lipids and some amino acids (Combs 1998). Deficiency of biotin is rare as it is widely distributed in foods and it is synthesised in the intestinal microflora. Conditions associated with biotin deficiency include dermatitis, anorexia and depression (Austin & Stroud 2007).

1.3.2.8. Folic acid

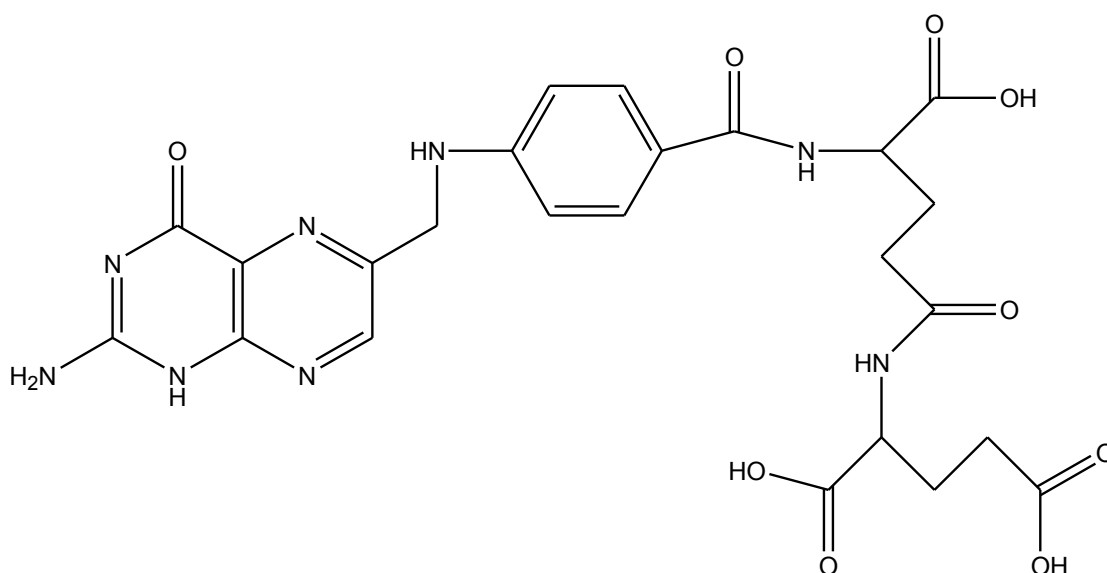


Figure 1.12. Chemical structure of folic acid

Folic acid (Figure 1.12) is used as a co-enzyme for many reactions such as the synthesis of nucleic acids and amino acid inter-conversions (Sobotka 2004).

Deficiency in folic acid results in impaired DNA and RNA biosynthesis causing reduced cell division, which can be seen clinically as poor growth, anaemia and

dermatological conditions (Combs 1998). Deficiency of folic acid can lead to an increase in homocysteine production, which increases the likelihood of coronary artery disease (Sobotka 2004; Austin & Stroud 2007).

1.3.2.9. Cyanocobalamin

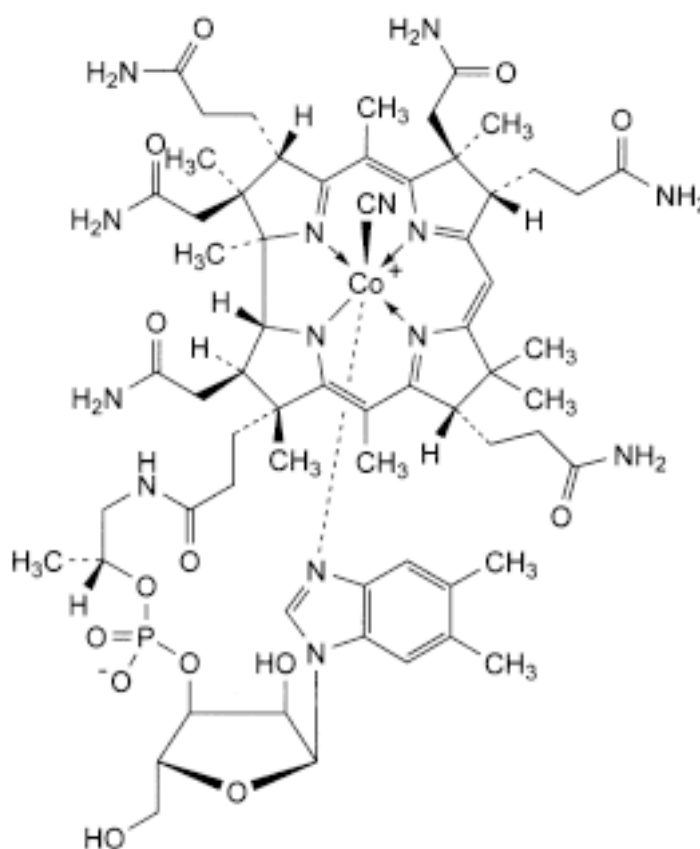


Figure 1.13. Chemical structure of cyanocobalamin (Anon. 2013)

Cyanocobalamin (Figure 1.13) is critical for cellular division and growth. Deficiency is not common and the vitamin can be stored in the body for long periods of time in conditions of deprivation (Combs 1998). Like folic acid,

deficiency of cyanocobalamin can result in anaemia and after a prolonged period, can produce neurological symptoms in a quarter of those affected (Austin & Stroud 2007; Sobotka 2004; Compher et al. 2002).

1.4. Trace elements

1.4.1. Zinc

Zinc is an essential component of the diet that has an important role in many enzymes, including DNA polymerase, RNA polymerase, reverse transcriptase and tRNA synthetase (Jeejeebhoy 2009). It is found routinely in PN admixtures not only as an addition but also as a contaminant. Potential sources of contamination include manufacturing methods, packaging and impurities of the chemicals used in the production of PN (Pluhator-Murton et al. 1999).

1.4.2. Copper

Copper is used by a wide array of enzymes involved in many metabolic processes. Copper deficiency is rare but can result in conditions such as neutropenia, anaemia and bone abnormalities (Shike 2009). It is an essential trace element, which like zinc, is found as a contaminant as well as an additive in PN admixtures (Pluhator-Murton et al. 1999).

1.4.3. Selenium

Selenium is required for the synthesis of an amino acid called selenocysteine. It is found in a number of proteins grouped as the selenoproteins, which have many functions within the body. The variety of functions it has means deficiency of selenium presents in a number of clinical conditions, such as skeletal myopathy, cardiomyopathy and abnormalities in skin and hair (Shenkin 2009).

1.4.4. Manganese

Manganese is an essential trace element, which is used in the body as a cofactor for a number of enzyme reactions. It is found in a number of metalloenzymes such as arginase and glutamine synthetase. Manganese deficiency is uncommon. PN treatment can cause hypermanganesia, due to large amounts of manganese being infused, contributing to PN-related cholestasis (Reimund et al. 2000). This may be an issue for long-term home treated and paediatric PN patients who may be more susceptible to manganese accumulation as they are only monitored every 3-6 months (NICE 2006). Hypermanganesia can cause side effects such as insomnia, headaches and loss of coordination (Hardy 2009).

1.4.5. Molybdenum

Whether molybdenum is an essential trace element is still undecided, however, it is known that it has a functional role in xanthine oxidase and sulfite oxidase. It has a low level of toxicity and deficiency is uncommon (Leung 1995).

1.4.6. Iron

Iron is found mainly in haemoglobin (around 60% of total iron in the body), which is vital for the transport of oxygen from the lungs to metabolic tissues. Iron is also present in myoglobin, cytochrome P-450 among a number of other enzymes. The most common side effect of low iron is anaemia (Forbes 2009).

1.4.7. Chromium

Chromium is involved in regulating insulin action. In its presence, less insulin is required as chromium increases its activity. It is routinely added to PN, however, there have been ongoing concerns with regards to infusion of excessive chromium. Increased concentrations of chromium are thought to have detrimental effects on the kidneys (Moukarzel 2009) and may contribute to chromium mutagenesis and carcinogenesis (Leung 1995).

1.4.8. Iodine

Iodine is found in hormones produced by the thyroid gland and is the main component of thyroxine (T4) and triiodothyronine (T3). Therefore, deficiency of iodine has multiple adverse effects on the growth and development of individuals and can cause conditions such as cretinism (Zimmermann 2009).

1.5. Vitamin, Trace Element and Electrolyte Requirements

The reference nutrient intakes for males and females between 19-50 years are described in Table 1.3. and 1.4.

Table 1.3. Reference nutrient intakes of vitamins for male and females between 19-50 years (Department of Health 2009).

Vitamin	Males 19-50 years	Females 19-50 years
Vitamin A	700 µg/day	600 µg/day
Vitamin D	-	-
Vitamin E	Above 4 mg/day	Above 3 mg/day
Vitamin K	1 µg/kg/day	1 µg/kg/day
Ascorbic Acid	40 mg/day	40 mg/day
Thiamine	1 mg/day	0.8 mg/day
Riboflavin	1.3 mg/day	1.1 mg/day
Niacin	17 mg/day	13 mg/day
Pantothenic acid	3-7 mg/day	3-7 mg/day
Pyridoxine	1.4 mg/day	1.2 mg/day
Biotin	10-200 µg/day	10-200 µg/day
Folate	200 µg/day	200 µg/day
Cyanocobalamin	1.5 µg/day	1.5 µg/day

Table 1.4. Reference nutrient intake for trace element and electrolytes in males and females aged between 19-50 years (Department of Health 2009)

Trace elements & Electrolytes	Males 19-50 years	Females 19-50 years
Calcium	17.5 mmol/day	17.5 mmol/day
Phosphorus	17.5 mmol/day	17.5 mmol/day
Magnesium	12.3 mmol/day	10.9 mmol/day
Sodium	70 mmol/day	70 mmol/day
Potassium	90 mmol/day	90 mmol/day
Chloride	70 mmol/day	70 mmol/day
Iron	160 µmol/day	260 µmol/day
Zinc	145µmol/day	110 µmol/day
Copper	19 µmol/day	19 µmol/day
Selenium	0.9 µmol/day	0.8 µmol/day
Iodine	1.0µmol/day	1.1 µmol/day

1.6. Stability and compatibility of vitamins and trace elements

1.6.1. Vitamins

1.6.1.1. Vitamin A

Retinol (vitamin A) is widely regarded as the most light-sensitive vitamin in PN admixtures. When exposed to light, retinol undergoes extensive photo-degradation (Dahl et al. 1986; DeRitter 1982; Allwood & Martin 2000; Billion-Rey et al. 1993; Allwood 1982b). The intensity and wavelengths of these light sources determines the rate of this degradation reaction (Allwood & Martin 2000; Allwood 1982b), however, the exact mechanism is still not fully understood (Allwood 1982b; Allwood & Plane 1984). Allwood and Plane (1986) reported that retinol was liable to degradation when subjected to wavelengths below 400 nm and that maximum degradation occurred in the UVA range between 330-350 nm. These wavelengths are commonly found in sunlight and not in artificial light sources, which only emit small amounts of wavelengths below 400 nm (Allwood 1982b). Since the introduction of AIO bags, there have been some investigations into protective effects of lipid emulsions against these types of light. There has been some division of opinions (Billion-Rey et al. 1993; Haas et al. 2002) but the addition of lipid emulsions seems to provide insufficient protection to photo-sensitive vitamins and the use of light protection is recommended (Allwood et al. 2000). It is important to note that since these studies were conducted lighting preferences have changed, with

hospitals and homes choosing to use energy saving bulbs and there has been no research into these new light sources.

Another stability issue that has been reported is the absorption of retinol onto the bag and administration set materials, which prevented patients from receiving their intended doses. This problem has been reduced through the use of a palmitate rather than an acetate ester (Allwood & Martin 2000). In addition, the introduction of new tubing materials containing polyolefin has further reduced absorption as it is free of PVC, plasticisers, adhesives or latex (Henton & Merritt 1990).

Peroxides generated by lipid emulsions are another potential source of degradation. Lipid emulsions that contain PUFAs are liable to peroxidation (Mühlebach & Steger 1998). Vitamin E scavenges these free radicals reducing their interaction with fatty acids (Skouroliahou et al. 2008), however, peroxidation can still occur to a certain extent. Guidetti and colleagues (2008) have investigated the impact of lipid emulsion compositions on peroxidation-mediated vitamin degradation. They found that following light protected storage at room temperature for 24 hours, retinol loss was significantly reduced in soybean-medium chain triacylglycerol oil-based emulsions when compared to olive/soybean oil-based emulsions and soybean oil-based emulsions. Further studies are needed to determine the relationship between lipid emulsion composition and vitamin stability.

1.6.1.2. Vitamin D

As discussed by Allwood and Kearney (1998), the current literature available on vitamin D is very limited. Only one study was found, which showed a 32% loss of vitamin D following a 24-hour infusion. Samples were taken at various sites within the infusion set-up and compared. The differences in concentration suggested that vitamin D may bind to the container and administration set materials (Gillis et al. 1983).

In recent years there has been a growing interest in vitamin D, particularly with the emergence of urban rickets. As a result, there have been calls to increase the Recommended Daily Allowance (RDA) of vitamin D especially in Canada and United States of America (DeLuca 2008). Increases in vitamin D dosing in PN may have important stability implications.

1.6.1.3. Vitamin E

Vitamin E reacts with oxygen in the presence of light. The concentration of oxygen as well the wavelength and intensity of light determines the rate of this reaction. Vitamin E is known to be more susceptible to degradation at wavelengths between 285 nm and 305 nm (Drott et al. 1991).

A number of studies have shown that if vitamin E is protected from light it remains relatively stable (Dahl et al. 1986; Allwood & Martin 2000; Lavoie et al. 2007; Billion-Rey et al. 1993). Allwood and Martin (2000) investigated light exposure on vitamin E concentration in multi-layered bags. Their experiments showed that regardless of light exposure, vitamin E was not significantly degraded when oxygen levels in the PN bag were reduced. In addition, vitamin E oxidation was reduced in the presence of ascorbic acid as the two vitamins compete for oxygen.

Guidetti et al. (2008) reported that lipid composition can also influence the rate of tocopherol degradation. Similar to retinol, the loss of both of the members of the tocopherol group examined (α -tocopherol and γ -tocopherol), were significantly reduced in admixtures containing soybean-medium chain triacylglycerol oil-based emulsions in comparison with olive/soybean oil-based emulsions and soybean oil-based emulsions.

As mentioned previously, vitamin E commonly presents as a mixture of isomers and the stability profiles for each of the isomers have not been determined (Kearney et al. 1998). In lipid emulsions containing fish oils there are larger amounts of tocopherol in an attempt to reduce oxidation. The use of these increased levels necessitates a thorough investigation on the stability of all tocopherol isomers (Waitzberg et al. 2006).

1.6.1.4. Vitamin K

Phylloquinone, or vitamin K₁, is known to be light sensitive but in PN it is considered to be relatively stable both in the absence and presence of lipid emulsions. Nevertheless, there is very little information regarding its stability in the literature. Billion-Rey and colleagues (1993) reported a 50% loss of phylloquinone following three hours of exposure to strong sunlight. Whereas Schmutz and colleagues (1992) found more conservative degradation, with 5.9-8.5 % loss over 4.5 hours in artificial daylight.

1.6.1.5. Ascorbic acid

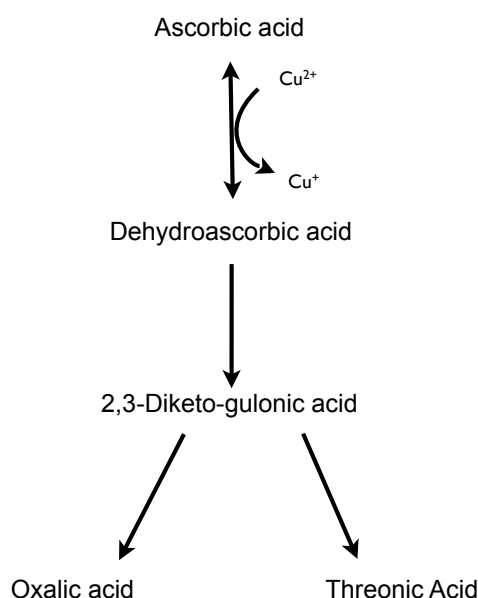


Figure 1.14. The degradation cascade of ascorbic acid in PN adapted from Allwood and Kearney (1998)

Ascorbic acid degradation occurs as described in Figure 1.14. In the presence of oxygen, ascorbic acid is converted in a reversible reaction to an equally biologically active product called dehydroascorbic acid (Allwood 1999). Dehydroascorbic acid can be degraded further by means of a hydrolysis reaction producing 2,3-diketo-gulonic acid, which is considered to be biologically inactive. Further hydrolysis of this intermediate produces oxalic and threonic acid (Allwood 1984b). The amount of oxygen in the medium directly influences the rate of ascorbic acid degradation (Dahl et al. 1986). Removal of free oxygen from an admixture minimises the conversion of ascorbic acid to dehydroascorbic acid, hence limiting the resultant cascade (Allwood et al. 1992). Oxygen present in PN bags can originate from a number of sources such as, residual head space formed during the filling and sealing of bags, diffusion of oxygen through the bag walls during storage and dissolved oxygen in additives and infusions (Allwood & Kearney 1998). The use of multi-layered bags has significantly improved ascorbic acid stability as it reduces the amount of oxygen diffusion (Hardy & Mari 1988). However, this has not completely eliminated the problem as these multi-layered bags prevent oxygen, transferred into the bag during filling and sealing, from escaping and therefore leave it free to react with ascorbic acid.

Trace elements such as copper, and to a lesser degree, zinc, ferric and manganese ions can catalyse ascorbic acid oxidation to dehydroascorbic acid (DeRitter 1982). In the case of copper, the oxidation of ascorbic acid causes the concomitant reduction of copper from the cupric (II) to cuprous (I) form (Hardy

et al. 2009). The inclusion of these trace elements inevitably speeds up the degradation cascade producing threonic and oxalic acid. It is important to note that these ions can also be found as trace contaminants in other PN products, thus enhancing the reaction (Hardy et al. 2009). Allwood (1984b) reported that the catalytic activity of copper can be inhibited by the amino acid cysteine. The addition of cysteine to PN may help slow the degradation of ascorbic acid, however, there are reports of precipitates of copper sulphide when cysteine is added to admixtures (Allwood et al. 1998).

Physical conditions can also influence ascorbic acid degradation in PN. Increases in temperature can accelerate degradation rates (Gibbons et al. 2001; Proot et al. 1994; Smith, Canham, Kirkland, et al. 1988) and pH values in excess of 4 make ascorbic acid liable to oxidation (Smith, Canham & Wells 1988; Berger 2009).

The degradation products of ascorbic acid may also cause problems. Oxalic acid can interact with free calcium forming calcium oxalate precipitate (Ribeiro et al. 2011). The impact of its formation is unknown in adults but it can cause renal calcification in neonates (Allwood 1999). Further research is required on the risks posed by calcium oxalate precipitates.

1.6.1.6. Thiamine

Previously, thiamine was degraded predominantly through a reduction reaction. Sodium metabisulphite, an antioxidant regularly used in older amino acid formulations, reacted readily with thiamine (Allwood 1982a). In this reaction, thiamine is cleaved by sulphite into thiazole and pyrimidine (Scheiner et al. 1981). The discovery that the stability of thiamine was directly related to the sodium metabisulphite concentration resulted in its removal from amino acid infusions (Bowman & Nguyen 1983; Scheiner et al. 1981). Now, sodium metabisulphite is not routinely used, with no UK amino acid infusions containing it. Since its removal thiamine stability has improved (Gallitelli 1995).

1.6.1.7. Riboflavin

Riboflavin is photo-sensitive vitamin, degrading readily on exposure to light sources (DeRitter 1982; Smith, Canham & Wells 1988; Gallitelli 1995; Allwood 1984a; Chen et al. 1983). Riboflavin is converted predominantly to luminochromo and luminoxanthin in the presence of light and oxygen (Ribeiro et al. 2011). A recent study by Mirkovic and colleagues (2011) has shown some significant degradation of riboflavin following 12 hours of exposure to sunlight. In contrast, other studies have shown very limited degradation on exposure to light sources over longer periods of time (Dahl et al. 1986; Ribeiro et al. 2011),

however, the illumination of the room has not been stated in any of these studies therefore the results are not quantifiable.

Another characteristic of riboflavin is that it can also act as a photochemical sensitizer (Silva et al. 1994). Riboflavin can be excited to its triplet state on exposure to light sources and can then react with substrates directly, or produce reactive oxygen species. The production of these reactive oxygen species may result in the oxidation of other PN components. Investigations into riboflavin's role as a photosensitiser in PN are on going (Huvaere et al. 2010; de La Rochette et al. 2000; Natera et al. 2011; Shen & Ji 2008; Silva et al. 1998).

1.6.1.8. Pyridoxine

Pyridoxine is another light sensitive vitamin, although, there is a limited amount of information in the literature on its stability in PN (DeRitter 1982; Smith, Canham & Wells 1988). Dahl and colleagues (1986) have reported that pyridoxine is stable in light-protected admixtures when stored at 2-8 °C for up to 96 hours. Similarly, a recent study reported that pyridoxine was stable when stored with and without light protection between 4°C and 25°C (Ribeiro et al. 2011). Conversely, another study reported an 86% loss following storage in direct sunlight for a period of 8 hours (Chen et al. 1983). Once again, the room illuminations in these experiments were not stated therefore it is difficult to deduce the extent degradation caused by these lights.

1.6.1.9. Folic acid

From the limited research available on folic acid stability it seems the main route of instability occurs when pH is adjusted. Folic acid injections are formulated at pH 8.0 because there is a risk of precipitation at lower pHs (Allwood 1984a). One study reported that provided folic acid is subjected to pHs in excess of 5.0 it remains in solution (Barker et al. 1984). PN admixtures are usually formulated between pH 5.0-6.0, therefore, folic acid should not precipitate.

There was some suggestion that folic acid may adsorb onto bag materials and one study found a 33% loss following 42 days storage in PVC bags (Lee et al. 1980). However, following studies found that folic acid was compatible in PVC (Barker et al. 1984) and ethyl vinyl acetate (EVA) bags (Dahl et al. 1986).

1.6.1.10. Cyanocobalamin, nicotinamide, pantothenic acid and biotin

There is virtually no information available in the literature regarding the stability of cyanocobalamin, nicotinamide, pantothenic acid and biotin. Only one study was found, reporting that all four vitamins are stable in PN when stored for a period of 96 hours (Dahl et al. 1986). Further research into these vitamins is required.

1.6.2. Trace elements

1.6.2.1. Zinc

Zinc is thought to be compatible with PN admixtures for 24 hours at between 2-6 °C (Allwood 1983). However, the information on its compatibility is limited.

1.6.2.2. Copper

Copper shows some incompatibility with certain amino acid mixtures and it is thought that copper can react with cysteine to form copper sulphide precipitate (Allwood et al. 1998). As previously discussed, copper can also act as a catalyst and increase the oxidation of vitamin C.

1.6.2.3. Selenium

Selenium is supplied in PN as anhydrous sodium selenite. At low pH (less than 5) ascorbic acid can change selenite ions to insoluble elemental selenium, thereby making it biologically unavailable in PN admixtures (Allwood & Greenwood 1992).

1.6.2.4. Manganese

A study by Allwood (1983) showed that after 24 hours the amount of manganese was the same as the standard when stored between 2-6°C and protected from light. Allwood also reported that after four months there was no evidence of precipitation when Synthamin® was used as an amino acid source, suggesting manganese is soluble and compatible with PN admixtures (Allwood 1983).

1.6.2.5. Molybdenum

Information on the compatibility of molybdenum is scarce. It seems very little is known about its compatibility in PN solutions.

1.6.2.6. Iron

There is limited information on the compatibility of iron within PN. There have been reports of precipitation when iron is added to PN mixtures. However, other reports show iron to be compatible with PN mixtures for a duration of between 18 and 24 hours (Kwong & Tsallas 1980). There is a suggestion that iron precipitation is dependent on the amount of ascorbic acid present in PN (Allwood 1984a). Further investigation is required. Iron may also affect lipid stability as it is a trivalent ion, which can destabilise emulsions (Forbes 2009;

Manning & Washington 1992). This may also occur to a lesser extent with other divalent ions (Skouroliakou et al. 2008) such as zinc and copper.

1.6.2.7. Chromium

It has been reported that chromium is compatible with PN admixtures for 48 hours at ambient temperature (Allwood et al. 1998). When stored between 2-6°C chromium was found to be soluble and compatible for 4 months using Synthamin as the amino acid source (Allwood 1983).

1.6.2.8. Iodine

Iodine has been shown to be compatible with PN mixtures after being stored for 24 hours at either ambient temperature or 4°C (Allwood & Kearney 1998). There is limited information on the duration iodine is compatible with PN admixtures.

1.7. Methods developed to increase stability and compatibility of micronutrients in PN

An advancement that has decreased Vitamin E and C loss is the production and use of multilayered bags. EVA (Ethyl Vinyl Acetate) bags are known to be porous to oxygen, which reacts with Vitamin E and Vitamin C as described previously. The introduction of multilayered bags has reduced the amount of oxygen interacting with these vitamins, thereby improving stability (Allwood & Martin 2000; Dupertuis et al. 2005).

A number of measures have been taken in response to the discovery of the light sensitivity exhibited by some constituents of PN (e.g. retinol and riboflavin). Various coloured bag covers are available, which prevent known problematic light wavelengths from coming into contact with the PN admixture. In practice, these are not always used and may not provide universal protection of all vitamins.

As described earlier, vitamin loss due to photodegradation occurs not only in the PN bags but also the giving sets. One paper (Kirk 1985) examines the use of a prototype amber giving set that reduces the vitamin A degradation significantly. However, it is important to be able to visibly see the contents when administering PN to look for any formulation problems, such as precipitates, therefore, an amber giving set may not be appropriate. Other

measures such as timing when PN is administered or positioning away from direct sunlight decreases the degradation of light sensitive vitamins (Allwood & Martin 2000).

There has been a drive to use different types of micronutrients in order to ensure stability. Most notably is the use of retinol palmitate instead of retinol acetate which has increased the amount of vitamin A received by the patient as it is not being absorbed onto the bags and giving sets (Allwood 1982b).

1.8. Thesis Aim and Objectives

1.8.1. Thesis aim

To investigate and understand the stability issues associated with vitamins in PN admixtures when exposed to types of artificial light commonly found in hospitals and homes, thereby improving delivery systems and current clinical practices.

1.8.2. Thesis objectives

- To design and validate a stability-indicating High-Pressure Liquid Chromatography (HPLC) assay, which can be used for the simultaneous identification and quantification of both fat and water-soluble vitamins.
- To determine the physico-chemical stability of vitamins when exposed to artificial light sources over 24 hours at room temperature.
- To determine physico-chemical stability of vitamins exposed to artificial light over 24 hours at room temperature following 6 days of light-protected storage at 2-8 °C.
- To investigate whether commonly used lipid emulsions have any protective effects on light-sensitive vitamins instability.
- To determine the extent of vitamin degradation occurring in administration sets during simulated delivery of PN when exposed to artificial light sources.
- To investigate the protective effects of lipid emulsions on vitamin degradation when exposed to artificial light sources during simulated delivery of PN through administration sets.
- To understand the mechanisms underlying any protective effects on vitamin degradation exhibited by lipid emulsions.

CHAPTER TWO

Designing a Novel Validated Stability-Indicating
HPLC assay for Fat and Water-Soluble Vitamins

2.1. Introduction

This chapter is concerned with the development of a HPLC assay, which will be used for the simultaneous quantification of fat and water-soluble vitamins to determine whether or not they have degraded.

2.2. HPLC

HPLC separates analytes by partitioning compounds between a mobile and stationary phase. Commonly, reversed phase HPLC is used, which has a non-polar stationary phase and a polar mobile phase. As the analyte passes through the column under high pressure, each of the compounds in the analyte will partition between the mobile and stationary phase and move through the column at different speeds. Once the compounds pass through the column they can be identified using various detectors (Snyder et al. 1997).

Several methods have been used in the past to quantify fat and water-soluble vitamins found in various mediums (Dionex 2009; Moreno & Salvadó 2000; Khan et al. 2010; Chen et al. 2006; Heudi et al. 2005; Papadoyannis et al. 1997; Ciulu et al. 2011; Momenbeik et al. 2010; Supelco 2000; Klejdus et al. 2004). However, there are very few simplistic methods that identify vitamins in PN formulations (Allwood et al. 1992; Drott et al. 1991; Dahl et al. 1986; Kearney et al. 1995), with even fewer able to identify fat and water soluble vitamins simultaneously. Many of these assays use a number of different solvents and

require expensive detectors that may not be accessible to the majority of researchers and clinicians. Nevertheless, two appropriate methods were found, which use readily available materials and equipment. One separated fat-soluble vitamins (Allwood & Martin 2000) and the other separated water-soluble vitamins (Supelco 2000).

The Allwood & Martin (2000) method uses a C18 column with methanol as the mobile phase at an isocratic flow rate of 1.5 ml/min over a period of 15 minutes. This method was able to detect vitamin E and vitamin A using a UV detector at 292 nm and 325 nm respectively. The Supelco application note (2000) also uses the readily available C18 column, however, in contrast to the previous assay it uses a gradient elution over a period of 25 minutes. The aqueous phase (A) consists of a 50 mM phosphate buffer (pH 7) and the organic phase (B) is methanol. The percentage of A is decreased from 99% to 70% over a 25 minute run at a flow rate of 1 ml/min. Ascorbic acid, thiamine, nicotinamide, pyridoxine, folic acid, pantothenic acid and riboflavin are detected using a UV detector at a wavelength of 265 nm. Sufficient similarities exist between the equipment used in these methods to combine them, allowing the simultaneous identification and quantification of fat and water-soluble vitamins in one run.

2.2.1. HPLC system

Development of a HPLC assay for the analysis of vitamins was performed using a Spectra System® from Thermo Finnigan (Thermo Scientific, West Palm Beach, United States). The system is comprised of a SCM1000 vacuum membrane degasser, P2000 gradient pump, AS3000 autosampler and a UV1000 UV detector. The software used to measure the results was Chromquest® version 4.2.

2.2.2. Water-soluble vitamins

Solivito® N is a yellow powder for reconstitution and contains in each vial: thiamine nitrate 3.1 mg, sodium riboflavin phosphate 4.9 mg, nicotinamide 40 mg, pyridoxine hydrochloride 4.9 mg, sodium pantothenate 16.5 mg, sodium ascorbate 113 mg, biotin 60 µg, folic acid 0.4 mg and cyanocobalamin 5.0 µg. The inactive ingredients in each vial consist of: methyl parahydroxybenzoate 0.5 mg, glycine 300 mg and disodium edetate 0.5 mg.

2.2.3. Fat-soluble vitamins

Vitlipid® N Adult is a milky emulsion with each 10 ml vial containing: retinol (as palmitate) 990 µg, ergocalciferol 5 µg, dl-alpha-tocopherol 9.1 mg and phytomenadione (vitamin K₁) 150 µg. The inactive ingredients in each vial are:

fractionated soybean oil 1 g, fractionated egg phospholipids 120 mg, glycerol 225 mg, sodium hydroxide q.s. and water for injection to 10 ml.

2.2.4. Reference standards

The analytical grade fat and water-soluble vitamin standards (Sigma-Aldrich Corp, Louis, Missouri, USA) used were:

- Thiamine HCl
- Riboflavin
- Nicotinamide
- Pyridoxine HCl
- D-Pantothenic acid hemicalcium
- (+) Sodium L-Ascorbate
- D-Biotin
- Folic Acid
- Cyanocobalamin
- Retinol palmitate
- Ergocalciferol
- (±)-Alpha-tocopherol
- Phylloquinone

2.3. Development of a HPLC assay for the simultaneous analysis of fat and water-soluble vitamins

This assay used a gradient elution method as seen in Table 2.1. The mobile phase consisted of varying percentages of methanol and a phosphate buffer 50 mM solution at pH 7. This assay was performed at room temperature.

Column:

OmniSpher 5 C18 150x3mm, 5 μ m particle size (Varian, Palo Alto, USA)

Mobile Phase:

A: 50 mM phosphate buffer 99:1 Methanol

B: Methanol

Table 2.1. Method of stability-indicating HPLC assay

Time (minutes)	A (%)	B (%)	UV (nm)	Flow Rate (ml/min)	
5	100	0	265	1.0	
20	70	30			
25	70	30			
26	0	100			
28	0	100	292	1.3	
33	0	100			
42	0	100	325		1.3
43	100	0			
47	100	0		1.0	

2.3.1. Preparation of phosphate buffer

The mobile phase A consisted of phosphate buffer (99%) and methanol (1%). 1000 ml of 50 mM phosphate buffer was freshly made by dissolving 6.80 g of KH_2PO_4 in ultra-filtered deionised water. After dissolving the phosphate in ultra-filtered deionised water the solution pH was adjusted to pH 7 (± 0.05 units) through drop-wise addition of sodium hydroxide. The solution was then filtered under vacuum to remove any particulates using a 0.20 μm nylon membrane filter.

2.3.2. Preparation of water and fat-soluble vitamins

Samples were prepared away from sunlight to prevent photo-degradation of the light sensitive vitamins. A vial of Solivito[®] N was reconstituted using 10 ml of Vitlipid[®] N Adult and transferred into a 100 ml volumetric flask. The 100 ml volumetric flask was then filled up to volume using ultra-filtered deionised water. The volumetric flask was inverted 20 times to ensure adequate mixing. The solution was then transferred immediately to light protecting amber HPLC vials.

2.3.3. Identification, calibration and stability-indicating tests for water and fat-soluble vitamins

The peaks in this assay were individually identified through spiking solutions with individual vitamin standards.

In order to validate the assay for fat and water-soluble vitamins, calibration curves were made for each of the vitamins detected. Five standard solutions were made at concentrations of 40%, 60%, 80%, 100% and 120% of the original Solvito® N and Vitlipid® N Adult solution prepared in 2.3.2. Each concentration level was analysed in triplicate. Calibration curves for each vitamin were generated by plotting mean absorbance values against concentration. The calibration curves for each of the vitamins can be seen in Figures 2.1.-2.10.

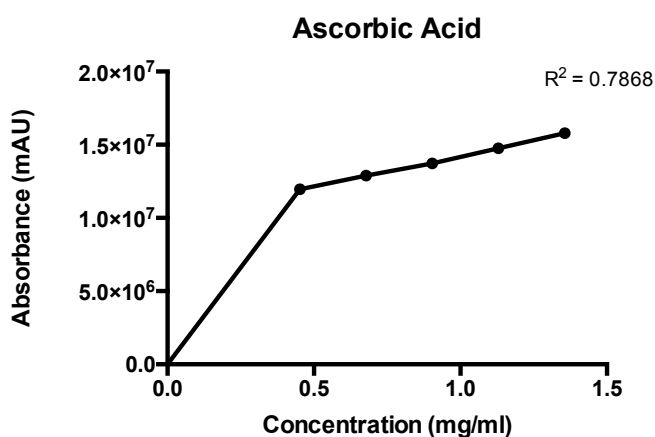


Figure 2.1. Calibration curve for Ascorbic acid, n=3

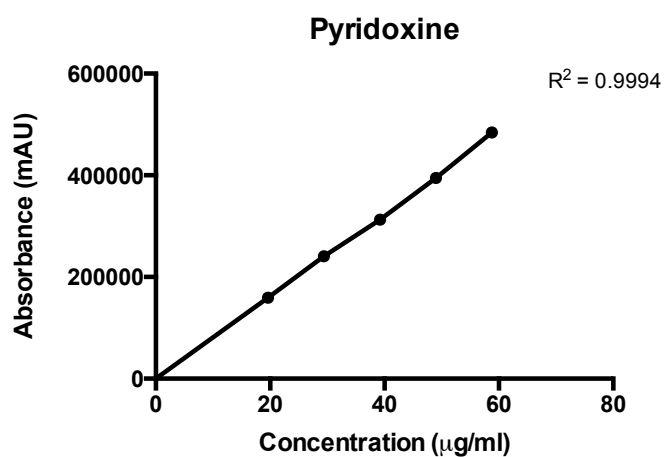


Figure 2.2. Calibration curve for Pyridoxine, $n=3$

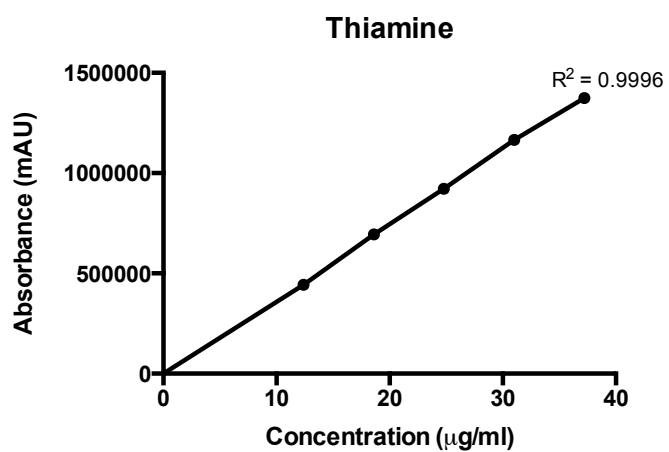


Figure 2.3. Calibration curve for Thiamine, $n=3$

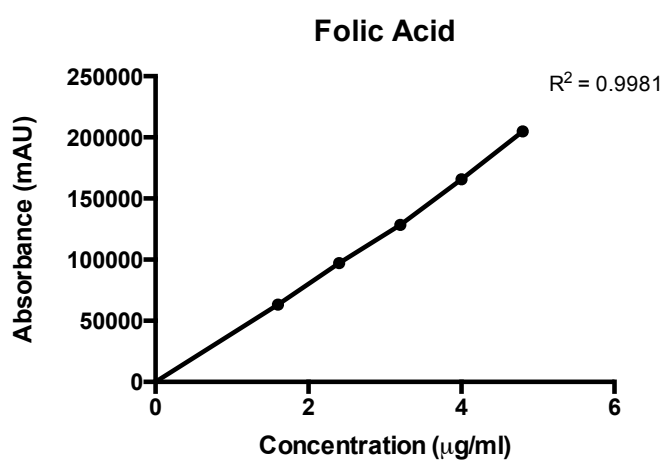


Figure 2.4. Calibration curve for Folic Acid, $n=3$

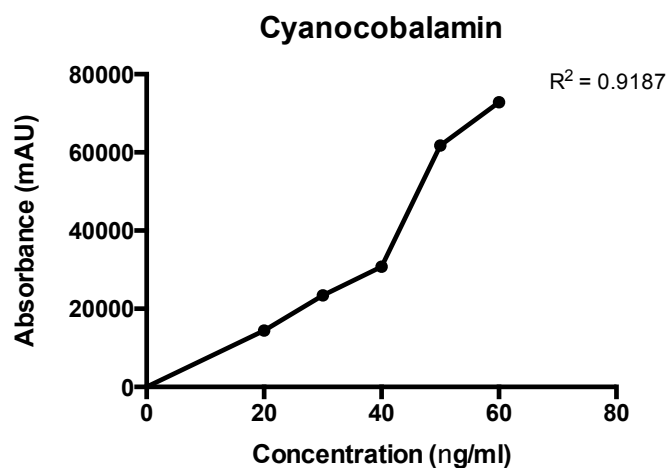


Figure 2.5. Calibration curve for Cyanocobalamin, n=3

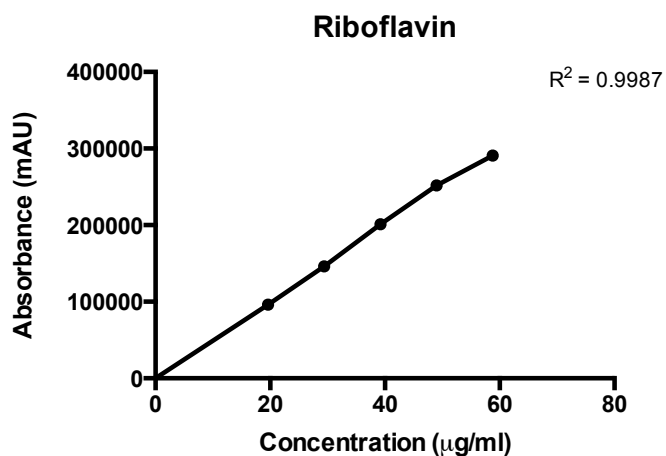


Figure 2.6. Calibration curve for Riboflavin, n=3

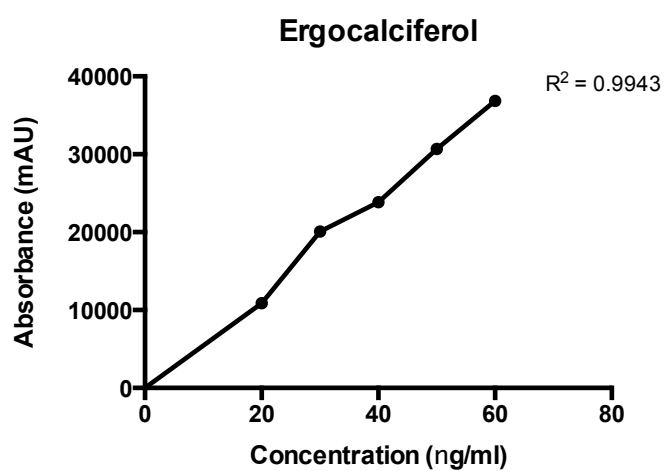


Figure 2.7. Calibration curve for Ergocalciferol, n=3

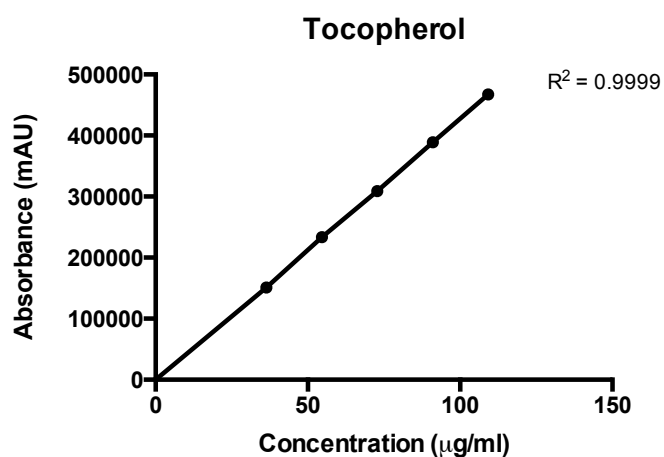


Figure 2.8. Calibration curve for Tocopherol, $n=3$

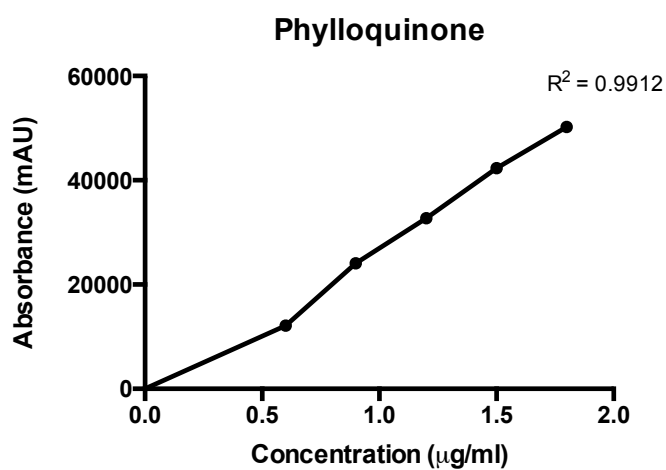


Figure 2.9. Calibration curve for Phylloquinone, $n=3$

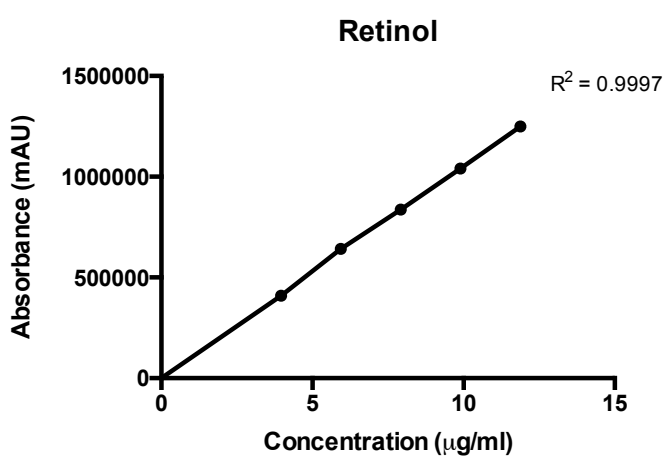


Figure 2.10. Calibration curve for Retinol, $n=3$

Separate samples of the Solivito® N and Vitlipid® N Adult solutions were subjected to the following conditions:

- (a) adjusted with sodium hydroxide solution to pH 12 or higher
- (b) adjusted with hydrochloric acid solution to pH 2 or lower
- (c) exposed to sunlight, by placing solutions by a south facing window for 6 hours
- (d) heated in a water bath for 2 hours at temperature of approximately 80°C

2.3.4. Results for HPLC assay identifying fat and water-soluble vitamins simultaneously

The HPLC assay described in 2.3 produced a chromatogram as shown in Figure 2.11. When spiking the sample with vitamin reference standards it became clear that the assay did not detect pantothenic acid, as there were no peak size increases even when spiking the sample with ten times the normal concentration. Nevertheless, peaks for cyanocobalamin, ergocalciferol and phylloquinone were identified.

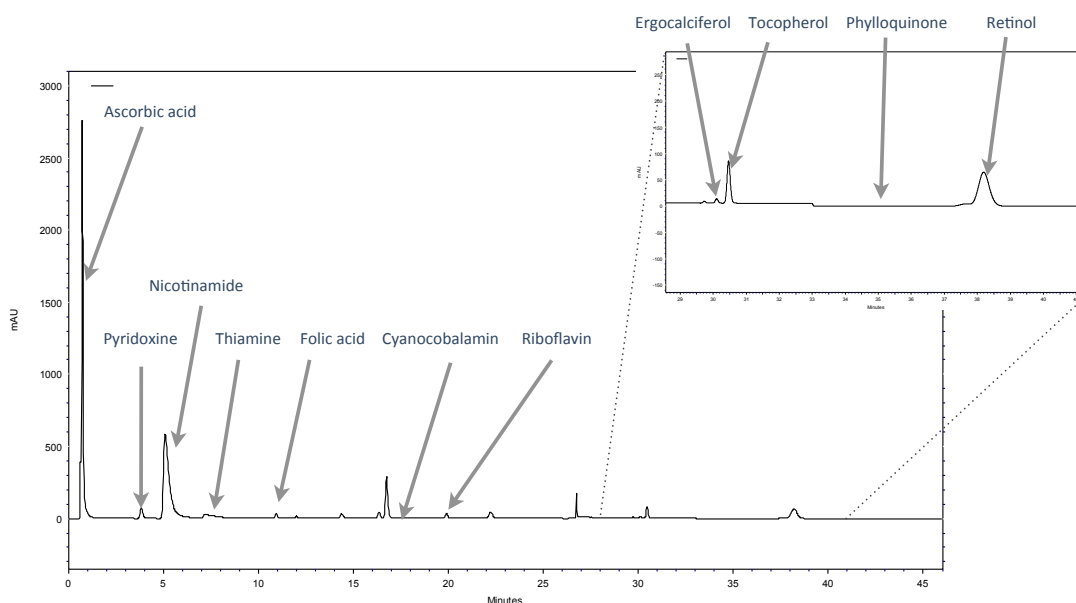


Figure 2.11. HPLC chromatogram identifying 11 water and fat-soluble vitamins

Table 2.2 shows the approximate retention times of the vitamins identified in the stability-indicating HPLC assay. Water-soluble vitamins are first to be identified, as initially there is a high level of aqueous mobile phase. Fat-soluble vitamins follow as higher percentages of organic mobile phase are used.

Table 2.2. The peaks and approximate retention times identified on the HPLC chromatogram.

Vitamin	Minutes
Ascorbic acid	0.74
Pyridoxine	3.84
Nicotinamide	5.12
Thiamine	7.23
Folic acid	10.97
Cyanocobalamin	17.58
Riboflavin	20.03
Ergocalciferol	30.13
Tocopherol	30.48
Phylloquinone	35.05
Retinol	38.36

Following individual identification of the various peaks in the assay, calibration curves for all the vitamins were generated by measuring concentrations of 40%, 60%, 80%, 100% and 120% in triplicate. To construct the calibration curves, concentrations were plotted against peak areas producing linear plots. Using these data, the relative standard deviation (RSD) and R^2 (correlation coefficient) values were calculated and can be seen in Table 2.3. The RSD shows the consistency of the data and low values indicate a precise assay. The limit of RSD in an assay should not usually exceed a value of 10 % (Snyder et al. 1997). R^2 values demonstrate the linearity of the assay. Acceptable R^2 values for stability indicating HPLC assay need to be in excess of 0.999 for most methods (Snyder et al. 1997). As this assay was simultaneously quantifying a number of vitamins R^2 values in excess of 0.99 were deemed acceptable. From these data all the

vitamins identified had acceptable R^2 value and RSD values apart from cyanocobalamin and ascorbic acid. Cyanocobalamin had a R^2 value of 0.9389 and RSD value of 0.1694. This indicates that this assay is not a sufficiently accurate method for measuring cyanocobalamin concentration. Ascorbic acid could also not be used as it had a R^2 value of 0.7868, which shows poor linearity. The rest of the vitamins examined had an acceptable RSD and R^2 value.

Table 2.3. Relative standard deviation and R^2 values of calibration curves for water and fat-soluble vitamins.

Vitamin	R^2	RSD (%)
Ascorbic Acid	0.7868	0.74
Pyridoxine	0.9994	0.64
Nicotinamide	0.9998	0.12
Thiamine	0.9996	2.74
Folic Acid	0.9981	1.88
Cyanocobalamin	0.9187	16.94
Riboflavin	0.9987	3.64
Ergocalciferol	0.9943	6.49
Tocopherol	0.9999	1.83
Phylloquinone	0.9912	3.94
Retinol	0.9997	0.37

2.3.5. Discussion

This method combines two assays (Supelco 2000; Allwood & Martin 2000) and required a number of adjustments to ensure that robust chromatograms with good separation were produced.

During the early stages of assay development, there was a noticeable increase in retention time on subsequent runs later in the day. This was problematic as the more lipophilic compounds, for example retinol, were gradually lost from the chromatogram following repeat runs. It was thought that this problem was due to a short equilibration time, preventing the column from returning to its original conditions before subsequent runs. This was resolved through increasing the equilibration time between runs. Equilibrating the column for 5 minutes between each run allowed the column to return to its initial condition, thus, preventing an increase in retention whilst ensuring that run time was not too long.

In an attempt to reduce run time, to increase throughput and reduce the volume of solvent required, the idea of using an ultra high-pressure liquid chromatography (UHPLC) column was considered. To achieve this, acetonitrile would need to replace methanol as the organic solvent as it produces much less backpressure. However, the use of acetonitrile in the presence of high levels of phosphate buffer produced precipitates on the HPLC columns and prevented any further developments in this area.

Precipitation was identified as a problem for progression of the assay development even when using methanol as the organic solvent. Further investigation provided an alternative phosphate salt, ammonium dihydrogen orthophosphate, which was slightly more soluble in organic mobile phase than the previously used buffer, potassium dihydrogen orthophosphate. However, after further investigation, the effects of the solubility differences between the two salts were negligible in this instance.

Another problem encountered during the assay development was the very high levels of backpressure during the assay, particularly between 26 and 28 minutes. The change in mobile phase composition alongside an initial flow rate of 1.5 ml/min caused a backpressure of around 4800 psi. The danger of having such a large backpressure is that it can damage the column and damage the pump. Ideally, backpressure should not exceed 4000 psi and if it does, it should only be momentary. With this in mind, a number of flow rates were trialed. 1.3ml/min was the most suitable flow rate, as it produced a lower backpressure but did not have any impact on peak shape. However, the lower flow rate led to increased retention of the fat-soluble vitamins on the column, therefore the run time was increased from 45 minutes to 47 minutes.

Examining the HPLC assay trace seen in Figure 2.2, it is evident that the peak for phyloquinone at 35.05 min is not easily identifiable. The limit of detection is defined as a peak with a signal to noise ratio (S/N) of at least 3:1 (Snyder et al. 1997). Therefore, the phyloquinone peak is not sufficiently large to be used in

this stability-indicating assay as it will not give a reliable indication of vitamin concentration.

The R^2 and RSD values for the results obtained are described in Table 2.3. From these data we can see that this is a robust assay that can separate a number of water and fat-soluble vitamins simultaneously. Cyanocobalamin, as shown in Table 2.3, has a large RSD value suggesting a lack in precision in the measurement of its peaks. It also has a small R^2 value, suggesting that it will not predict cyanocobalamin concentration accurately. As the cyanocobalamin peak was very close to other peaks in the chromatogram, it is probable that co-elution has occurred producing inaccurate absorbance values. As a result of these data, the peak for cyanocobalamin cannot be used in these stability-indicating studies. Ascorbic acid could not be used in this thesis as it had a small R^2 value indicating poor linearity. In this assay ascorbic acid is present in very high concentrations that are outside of the linear range. Further dilution of ascorbic acid was not possible as the aim of this assay was to identify as many vitamins as possible in one HPLC run and diluting further would limited detection of other vitamins in smaller concentrations. Nevertheless, the other eight vitamins can be identified by the assay and give an accurate quantitative representation.

There are a few reasons why this stability-indicating assay did not identify all the vitamins added to PN admixtures. One of the reasons is that some of the vitamins were added in very small quantities, such as biotin (60 μ g), which

made them more difficult to detect. In addition, some of the vitamins either co-eluted with other components of the admixture or are difficult to identify using a UV detector. Nevertheless, the vitamins identified in this assay are generally considered the most sensitive to degradation, therefore, degradation associated with these vitamins would give a strong indication of what impact storage conditions would have on these other less sensitive vitamins.

In conclusion, this stability-indicating assay is a reproducible and robust HPLC method, as illustrated by the RSD and R^2 values, showing good shape and separation of chromatographic peaks. It can identify eight vitamins (pyridoxine, nicotinamide, thiamine, folic acid, riboflavin, ergocalciferol, tocopherol and retinol) and accurately predict their concentration in solution. This is a novel method for identifying fat and water-soluble vitamins on standard HPLC machinery.

CHAPTER THREE

Stability of Vitamins in Artificial Light Sources Over 24 Hours

3.1. Introduction

This chapter is concerned with the sensitivity of vitamins to light. This chapter will use the stability-indicating HPLC assay developed in Chapter Two as well as physical studies to help determine the stability of eight water and fat-soluble vitamins when exposed to different types of light over a 24-hour period. The types of light that the PN will be exposed to will be warm white light (found in homes), cool white light (found in hospitals) and UVA light (present in sunlight and known to degrade riboflavin and retinol (Allwood & Kearney 1998; Allwood & Plane 1986). The methods generated in this chapter provide an accurate and reproducible way of measuring vitamin degradation when exposed to light sources.

3.2. Types of Light

In practice PN can come into contact with many different types of light. Depending on where PN formulations are made and used, they can come into contact with warm white light, cool white light and sunlight. This is especially true when compounded PN is administered to patients at home. Each type of light has a different spectrum that emits different wavelengths of light. Sunlight is the most problematic type as it emits the widest spectrum of light at different intensities, as seen in Figure 3.1.

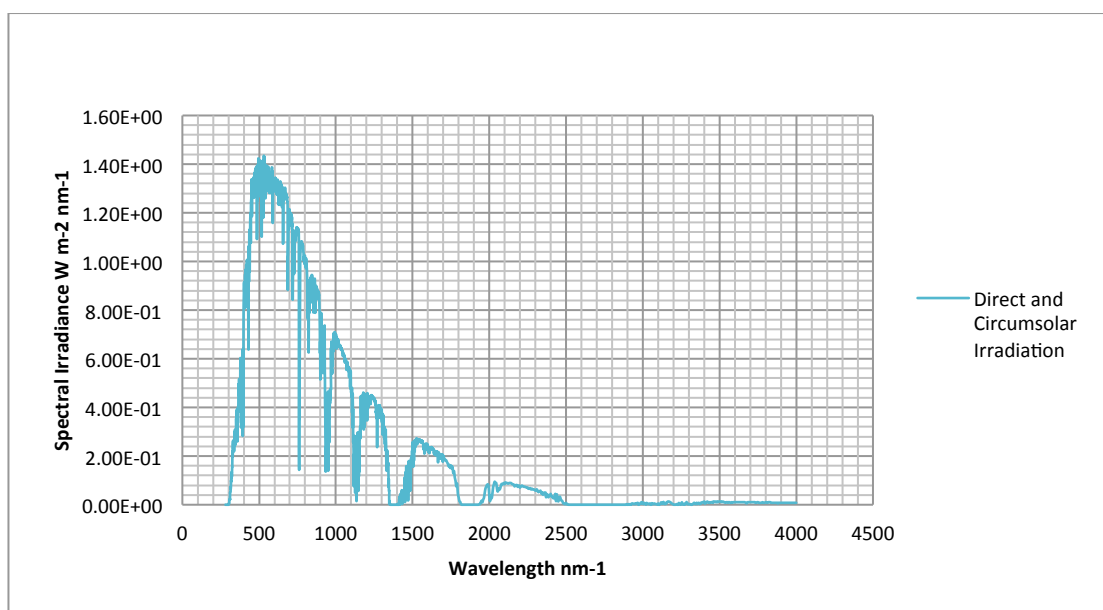


Figure 3.1. Sunlight spectra in the northern hemisphere recorded in North America (National Renewable Energy Laboratory, 2003)

3.3. Vitamins sensitive to light

Light can interact with various components of PN, such as vitamins. Some vitamins are known to be more photosensitive than others. As discussed in Chapter One, vitamins known to be sensitive to light include retinol, tocopherol, phyloquinone, pyridoxine and riboflavin (Allwood & Kearney 1998; Allwood & Plane 1984; DeRitter 1982; Smith, Canham, & Wells 1988), however, photostability has not been extensively tested for many vitamins. Therefore, there is a need for further research in this area. The vitamins known to be light-sensitive that will be examined in this experiment will be: pyridoxine, riboflavin, tocopherol and retinol. In addition, the intensity of light has not been routinely monitored and so the extent of degradation seen in other studies can not be

extrapolated to a clinical setting (Allwood & Martin 2000; Ribeiro et al. 2011). For this reason, lux and UV meters were used to measure the visible and UV light respectively throughout this thesis. Other vitamins that were analysed using the HPLC assay described in Chapter Two were nicotinamide, folic acid, thiamine and ergocalciferol. These vitamins are not known to be light sensitive, but there is limited information available for some of them, particularly nicotinamide, folic acid and ergocalciferol. Therefore, further investigation into their stability profiles may provide useful information.

3.4. Materials and methods

The materials used in this chapter are listed below.

Materials	Manufacturer
Methanol HPLC grade	Fisher Scientific UK Ltd, Loughborough, Leicestershire
Fistreem Multipure Grade 2 water filter	SANYO Gallenkamp PLC, Monarch way, Belton Park, Loughborough, Leicestershire
Potassium dihydrogen orthophosphate (Analytical grade)	Fisher Scientific UK Ltd, Loughborough, Leicestershire
Solivito N Adult injection	Fresenius Kabi Ltd, Birchwood, Warrington
Vitlipid N Adult injection	Fresenius Kabi Ltd, Birchwood, Warrington
Vitamin Reference standards	Sigma-Aldrich Corp, Louis, Missouri, USA
Sodium hydroxide analytical grade	Fisher Scientific UK Ltd, Loughborough, Leicestershire
Autosampler 2.0 ml amber vials Autosampler PTFE/ silicon vial seals Black 8mm screw caps	Fisher Scientific UK Ltd, Loughborough, Leicestershire
Sterile plastic syringes 10ml, 20ml, 50ml BD Plastipak® (Polypropylene)	Becton Dickinson, Madrid, Spain
Sterile disposable needles, 19G/21G BD Microlance™ 3	Becton Dickinson, Madrid, Spain
Magnetic stirrer	Fisher Scientific UK Ltd, Loughborough, Leicestershire
Orion 420A pH meter	Orion Research Inc., Beverly, Massachusetts
Ultra Low Freezer	Sanyo Electric Biomedical Co., Moriguci city, Osaka
Pharmaceutical refrigerator	Lec Refrigeration, Prescott, Merseyside
Plastic disposable pipettes 145 mm	Fisher Scientific UK Ltd, Loughborough, Leicestershire
Nylon Membrane filters, 47 mm, 0.2 µm	Whatman International Ltd, Maidstone, England
Multi-Ray Lamp with Cool white, warm white and UVA light bulbs	UVP, UK
Pharmaceutical Stability Chamber PSC 062	SANYO® Gallenkamp PLC, Leicestershire, England

3.4.1. Physical analysis

Physical analysis was performed during these experiments to monitor the effects that vitamins had on the other constituents in PN admixtures. Through using tests such as visual analysis, microscope analysis, laser diffraction, pH and osmolality we could determine whether the admixture was safe for administration in a clinical setting.

3.4.1.1. Visual examination

The first physical test that was performed on the samples was a visual examination to look for any signs of instability. The colour of the emulsion was recorded, as changes may be indicative of instability. Creaming was another sign that was looked for, which is when an emulsion has a higher concentration of disperse phase in one area than another. Although reversible on shaking the emulsion, creaming increases the likelihood of an irreversible process known as 'cracking' or coalescence, as globules are in closer proximity to one another (Billany 2004). Coalescence signifies the breaking down of globule structure, which releases free oil into the emulsion (Billany 2004). As free oil is indicative of a cracked emulsion it was also looked for during visual examination.

3.4.1.2. Microscopy

Large changes in physical stability can be seen with the naked eye, however, instability can be seen at a much earlier stage when examined with a microscope. In these experiments an Olympus BH-2 light microscope (Tokyo, Japan) was used and globules were examined at 100 times magnification. Samples were placed onto a Thoma hemocytometer and covered with a disposable cover slip ensuring that no bubbles were introduced to the slides. The globules' size and shape were examined. Largest globule size, numbers of globules greater than 10 μm and numbers of globules sized between 7.5 and 10 μm were recorded using the hemocytometer as shown in Figure 3.2. The shape of the globules (round, amorphous or crystalline) and their arrangement (single, flocculated and aggregated) were also recorded. For the purpose of these experiments flocculated is described as globules within close proximity of each other (but not touching) in defined groups (Billany 2004). Aggregated is defined as groups of globules touching one another with the globule walls intact.

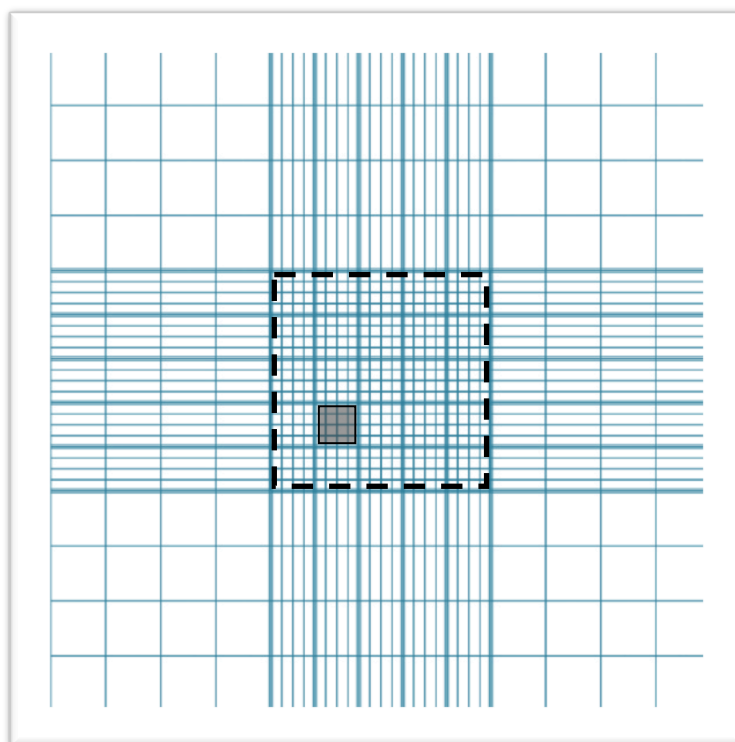


Figure 3.2. Thoma hemocytometer. Dashed box indicates the overall area to be considered. Grey shading shows one of the counting areas within the dashed box.

3.4.1.3. Laser diffraction

Globule size was also analysed using a Malvern Mastersizer 2000 (Malvern, UK). The Malvern Mastersizer 2000 was attached to a small volume sampler (SVS), which was connected to a flow measuring cell and was filled with ultra-filtered deionised water. Care was taken to ensure the SVS was cleaned thoroughly between samples and all air was expelled from the system, as this would interfere with results generated. Samples were added to the SVS until approximately 20% light obscuration was achieved and circulated through the system at a constant flow rate. The samples were passed through the instrument and a laser was shone upon them. The light emitted from the laser

was then scattered following contact with the globules and the resultant scattered light was detected. The angle of this scattering is indicative of the size of the globule. A basic outline of how the Malvern Mastersizer 2000 analyses globule size can be seen in Figure 3.3. The results were then analysed using Malvern Mastersizer 2000 software package version 5.00. The values recorded were the D[4,3] and the 'Size under'. The D[4,3] is the volume mean diameter and the 'Size under', in these experiments, is the smallest size in which 100 % of the sample globules is below (Anon. 1999).

It is important to note that this instrument does not report oversized molecules unless there is a significant volume of them in the sample. In addition, it may not give accurate results if the globules are irregularly shaped as it assumes that all globules are spherical. Due to the lack of sensitivity exhibited by this instrument, further globule size analysis using a microscope is necessary (Akasah 2002).

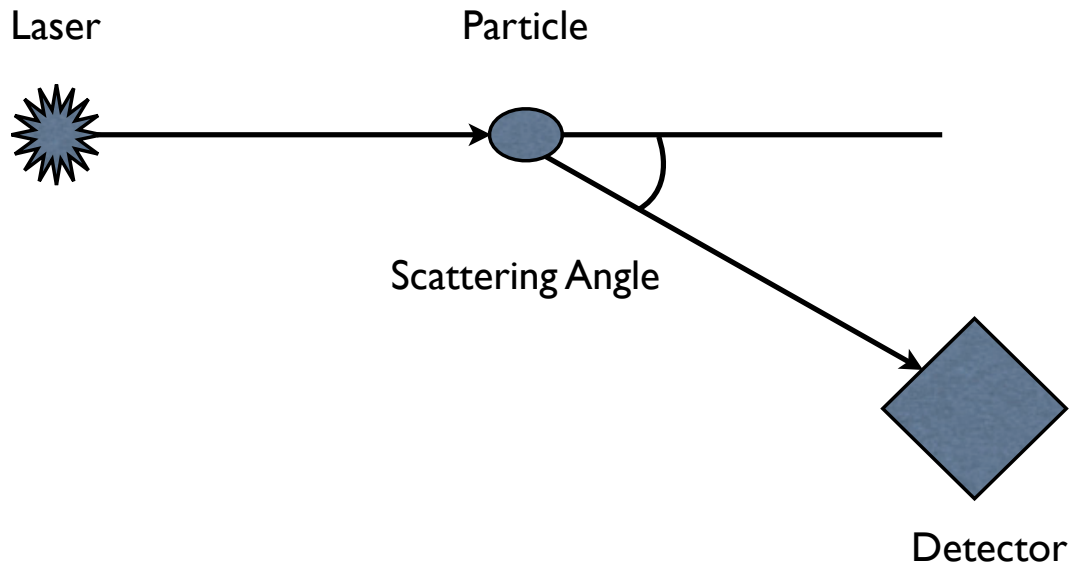


Figure 3.3. Scattering process adapted from the Malvern reference manual (Anon. 1993).

3.4.1.5. Osmolality

Osmolality is a common unit of concentration and is an important consideration with parenteral products. It is defined as the concentration of a solution, which is expressed in terms of osmoles of solute per kilogram of solvent. Osmolality was measured using The Advanced™ Osmometer (Model 3D3, Norwood, Massachusetts, USA). This instrument can accurately determine the concentration of a solute through calculating its freezing point depression in solution (Anon. 1996).

Samples (0.25 ml) were added to sample tubes using a Volac® minipipet (Poulten & Graf, Barking) and placed into the cooling compartment of the

osmometer. On activation of the machine a freeze/stir wire was lowered automatically into the sample. The freezing point depression was measured through super-cooling the sample to several degrees below the freezing point and then mechanically inducing the sample to freeze using the freeze/stir wire. The resultant heat of fusion liberated from the sample crystallizing increases the temperature to a temporary plateau and this is the sample's freezing point. The instrument converts this measurement to a value in milliosmoles per kilogram (mOsm/kg), which is displayed and subsequently recorded (Anon. 1996). The osmolality of test and control samples were measured once at the end of each experiment as it does not change over time. The reason for measuring osmolality is that values in excess of 1000 mOsm/kg increase the risk of thrombophlebitis in peripheral PN patients (Madan et al. 1992). Therefore, it is important to measure the osmolality to ensure that the admixture can be tolerated by the patient.

3.4.1.6. pH

The pH of the samples was measured using an Orion Benchtop pH/ISE Meter (Model 420A, Orion Research Inc., Massachusetts) with a Fisher semi-micro pH electrode (Fisher Scientific UK Ltd, Loughborough). Samples were measured at the start and end of each experiment, rather than at every time point due to the lack of sample available. The test samples were compared to controls, with

substantial differences in pH (greater than 0.5 pH units) over the testing period indicative of instability.

3.4.2. Experimental design

In this series of experiments, syringes containing fat and water-soluble vitamins mixed with Intralipid® 20 (IL20) were exposed to either cool white, warm white or UVA light. The chemical stability of the vitamins and the physical stability of the emulsion were monitored through comparison with control syringes protected from light. A photograph of the setup for the first two experiments can be seen in Figure 3.4. Temperature in the presence and absence of this light remained relatively unchanged. The third experiment was conducted in a SANYO® stability chamber to measure UVA emissions.



Figure 3.4. The experimental set up for the 50 ml BD syringes exposed to either cool white or warm white light.

3.4.2.1. Sample preparation

A vial of Solivito® N was reconstituted with 10 ml of Vitlipid® N, transferred to a 50 ml Becton Dickinson® (BD) syringe and made up to 50 ml with IL20. Ensuring excess air was removed, the syringes were end-capped using Multi-Ad® Luer Lock Syringe Caps (B. Braun Medical Inc. Bethlehem, USA) and inverted 20 times to evenly distribute the vitamins. For each experiment, five syringes were made; three syringes were exposed to either warm white, cool white or UVA light while two syringes were used as controls and protected from light.

3.4.2.2. Cool and warm white light experiments

For each experiment three syringes were exposed to either cool or warm white light emitted from a multi-ray lamp at room temperature for a period of 24 hours. The remaining two syringes (controls) were protected from light and stored at room temperature. Temperature and illuminance were measured throughout this experiment. Illuminance in these experiments was measured in Lux, which is the amount of luminous flux per square meter (Kenyon 2008). The levels throughout this experiment were set at approximately 5,000 lux. The spectra for the cool and warm white light emitted can be seen in Figure 3.5 and Figure 3.6 respectively. The spectra showing cool white light (Figure 3.5) has a higher proportion of light with a shorter wavelength and emits more blue light. This type of light is typically used in an office and hospital setting to aid

concentration. The warm white light spectrum (Figure 3.6) has a higher proportion of light with a longer wavelength. This light appears more yellow and orange and is more readily found in homes.

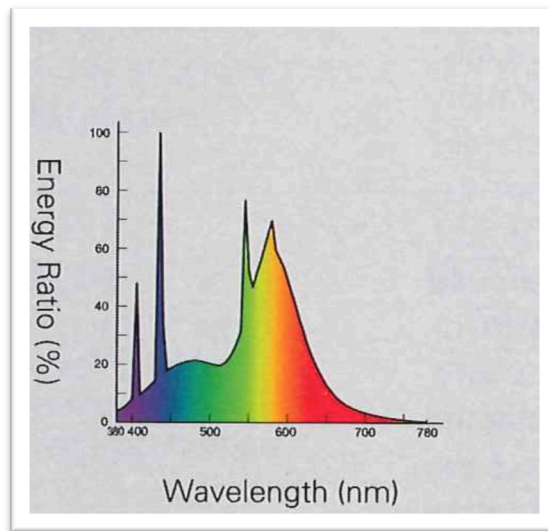


Figure 3.5. Spectrum of cool white light using a multi-ray lamp (Hatley 2012)

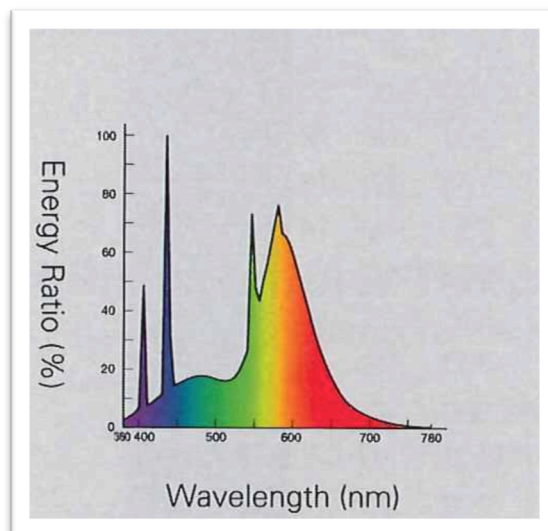


Figure 3.6 Spectrum of warm white light using a multi-ray lamp (Hatley 2012)

3.4.2.3. UVA light experiment

Three syringes were stored in a SANYO® stability chamber and exposed to UVA light at room temperature for a period of 24 hours. The remaining two syringes (controls) were protected from light and stored at room temperature. Temperature and light intensity were measured throughout this experiment. As lux is only a measure of visible light, different units need to be used for UVA light. In these experiments light intensity of UVA light was measured in watts per squared meter (Wm^{-2}). The levels of UVA light set throughout this experiment were approximately 1.2 Wm^{-2} . The spectra for this light source can be seen in Figure 3.7. UVA light is found in very small proportions in most artificial lights, however, sunlight and phototherapy treatments that have much higher quantities of UV light. Therefore, it is useful to investigate the impact of these types of light on vitamins as bags could be exposed regularly to these types of light either on the ward or in a home setting. UVA is characterised as light with a wavelength between 315nm and 400nm.

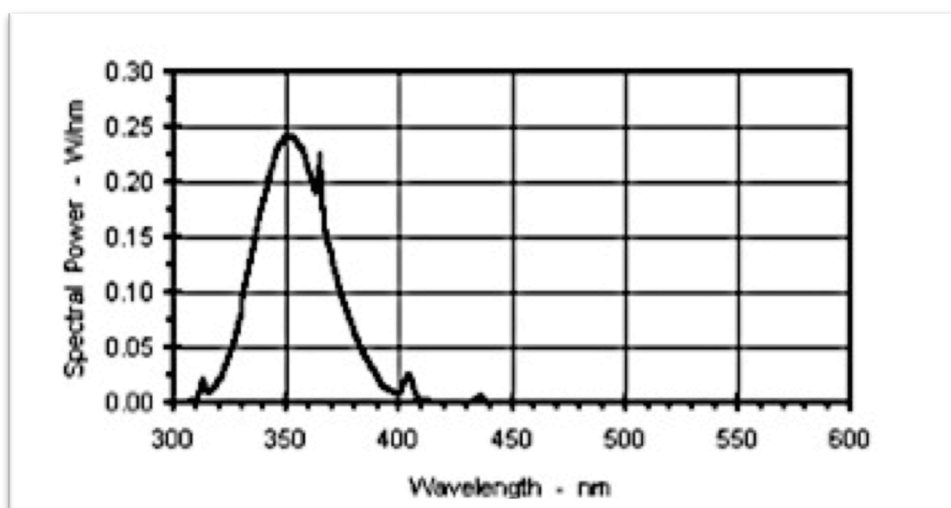


Figure 3.7. Spectrum of UVA light in SANYO® stability chamber (Grummett 2012)

3.4.2.4. Sampling of syringes

Chemical tests were performed on two of the syringes exposed to the light source and one of the controls (protected from light) using a stability indicating HPLC assay described in Chapter Two. Substantial degradation is defined as >10% over the 24 hour period. Samples were taken at 0, 8, 16 and 24 hours.

Physical tests were conducted on the remaining syringes (one test and one control). Visual examination, microscopy, laser diffraction and pH were performed at 0 and 24 hours. Osmolality was examined at 24 hours only.

3.5. Results

3.5.1. Visual examination

All the syringes examined had a yellow/cream colour, which did not change when exposed to cool white, warm white or UVA light. There were no other changes that could be seen with the naked eye, with no creaming or free oil visible.

3.5.2. Microscopy

All samples were examined using a microscope to monitor globule size, shape and arrangement. Microscopy results can be seen in Tables 3.1-3.3.

Following exposure to cool white light there was a very slight increase in the amount of globules sized over 7.5 μm when compared to the control, however, none were larger than 10 μm . The globules were singles and had a spherical morphology indicating that the emulsion was stable.

The syringe that was exposed to warm white light was affected to a greater extent than the syringe exposed to cool white light. There was a large increase in the amount of globules sized over 7.5 μm when compared to the control. In

addition, the largest globule size increased from 10 µm at 0 hours to 40 µm at 24 hours.

The syringe exposed to UV light showed very similar results to those obtained for cool white light. Following exposure, the globules remained the same size and kept their spherical morphology and single uniformity.

Table 3.1. Microscope analysis of control and test syringes exposed to cool white light.

	0 Hours		24 Hours	
	Control	Test	Control	Test
Colour	Yellow/Cream	Yellow/Cream	Yellow/Cream	Yellow/Cream
Largest (µg)	7.5	10	20	10
>10 µg	0	1	4	4
7.5-10 µg	13	4	5	8
Round/Crystalline/Amorphous	Round	Round	Round	Round
Single/Flocculated/Aggregated	Single	Single	Single	Single

Table 3.2. Microscope analysis of control and test syringes exposed to warm white light.

	0 Hours		24 Hours	
	Control	Test	Control	Test
Colour	Yellow/Cream	Yellow/Cream	Yellow/Cream	Yellow/Cream
Largest (µg)	10	20	10	15
>10 µg	2	3	1	1
7.5-10 µg	10	8	6	7
Round/Crystalline/Amorphous	Round	Round	Round	Round
Single/Flocculated/Aggregated	Single	Single	Single	Single

Table 3.3. Microscope analysis of control and test syringes exposed to UVA light.

	0 Hours		24 Hours	
	Control	Test	Control	Test
Colour	Yellow/Cream	Yellow/Cream	Yellow/Cream	Yellow/Cream
Largest (μg)	7.5	20	30	20
>10 μg	0	8	8	4
7.5-10 μg	3	20	7	5
Round/Crystalline/Amorphous	Round	Round	Round	Round
Single/Flocculated/Aggregated	Single	Single	Single	Single

3.5.3. Laser diffraction

Laser diffraction measured the D(4,3) and 'size under' at 0 and 24 hours in test and control syringes. As shown in Table 3.4 the test syringe exposed to cool white light and its corresponding control protected from light had a D[4,3] ranging between 0.181 μm and 0.196 μm . The maximum size of the globules for either the test or the control syringe did not exceed 0.955 μm . The small globule size is important as it signifies a stable emulsion. In addition, small globules that are less than 5 μm in diameter are less likely to cause occlusion of the microvasculature (Driscoll 2006).

The syringe exposed to warm white light had very similar values to its control. The D[4,3] for the control and test syringes ranged from 0.181 μm to 0.183 μm . The globule size did not exceed 0.955 μm for either syringe.

Finally, the syringe exposed to UVA light and its corresponding control had a D[4,3] ranging between 0.181 μm and 0.196 μm . As with the other syringes the globule size for control and test syringes did not exceed 0.955 μm .

Table 3.4. Means and Standard Deviation (SD) of D[4,3] (μm) and 'Size Under' (μm) in control and test samples when exposed to cool white, warm white and UVA light over 24 hours, N=3.

		0 Hours				24 Hours			
		D[4,3] (μm)		'Size Under' (μm)		D[4,3] (μm)		'Size Under' (μm)	
		Control	Test	Control	Test	Control	Test	Control	Test
Cool White	Mean	0.304	0.303	1.259	1.205	0.304	0.306	1.150	1.150
	SD	0.005	0.003	0.000	0.094	0.003	0.003	0.094	0.094
Warm White	Mean	0.303	0.303	1.150	1.096	0.306	0.305	1.205	1.205
	SD	0.000	0.002	0.000	0.000	0.002	0.002	0.000	0.000
UVA	Mean	0.283	0.282	1.096	1.096	0.283	0.284	1.096	1.096
	SD	0.000	0.002	0.000	0.000	0.002	0.002	0.000	0.000

3.5.4. Osmolality

The osmolality was very similar for all test syringes exposed to the three types of light and their corresponding control syringes as shown in Table 3.5. Values ranged from 460 and 480 mOsm/kg. The largest difference in osmolality between test and control samples was 5 mOsm/kg.

Table 3.5. Osmolality values for control and test syringes.

	Cool White		Warm White		UVA	
	Control	Test	Control	Test	Control	Test
Osmolality (mOsm/kg)	478	480	460	465	474	478

3.5.5. pH

The pHs for all test and control samples following exposure were very similar as shown in Table 3.6. pH differences in red indicate decreases in pH and those in green represent increases in pH over the 24 hour period. The pH for all syringes tested ranged between 5.88 and 6.24 pH units. The largest difference between control and test samples was 0.05 pH units.

Table 3.6. pH values for control syringes and test syringes exposed to cool white, warm white and UVA light.

Time (Hours)	Cool White		Warm White		UVA	
	Control	Test	Control	Test	Control	Test
0	6.24	6.21	6.23	6.20	6.11	6.10
24	5.91	5.88	6.04	6.00	6.00	5.95
pH difference	- 0.33	- 0.33	- 0.19	- 0.20	- 0.11	- 0.15

3.5.6. HPLC

3.5.6.1. Summary of HPLC results

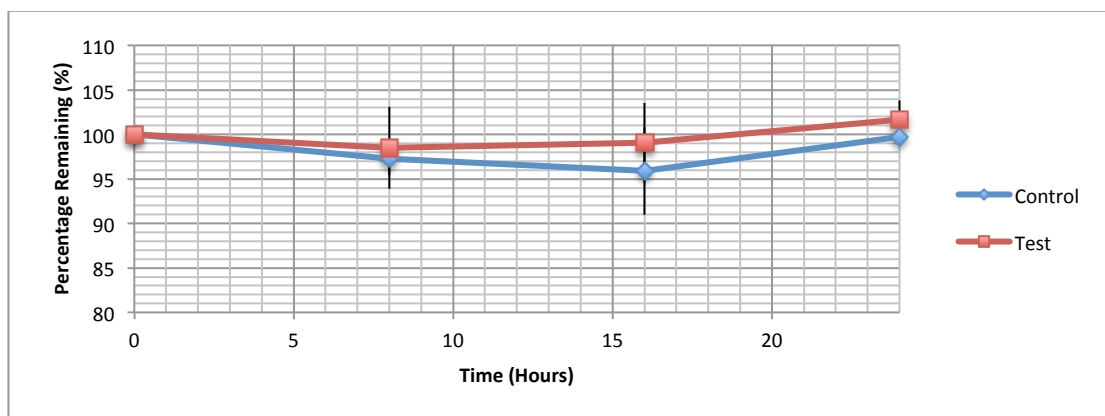
The HPLC results in Table 3.7 summarise the photo-degradation of vitamins when exposed to artificial light sources. Figures 3.8-3.12 show the degradation of pyridoxine, nicotinamide, riboflavin , tocopherol and retinol respectively.

Table 3.7. Percentage of vitamins remaining \pm SD in control syringes that were protected from light and test syringes (mean) that were exposed to light for 24 hours.

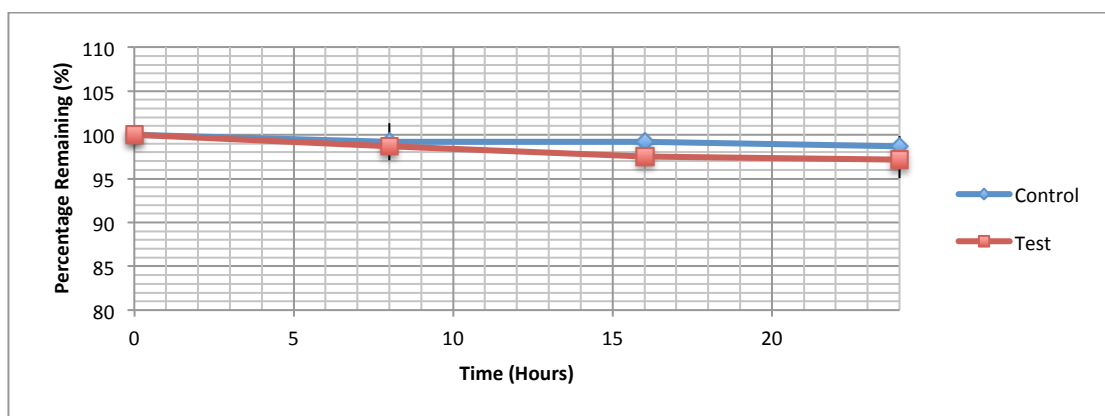
Vitamin	Cool White Light		Warm White Light		UVA Light	
	Control	Test	Control	Test	Control	Test
Pyridoxine	99.71 \pm 0.45	101.65 \pm 2.18	98.66 \pm 1.20	97.17 \pm 2.10	101.72 \pm 1.54	100.36 \pm 1.85
Nicotinamide	97.95 \pm 0.78	101.49 \pm 1.49	101.02 \pm 1.01	99.91 \pm 0.17	98.89 \pm 0.81	100.09 \pm 0.27
Thiamine	116.50 \pm 4.39	100.33 \pm 0.81	103.78 \pm 10.86	110.09 \pm 1.35	110.45 \pm 3.70	98.55 \pm 1.28
Folic acid	93.66 \pm 1.12	102.39 \pm 4.26	98.72 \pm 2.06	103.68 \pm 1.87	98.11 \pm 1.07	103.33 \pm 0.80
Riboflavin	93.85 \pm 5.10	75.77 \pm 4.25	96.71 \pm 3.97	80.76 \pm 0.41	96.58 \pm 2.29	94.77 \pm 0.82
Ergocalciferol	100.80 \pm 1.48	100.80 \pm 0.75	101.81 \pm 1.03	100.04 \pm 0.87	99.51 \pm 3.42	99.73 \pm 1.54
Tocopherol	100.49 \pm 0.27	101.71 \pm 1.04	102.37 \pm 1.27	100.20 \pm 0.67	100.06 \pm 1.58	99.56 \pm 0.76
Retinol	100.56 \pm 0.32	88.93 \pm 0.45	102.08 \pm 0.77	90.58 \pm 0.89	99.09 \pm 0.63	76.69 \pm 0.79

3.5.6.2. Pyridoxine

(a)



(b)



(c)

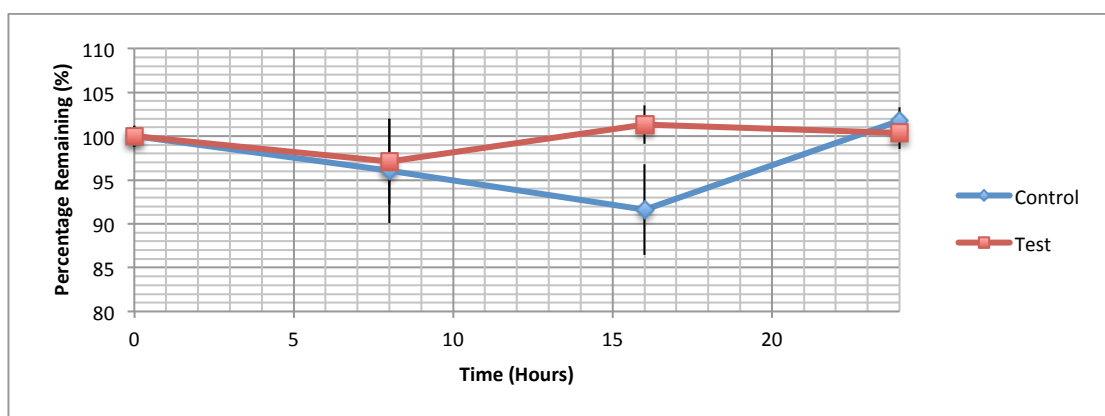
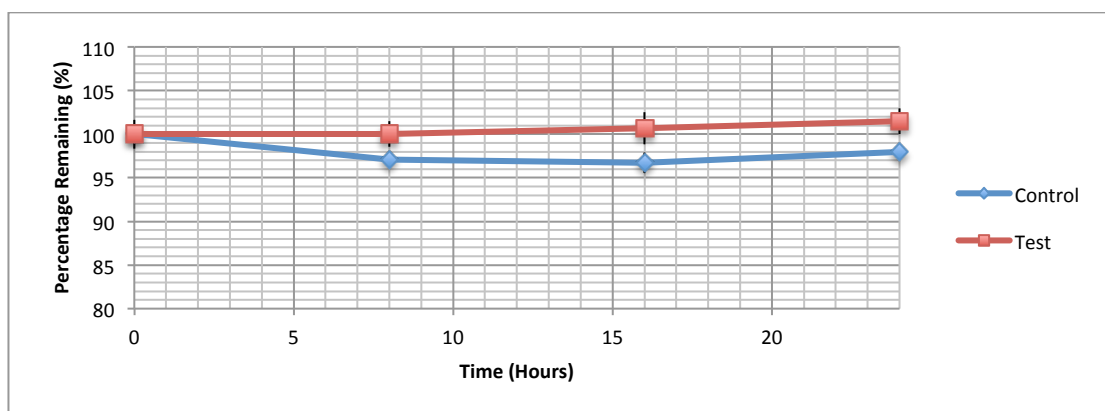


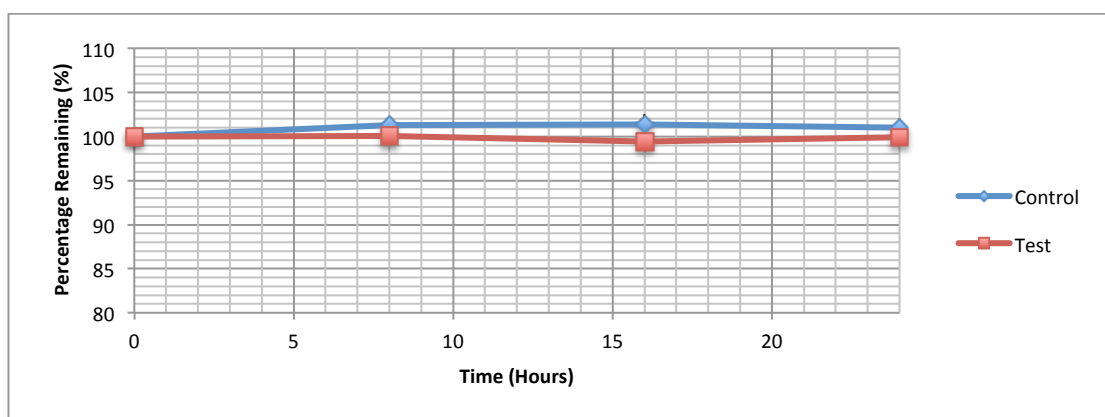
Figure 3.8. Degradation of pyridoxine when exposed to (a) cool white light, (b) warm white light and (c) UVA light

3.5.6.3. Nicotinamide

(a)



(b)



(c)

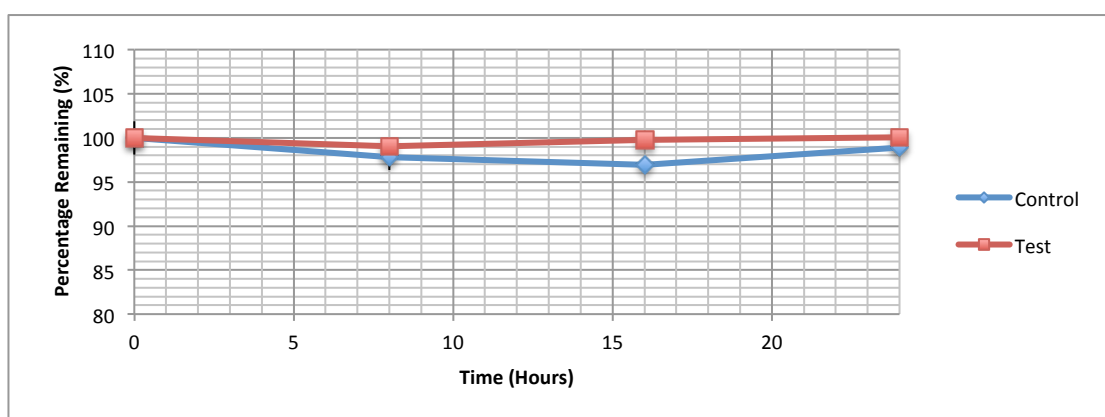
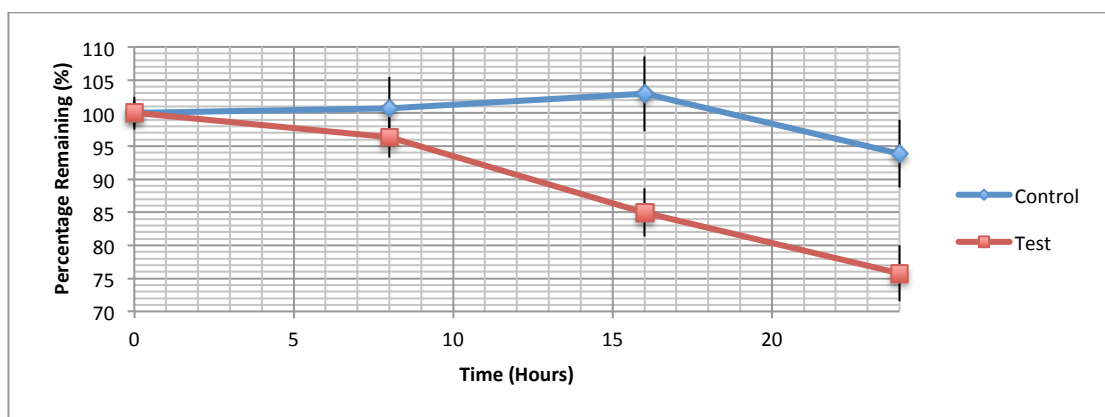


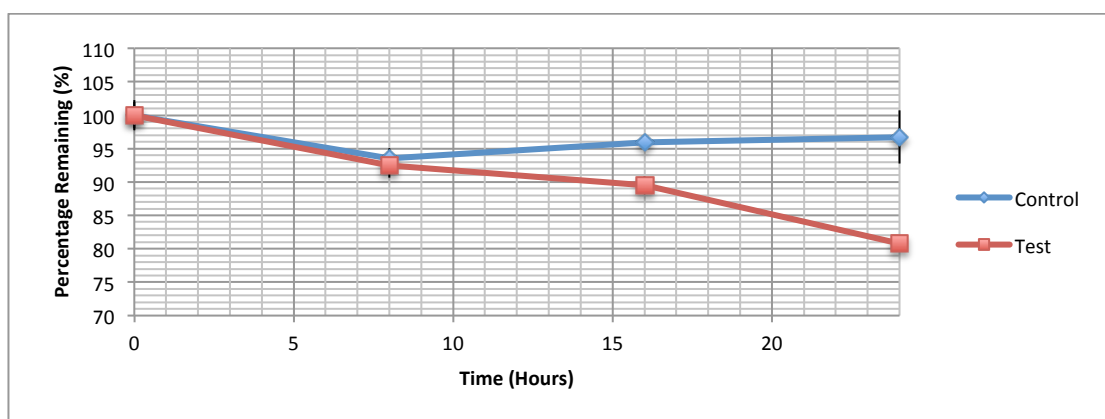
Figure 3.9. Degradation of nicotinamide when exposed to (a) cool white light, (b) warm white light and (c) UVA light

3.5.6.4. Riboflavin

(a)



(b)



(c)

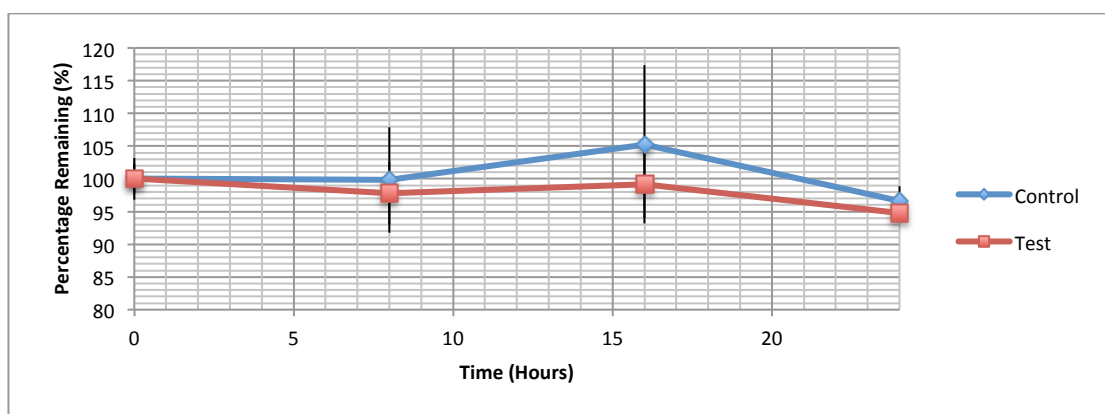
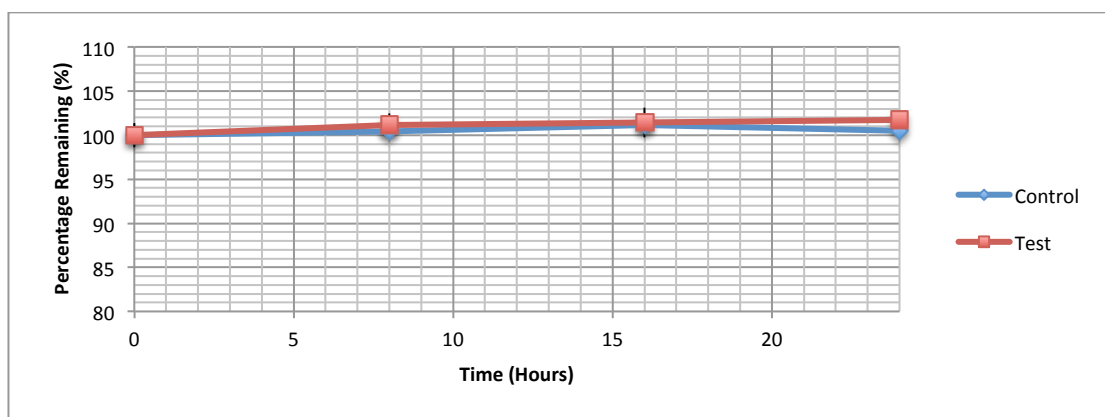


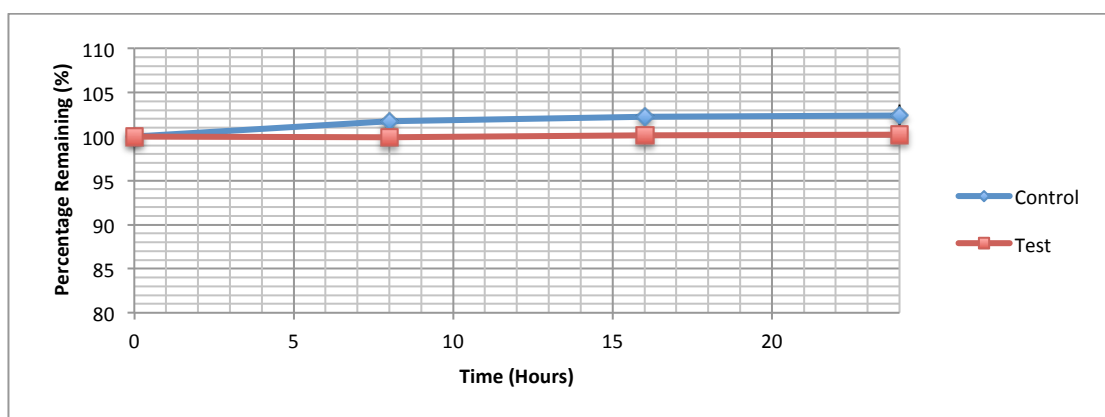
Figure 3.10. Degradation of riboflavin when exposed to (a) cool white light, (b) warm white light and (c) UVA light

3.5.6.5. Tocopherol

(a)



(b)



(c)

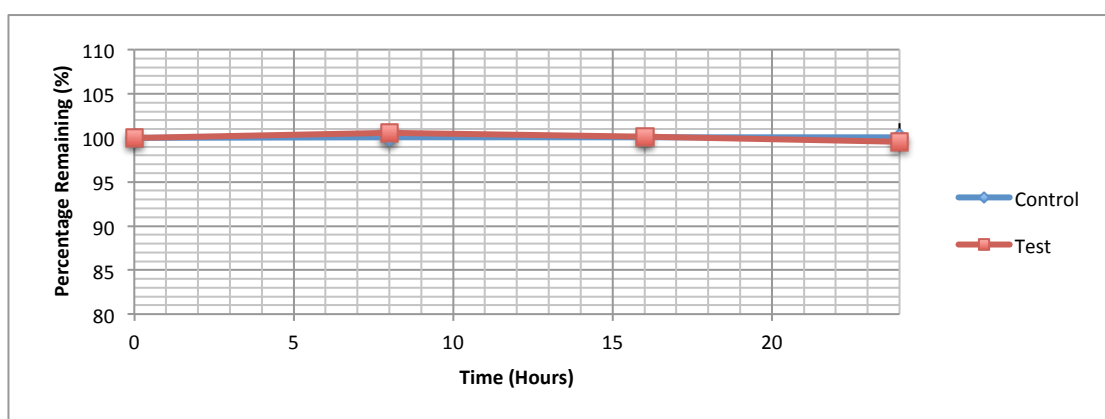
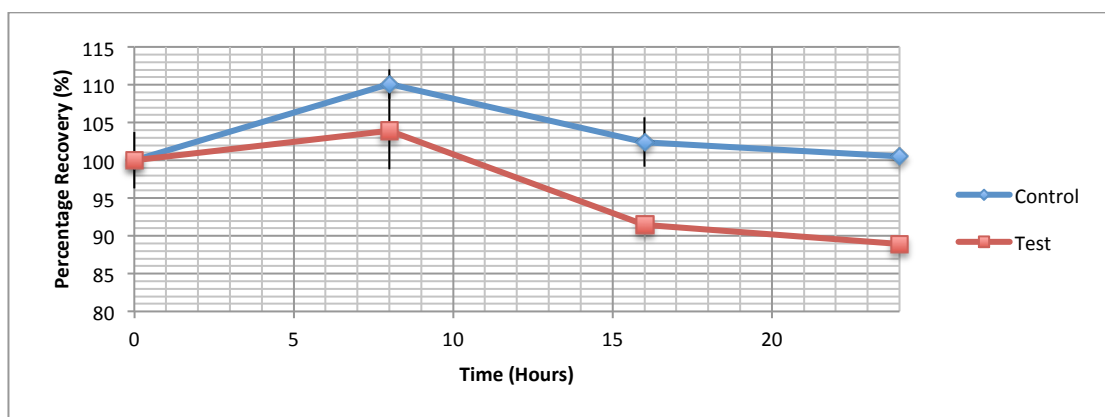


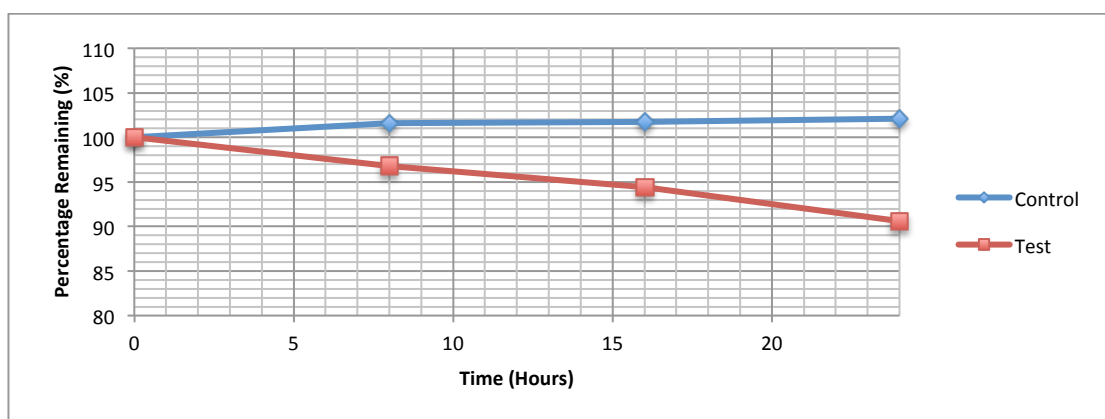
Figure 3.11. Degradation of tocopherol when exposed to (a) cool white light, (b) warm white light and (c) UVA light

3.5.6.6. Retinol

(a)



(b)



(c)

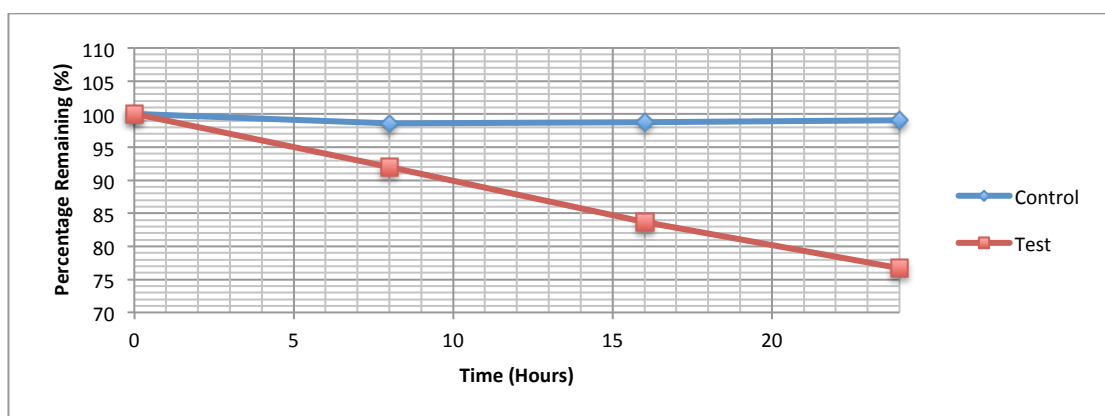


Figure 3.12. Degradation of retinol when exposed to (a) cool white light, (b) warm white light and (c) UVA light

Out of the eight vitamins examined retinol and riboflavin were the only vitamins to display substantial degradation when exposed to cool and warm white light. Riboflavin showed approximately a 20% loss whereas retinol loss was approximately 10% in both types of lights when compared to a control over the 24-hour period. The other vitamins examined showed no degradation and were considered stable in these conditions.

Retinol was the only vitamin that was substantially degraded by UVA light over the 24-hour period. Approximately 25% of retinol was degraded over this period of time. Riboflavin displayed no substantial degradation in UVA light.

3.6. Discussion

For the most part, the test syringes and their corresponding control syringes show good physical stability. Visual examination, pH, osmolality and laser diffraction show consistent results between 0 hours and 24 hours for the test syringes and this data mimics the results seen with the control syringes. However, examination of the test syringe under warm white light over 24 hours shows an increase in largest globule size and number of globules sized over 7.5 μm . Unlike the test syringe the corresponding control syringe did not show any increases in globule size. The reduced physical stability in this type of light seems strange considering no problems were encountered with the other types of light. Further investigation into this physical instability is required.

From these data it is evident that the most sensitive vitamins to light are clearly riboflavin and retinol. Substantial degradation has occurred in all three types of light with retinol and riboflavin was substantially degraded in cool white and warm white light. In these experiments, riboflavin is predominantly degraded in visible light, which contradicts some of the literature available, which suggests that riboflavin is more susceptible to degradation in UVA light (Allwood & Kearney 1998). Retinol displayed modest degradation in cool and warm white light and approximately a 10% loss was observed. However, in UVA light retinol is degraded to a much higher degree, with almost a third of the total quantity of retinol being lost over a 24-hour period.

The other vitamins examined do not show any substantial degradation. This is interesting especially for pyridoxine, which according to the literature should show some light degradation (DeRitter 1982; Gallitelli 1995; Smith et al. 1988). However, we can clearly see from this experiment that very little pyridoxine is lost during exposure to cool white, warm white and UVA light. In addition, no vitamin loss was seen with tocopherol, supporting previous work in the area (Allwood & Martin 2000). It is thought that tocopherol is oxidised in a reaction catalysed by light, therefore, if excess oxygen is prevented from entering the medium then degradation cannot occur.

Furthermore, out of all the vitamins examined, nicotinamide has the least stability data available in the literature. We can see that it was very stable in all three types of light following 24-hour exposure.

Some researchers believe that lipid emulsions may confer some sort of protection to vitamins in PN, however, as previously discussed this is certainly a contentious issue (Allwood & Martin 2000; Allwood 1982; Billion-Rey et al. 1993; Haas et al. 2002; Smith et al. 1988). In these experiments the presence of lipid did not prevent the degradation of retinol or riboflavin. Whether or not it slowed the rate of degradation is uncertain and would require further investigation.

3.7. Conclusion

Water and fat-soluble vitamins showed good stability in cool white, warm white and UVA light. Substantial degradation was seen with retinol and riboflavin, however, riboflavin showed some unexpected degradation in cool and warm white light. In fact, it showed more pronounced degradation in cool and warm white light than it did in UVA light. The sensitivity shown to these commonly used artificial lights suggests light protection is necessary at all times.

CHAPTER FOUR

Stability of Vitamins in Artificial Light Sources Over 6+1 Days

4.1. Introduction

There is an increased interest in administering PN in homes as a convenient alternative to hospitals aimed at reducing costs and improving patient care. To make home administration feasible, it should be stable for a minimum period of seven days, with six days storage in a refrigerator (2-8°C) and one day storage at room temperature while being administered to the patient. The purpose of this chapter is to investigate the impact of different types of light on PN after storage in a refrigerator. These experiments will mimic home administration of PN.

4.2. Materials and Methods

4.2.1. Experimental design

The materials and analysis methods used in these experiments are the same as described in Chapter Two and Three.

4.2.1.1. Sample preparation

Five syringes were made for each experiment. Five Solivito® N vials were reconstituted with 10 ml of Vitlipid® N Adult and transferred to five 50 ml Becton Dickinson® syringes. These syringes were made up to volume with IL20

removing any excess air. The syringes were then end-capped and inverted 20 times to ensure that the vitamins were evenly distributed. Three syringes were exposed to either warm white, cool white or UVA light while two syringes were used as controls and protected from light.

4.2.1.2. Cool and warm white light

In each experiment, five syringes were initially protected from light and stored between 2-8°C using a Lec Medical refrigerator (Bognor Regis, West Sussex) for a period of six days. Following this storage period, the syringes were removed and three of the syringes were exposed to either cool white (experiment one) or warm white light (experiment two) using a multi-ray lamp for a period of 24 hours at room temperature (25°C). The remaining two syringes in each experiment were used as controls and protected from light at room temperature. During these experiments the illuminance of cool and warm white light was set at approximately 5,000 lux.

4.2.1.3. UVA light

All five syringes were stored in a refrigerator (2-8°C) for 6 days. Following storage, three of the syringes were placed in a SANYO® stability chamber and exposed to UVA light for a period of 24 hours at room temperature (25°C). The remaining two syringes were used as controls and protected from light at room

temperature. During these experiments the light intensity of UVA was set at approximately 1.2 Wm^{-2} .

4.2.1.4. Physical and chemical testing

In each experiment, two of the test syringes that were exposed to artificial light sources and one of the control syringes protected from light were chemically analysed using the stability-indicating HPLC assay described in Chapter Two. Chemical testing was performed on day 0, 6 and 6+1. Substantial degradation is defined as loss of greater than 10% over the 7-day period.

The remaining test and control syringes had physical studies performed on them. Visual examination, microscope analysis and laser diffraction were carried out on day 0, 6 and 6+1, whereas, pH was performed on day 0 and 6+1. Osmolality was only performed on day 6+1. The physical and chemical tests were undertaken with different syringes to ensure that the volume of the syringes remained as close to 50 ml as possible. It is conceivable that changes in volume could have an impact on rate of degradation and emulsion stability. Reducing the volume of the syringe increases the surface area to volume ratio, thereby increasing the interaction of light with photosensitive components of PN potentially increasing degradation.

4.3. Results

4.3.1. Visual examination

At the start of all the experiments the syringes had a yellow/cream colour. This did not change following storage in the refrigerator and exposure to the three types of artificial light. No creaming or free oil was observed in any of the syringes indicating a stable emulsion. There were no other visual changes with any of the samples examined.

4.3.2. Microscopy

All samples were examined using a microscope to monitor globule size, shape and arrangement. These results can be seen in Tables 4.1-4.3. In all three experiments, there was little change in globule size and shape after six days of storage in the refrigerator.

Following exposure to cool white light, the sample changed little, with a slight increase in particles sized above 7.5 μm . The globules of the emulsion remained singular and maintained their spherical morphology throughout.

The syringe exposed to warm white light was similar to the results seen with cool white light. There was a slight increase in the number of particles sized between 7.5 – 10 µm, but no other changes were seen.

The syringe exposed to UVA light had some changes with regards to emulsion stability. The amount of globules sized over 7.5 µm was vastly increased and the maximum globule size was increased from 10 µm to 30 µm. The globules remained spherical, however, there were some flocculated arrangements visible.

Table 4.1. Microscope analysis of control and test syringes exposed to cool white light.

	0 Days		6 Days		6+1 Days	
	Control	Test	Control	Test	Control	Test
Colour	Yellow/ Cream	Yellow/ Cream	Yellow/ Cream	Yellow/ Cream	Yellow/ Cream	Yellow/ Cream
Largest	10	7.5	20	10	40	20
>10 µg	1	0	1	4	5	11
7.5-10 µg	5	4	3	5	10	12
Round/Crystalline/Amorphous	Round	Round	Round	Round	Round	Round
Single/Flocculated/Aggregated	Single	Single	Single	Single	Single	Single

Table 4.2. Microscope analysis of control and test syringes exposed to warm white light.

	0 Days		6 Days		6+1 Days	
	Control	Test	Control	Test	Control	Test
Colour	Yellow/ Cream	Yellow/ Cream	Yellow/ Cream	Yellow/ Cream	Yellow/ Cream	Yellow/ Cream
Largest	10	10	10	30	7.5	30
>10 µg	2	2	1	7	0	3
7.5-10 µg	12	7	5	12	3	13
Round/Crystalline/Amorphous	Round	Round	Round	Round	Round	Round
Single/Flocculated/Aggregated	Single	Single	Single	Single	Single	Single

Table 4.3. Microscope analysis of control and test syringes exposed to UVA light.

	0 Days		6 Days		6+1 Days	
	Control	Test	Control	Test	Control	Test
Colour	Yellow/ Cream	Yellow/ Cream	Yellow/ Cream	Yellow/ Cream	Yellow/ Cream	Yellow/ Cream
Largest	10	20	7.5	10	20	40
>10 µg	3	7	0	2	5	14
7.5-10 µg	13	29	2	7	9	14
Round/Crystalline/Amorphous	Round	Round	Round	Round	Round	Round
Single/Flocculated/Aggregated	Single	Single	Single	Single	Single	Single

4.3.3. Laser diffraction

The laser diffraction results for test and control samples are summarised in Table 4.4. When exposed to cool white light the globule size did not increase. The 'size under' of globules remained constant throughout the testing period for both control and test syringes. In both test and control syringes, the largest

mean 'size under' recorded was 1.150 μm . The D[4,3] of the test and control syringes remained relatively constant between the time points and the means ranged between 0.282 μm and 0.289 μm . There was a slight increase in this value over time.

In warm white light, the mean 'size under' value of the globules did not exceed 1.259 μm in both the control and test syringes. The mean D[4,3] ranged between 0.297 μm and 0.302 μm for both the test and the control syringes over all three time points.

The test and control syringes exposed to UVA light mean globule size did not exceed 1.096 μm . The D[4,3] for both the test and control syringes ranged between 0.284 μm and 0.290 μm over the three time points.

Table 4.4. Mean and SD of D[4,3] and 'size under' results in control (C) and test (T) samples at 0, 6 and 6+1 days.

		0 Days				6 Days				6+1 Days			
		D[4,3] (μm)		'Size under' (μm)		D[4,3] (μm)		'Size under' (μm)		D[4,3] (μm)		'Size under' (μm)	
		C	T	C	T	C	T	C	T	C	T	C	T
Cool White	Mean	0.282	0.282	1.096	1.150	0.282	0.282	1.096	1.150	0.288	0.289	1.096	1.096
	SD	0.001	0.001	0.001	0.094	0.001	0.001	0.000	0.094	0.003	0.002	0.000	0.000
Warm White	Mean	0.300	0.302	1.259	1.150	0.301	0.301	1.096	1.096	0.297	0.299	1.096	1.205
	SD	0.000	0.002	0.000	0.094	0.004	0.002	0.000	0.000	0.002	0.003	0.000	0.094
UVA	Mean	0.286	0.284	1.096	1.096	0.287	0.284	1.096	1.096	0.290	0.290	1.096	1.096
	SD	0.003	0.001	0.000	0.000	0.004	0.005	0.000	0.000	0.003	0.002	0.000	0.000

4.3.4. Osmolality

Osmolality was measured on day 6+1 and the results are summarised in Table 4.5. The osmolality of the test and control syringes were very similar in each of the experiments. The osmolality ranged between 456 and 475 mOsm/kg for all the syringes (control and test syringes) showing consistency between results.

Table 4.5. Summary of osmolality values recorded on day 6+1.

	Cool White		Warm White		UVA	
	Control	Test	Control	Test	Control	Test
Osmolality (mOsm/kg)	475	466	456	466	460	461

4.3.5. pH

The pH (Table 4.6) was measured on day 0 and 6+1 and test syringes were compared with the control. pH differences in red indicate decreases in pH and those in green represent increases in pH over the time period. The pH remained relatively unchanged regardless of exposure to different artificial light sources. The pH values ranged from 6.24 and 5.73. The largest difference in pH values following storage was 0.45 pH units in any of the syringes examined.

Table 4.6. pH of syringes exposed to cool white, warm white and UVA light at 0 and 6+1 days

Time (Days)	Cool White		Warm White		UVA	
	Control	Test	Control	Test	Control	Test
0	6.23	6.22	6.07	6.06	6.24	6.18
6+1	5.90	5.83	5.96	5.91	5.82	5.73
pH difference	- 0.33	- 0.39	- 0.11	- 0.15	- 0.42	- 0.45

4.3.6. HPLC

4.3.6.1. Summary of HPLC results

Table 4.7 summarises the percentage of vitamins remaining following 6 days storage in a refrigerator (2-8 °C) and then either exposure to artificial light (Test) or protection from light (Control) at 25 °C.

From these data it is evident that the majority of vitamins that were examined in these experiments were stable regardless of an initial storage period. Out of the eight vitamins examined, only riboflavin and retinol were substantially degraded in the artificial light sources. Retinol was degraded to a greater extent than riboflavin in UVA light, whereas in cool and warm white light riboflavin had larger losses. Other light sensitive vitamins such as tocopherol and pyridoxine had no substantial losses throughout the testing period. Nicotinamide, a vitamin

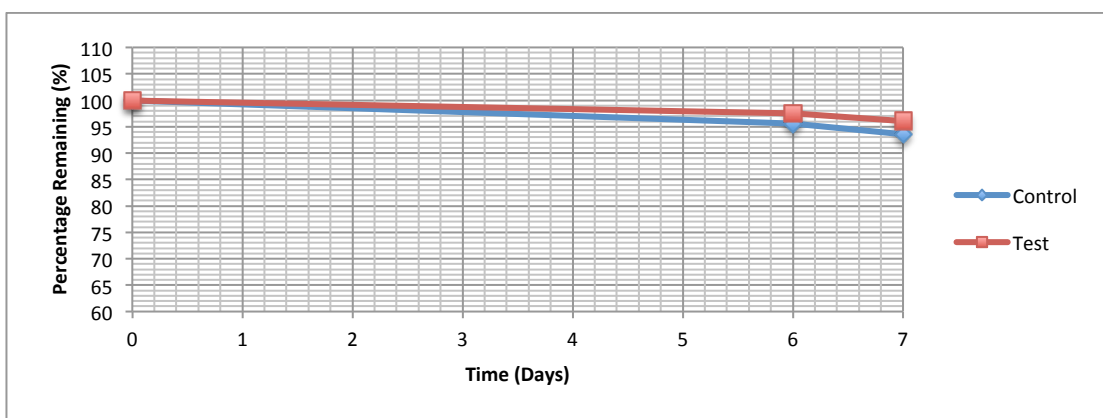
with very little stability information, remained stable throughout the testing period.

Table 4.7. Mean percentage \pm SD of vitamins remaining after 6+1 days in control syringes and test syringes.

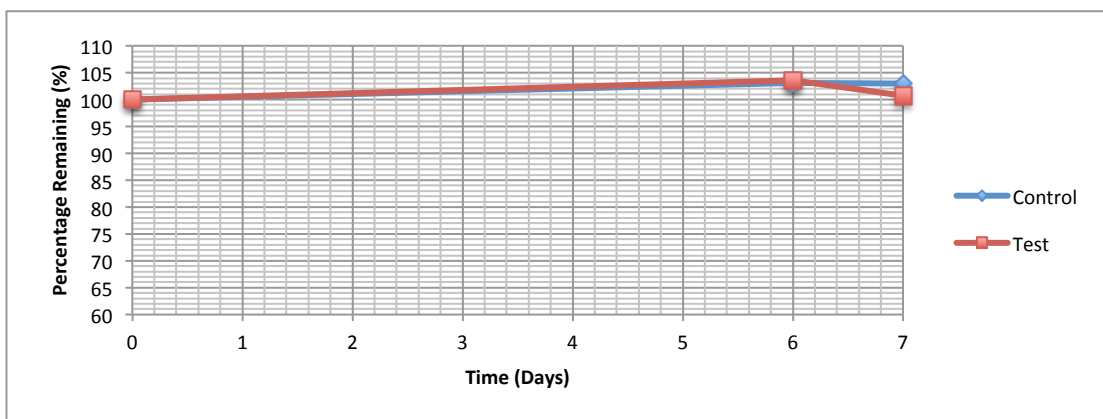
Vitamin	Cool White Light		Warm White Light		UVA Light	
	Control	Test	Control	Test	Control	Test
Pyridoxine	93.58 \pm 1.03	96.06 \pm 0.82	102.96 \pm 1.24	100.69 \pm 1.28	101.43 \pm 0.85	101.72 \pm 1.23
Nicotinamide	99.81 \pm 2.26	99.08 \pm 0.13	101.31 \pm 1.86	101.49 \pm 0.93	101.05 \pm 1.54	101.00 \pm 1.01
Thiamine	99.99 \pm 9.68	98.11 \pm 1.24	95.61 \pm 4.94	94.19 \pm 2.51	96.81 \pm 6.92	97.71 \pm 1.15
Folic acid	94.72 \pm 0.32	99.95 \pm 3.56	102.72 \pm 3.72	103.24 \pm 2.03	105.83 \pm 0.87	108.81 \pm 2.93
Riboflavin	104.69 \pm 2.92	84.48 \pm 2.73	100.17 \pm 6.82	74.14 \pm 4.29	94.39 \pm 3.66	87.67 \pm 1.70
Ergocalciferol	100.89 \pm 0.98	100.42 \pm 0.94	98.72 \pm 1.90	96.24 \pm 2.81	99.44 \pm 0.29	98.84 \pm 0.45
Tocopherol	100.20 \pm 0.73	100.71 \pm 0.33	100.11 \pm 1.70	99.30 \pm 2.28	99.93 \pm 0.20	99.46 \pm 0.39
Retinol	100.12 \pm 0.28	89.84 \pm 0.24	96.30 \pm 1.52	88.22 \pm 1.19	99.27 \pm 1.32	77.15 \pm 0.30

4.3.6.2. Pyridoxine

(a)



(b)



(c)

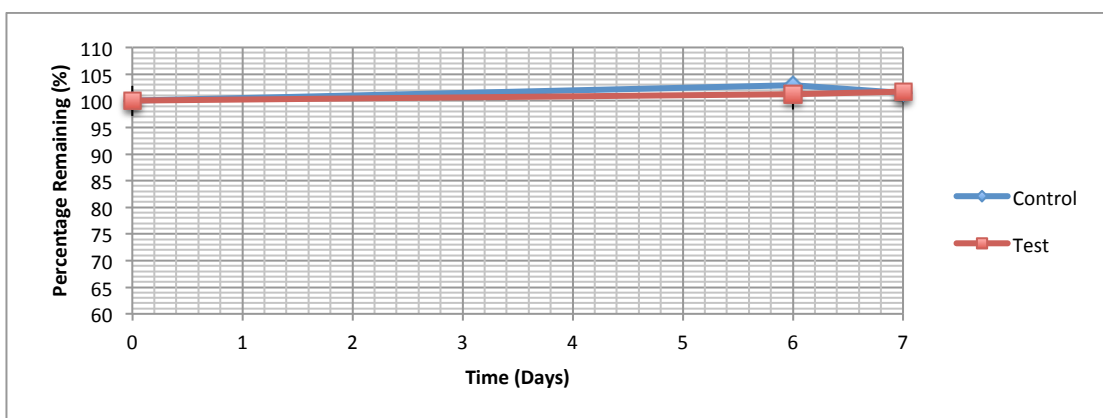
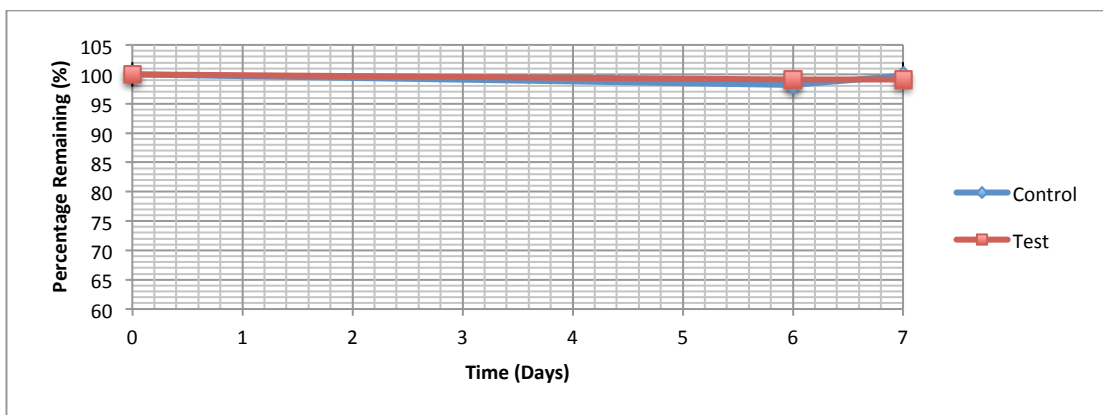


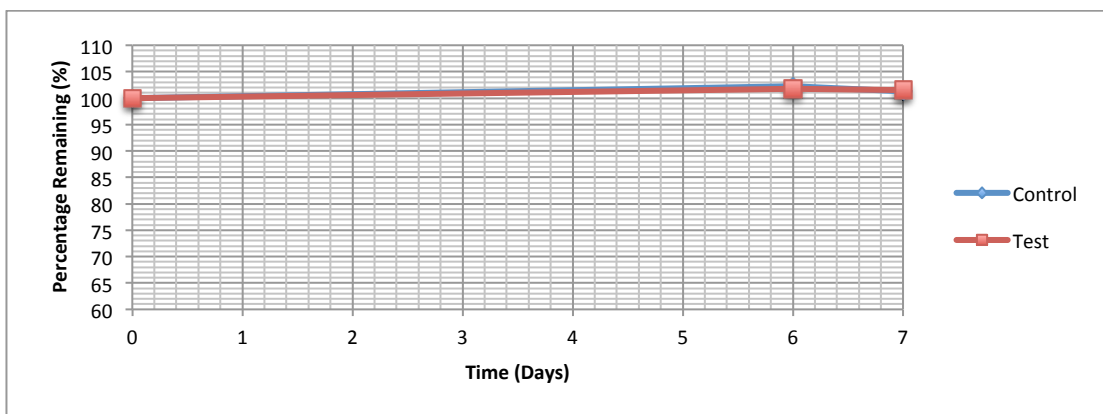
Figure 4.1. Degradation of pyridoxine when exposed to (a) cool white light, (b) warm white light and (c) UVA light following up to 7 days of storage

4.3.6.3. Nicotinamide

(a)



(b)



(c)

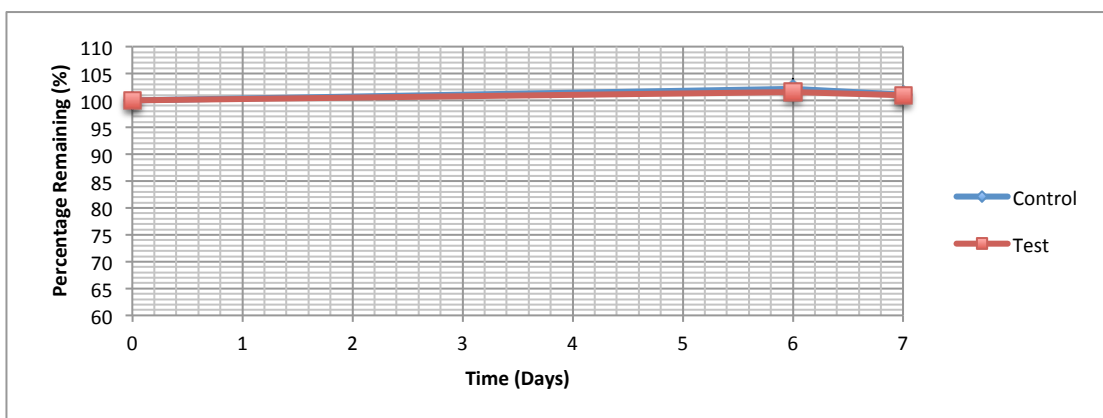
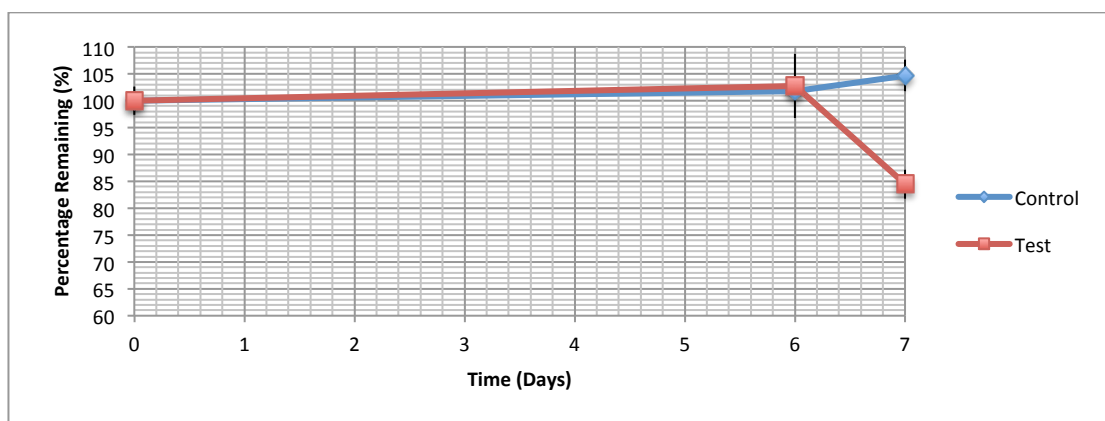


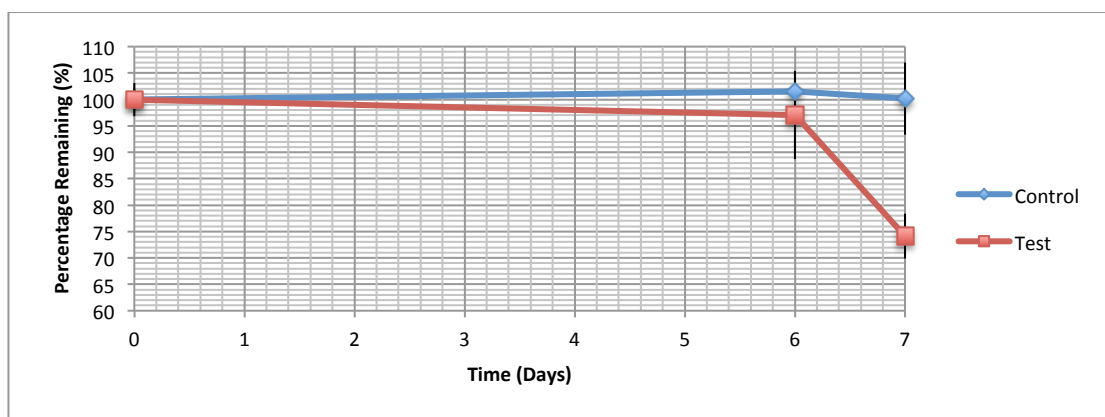
Figure 4.2. Degradation of nicotinamide when exposed to (a) cool white light, (b) warm white light and (c) UVA light following up to 7 days of storage

4.3.6.4. Riboflavin

(a)



(b)



(c)

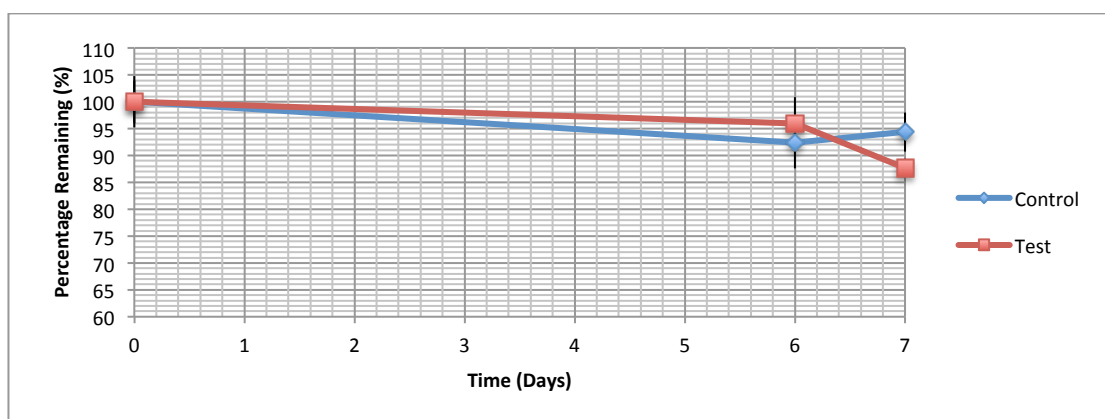
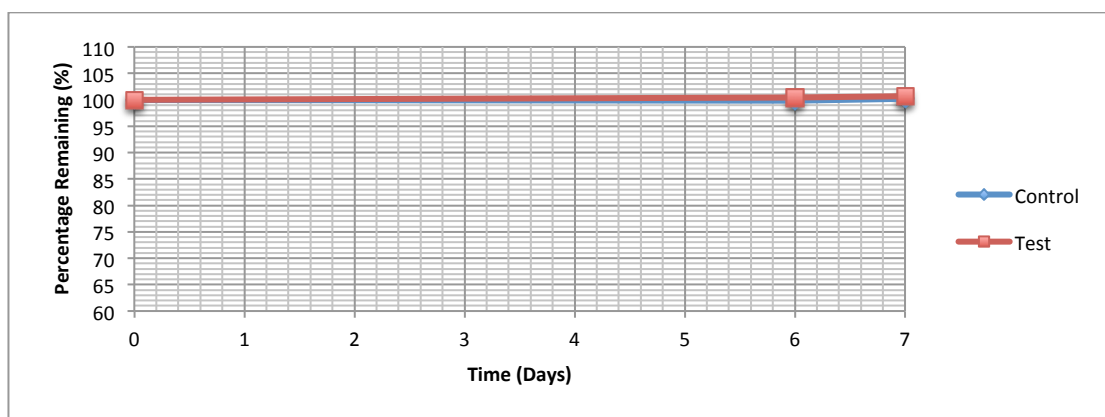


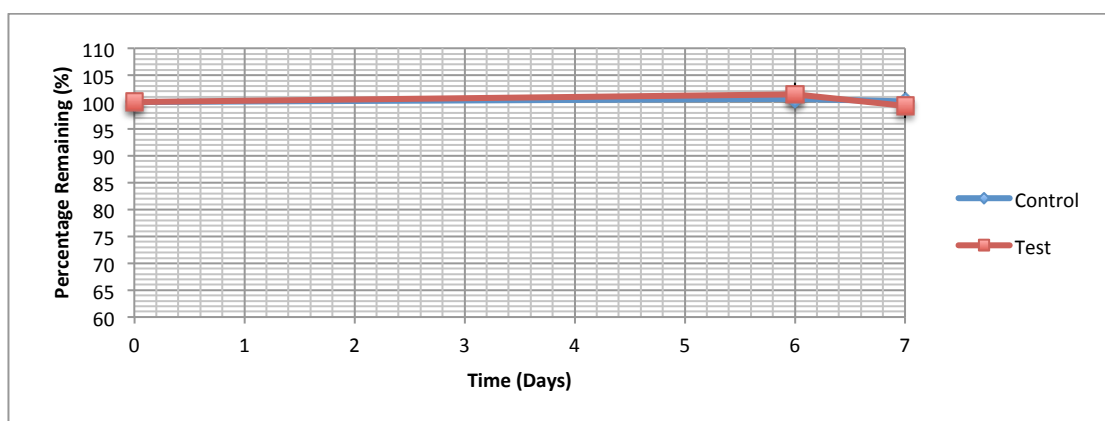
Figure 4.3. Degradation of riboflavin when exposed to (a) cool white light, (b) warm white light and (c) UVA light following up to 7 days of storage

4.3.6.5. Tocopherol

(a)



(b)



(c)

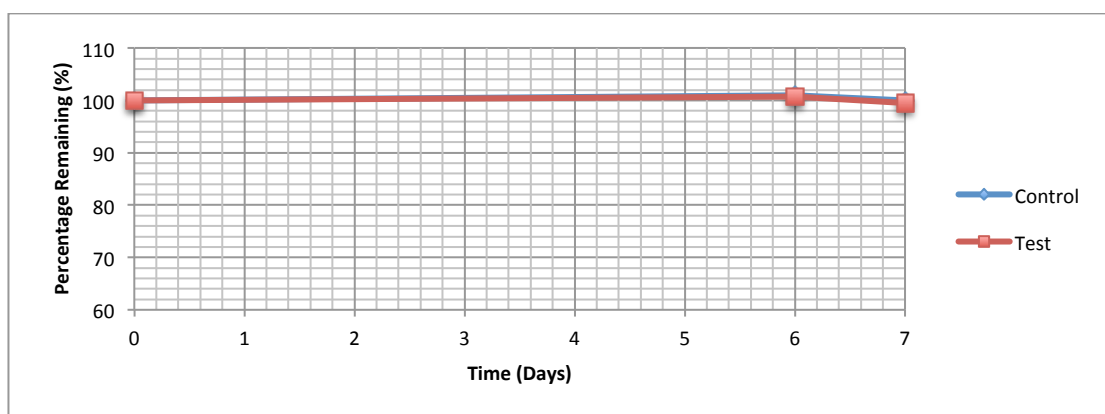
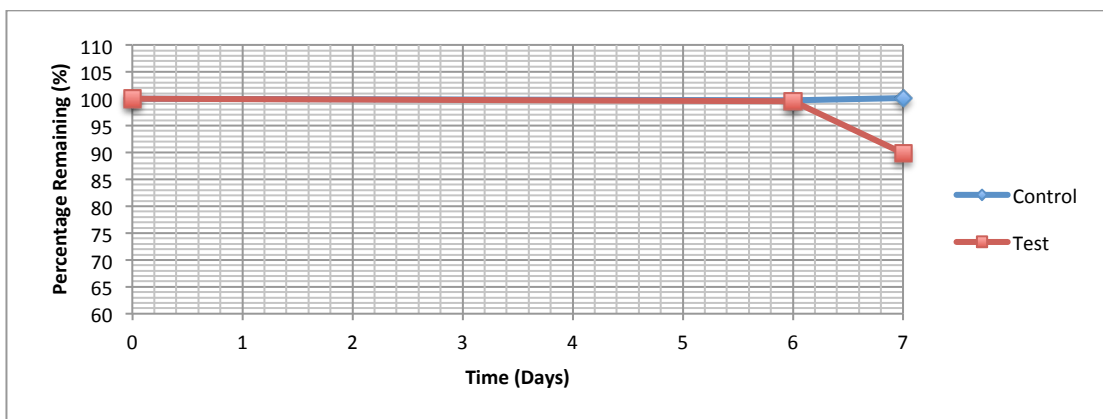


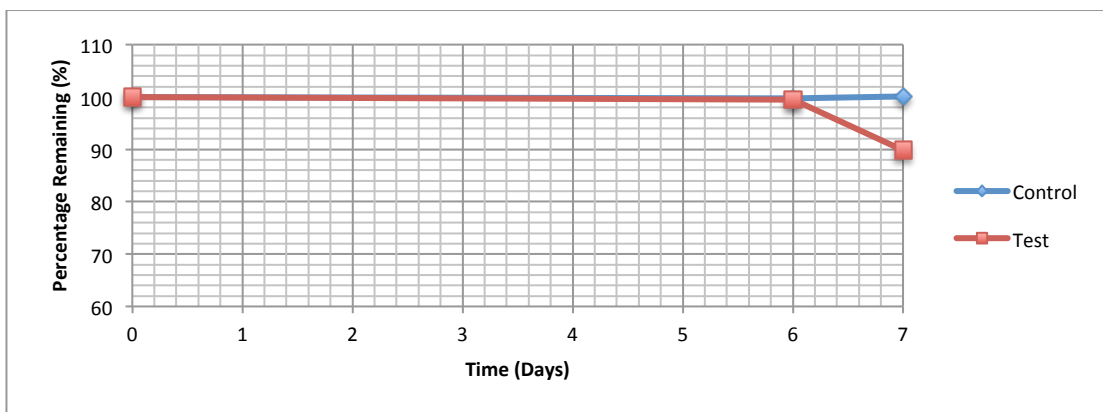
Figure 4.4. Degradation of tocopherol when exposed to (a) cool white light, (b) warm white light and (c) UVA light following up to 7 days of storage

4.3.6.6. Retinol

(a)



(b)



(c)

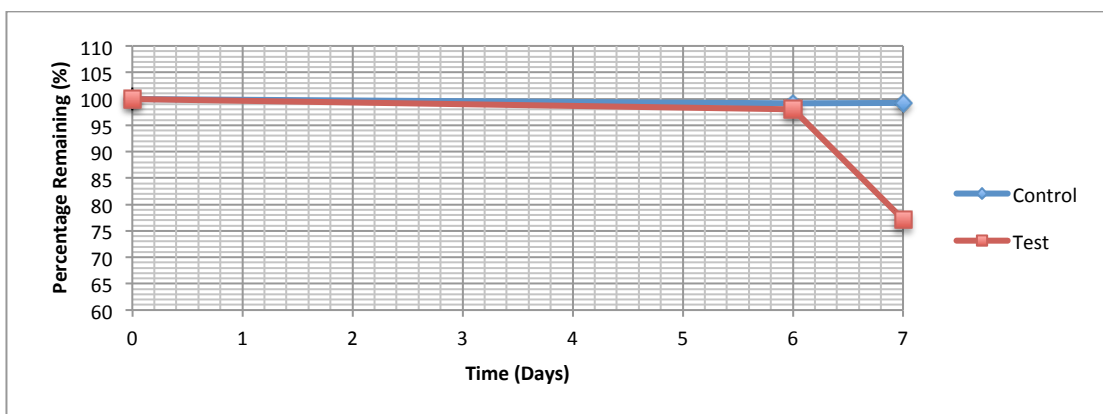


Figure 4.5. Degradation of retinol when exposed to (a) cool white light, (b) warm white light and (c) UVA light following up to 7 days of storage

4.4. Discussion

The physical studies have shown that following exposure of the samples to cool white and warm white light, there were no stability issues and the emulsion retained its integrity. Visual examination along with microscope, laser diffraction, osmolality and pH values of the test and control syringes remained constant throughout the time points. Samples that had been exposed to cool and warm white light would therefore be deemed physically safe for administration to a patient.

Examination of the emulsion exposed to UVA light revealed an increase in globule size and also some flocculated arrangements. This could be potentially dangerous for a patient as it could block a capillary and prevent blood from being transported in the body (Driscoll 2005). Apart from microscope examination, the physical tests performed showed little difference between the control and the test syringes.

All of the vitamins examined remained chemically stable when protected from light and stored for 6 days between 2-8°C. However, once the syringes were exposed to different artificial light sources at room temperature, degradation did occur. From the eight vitamins examined, riboflavin and retinol showed substantial sensitivity to light. When compared with day 0, approximately 25%, 15% and 13% of riboflavin was degraded on exposure to warm white, cool white and UVA light respectively. This supports data produced in Chapter Three,

which show a substantial decrease of riboflavin in cool and warm white light, but a much smaller loss in UVA light. This suggests that riboflavin is degraded more by light in the visible spectrum than previously thought (Allwood & Kearney 1998).

Retinol was less sensitive to cool and warm white light showing approximately a 10% loss in both artificial light sources in comparison with day 0. In UVA light, the degradation was more substantial, with an approximate loss of 23%. Retinol is known to primarily degrade in UVA light (Allwood & Plane 1986) and follows an expected pattern, with little loss in light sources containing small amounts of UVA light (cool and warm white light) and substantial losses in exclusively UVA light.

As described earlier both pyridoxine and tocopherol have been identified as light sensitive vitamins in previous studies (Allwood & Martin 2000; DeRitter 1982; Gallitelli 1995; Smith, Canham, & Wells 1988). Still, there was no substantial losses seen with either vitamin following 6 days of storage and exposure to any of the artificial light sources. This further supports the theory that tocopherol is oxidised in a reaction catalysed by light, as all excess oxygen was removed from the syringe during the sample preparation (see 4.2.1.1) this reaction was limited.

As described in Chapter One, nicotinamide stability in PN has not been thoroughly investigated, however, studies suggest that it is a robust vitamin,

which does not degrade readily (Dahl et al. 1986). When exposed to cool white, warm white or UVA light following a six-day storage period we can see that there was no vitamin loss.

4.5. Conclusion

From these data it is evident that most water and fat-soluble vitamins remain relatively stable following six days of storage and one day of exposure to either cool white, warm white or UVA light. Riboflavin and retinol were the most sensitive vitamins and substantial losses were seen when exposed to any of the artificial light sources investigated. Riboflavin had larger losses than expected in warm and cool white light compared to UVA light. These data agree with results obtained in Chapter Three and storage of the syringes in a fridge for six days has little impact on the degradation of vitamins. However, stability issues identified with retinol and riboflavin support the use of light protection at all times.

CHAPTER FIVE

Investigating the Photo-Protective Effects of
Lipid Emulsions on Light-Sensitive Vitamins

5.1. Introduction

Whether lipid emulsions provide protection to PN constituents is still a matter of debate. Some researchers believe that lipid emulsions provides very little protection to vitamins and advocate the use of light protection wherever possible (Allwood & Martin 2000; Drott et al. 1991). However, others have seen markedly decreased degradation rates in admixtures containing lipid emulsions (Billion-Rey et al. 1993; Haas et al. 2002). Further investigation into the protective effects of lipid emulsions on light sensitive vitamins is needed for two reasons: Firstly, the current literature available on this topic is limited and it has not been the primary focus of any of the investigations reported, and secondly, there are no recent studies in the literature investigating exposure from contemporary artificial light sources.

This chapter describes investigations into the protective effects of different types of commonly used lipid emulsions (Intralipid® 10 (IL10), Intralipid® 20 (IL20) and SMOFLipid® 20% (SMOF)) on vitamin degradation.

5.2. Materials and Methods

The materials and physical analysis methods used in these experiments are the same as described in Chapter Three.

5.2.1. Stability-indicating HPLC assay used for the quantification of fat-soluble vitamins

5.2.1.1. Stability-indicating HPLC assay method

The stability-indicating assay used in these experiments was developed by Allwood and Martin (2000) and the method can be seen in Table 5.1. This is 32 minutes shorter than the assay developed in Chapter Two, therefore, this allows the analysis of more samples in a shorter time period. This assay uses an isocratic elution method performed at room temperature.

Column:

OmniSpher 5 C18 150x3mm, 5µm particle size (Varian, Palo Alto, USA)

Mobile Phase:

A: Methanol

Table 5.1. Stability-indicating HPLC assay method

Time (minutes)	A (%)	UV (nm)	Flow Rate (ml/min)
7	100	292	1.5
15		325	

As seen in the chromatogram (Figure 5.1), this stability-indicating assay can identify ergocalciferol, tocopherol and retinol. However, in this chapter, we will primarily be concerned with tocopherol and retinol. In these experiments, retinol was chosen as it has been shown to degrade in cool white, warm white and UVA light sources, which was demonstrated in the studies in Chapter Three and Four. Tocopherol is also being investigated as light has been shown to catalyse its degradation (Allwood & Martin 2000). Ergocalciferol has not shown any light sensitivity in the literature or in previous experiments in Chapter Three and Four and so its data has not been recorded in this instance.

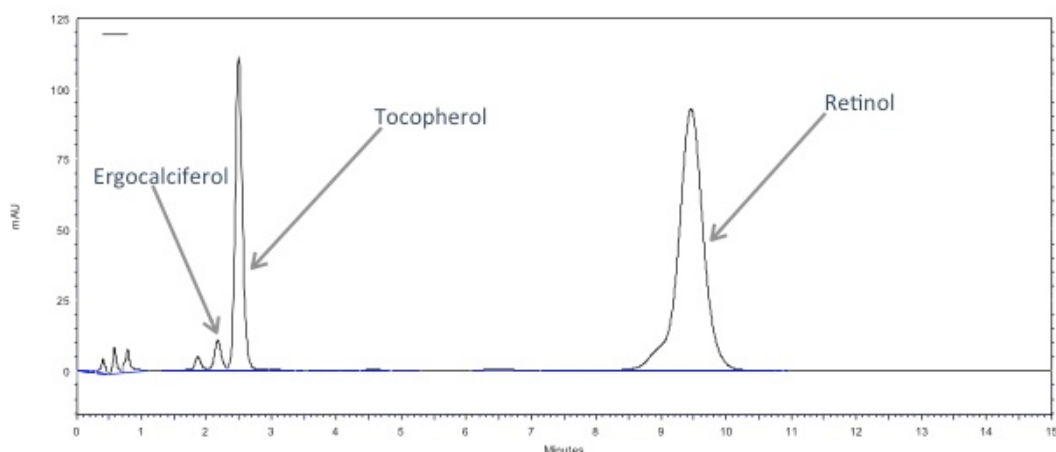


Figure 5.1. Chromatogram of Vitlipid® N Adult in ultra-filtered deionised water.

Peaks include: ergocalciferol, tocopherol and retinol.

5.2.1.2. Calibration, identification and stability-indicating tests for fat-soluble vitamins

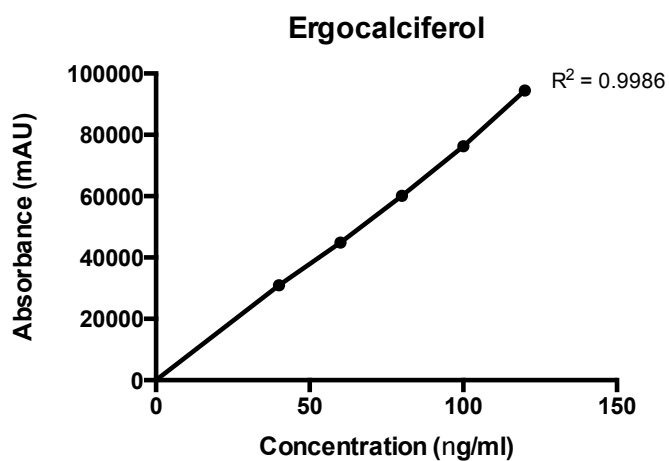
All peaks were identified individually by spiking the sample with reference standards (as seen in Chapter Two). The peaks identified in the chromatogram and their retention times can be seen in Table 5.2.

Table 5.2. Retention times of fat-soluble vitamins in a chromatogram generated by the validated stability-indicating HPLC assay described.

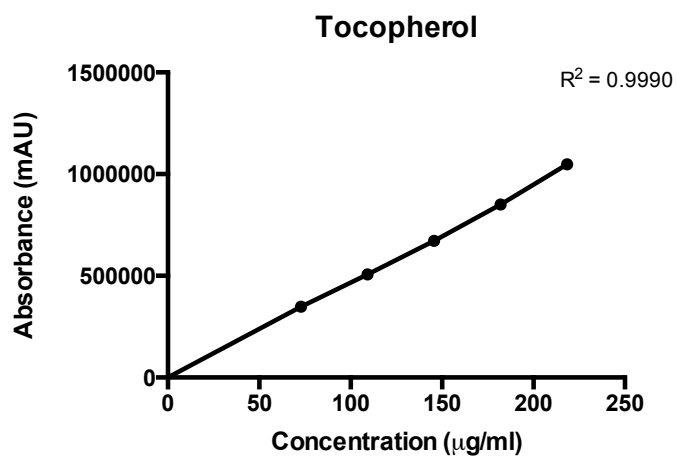
Fat-soluble vitamin	Time (minutes)
Ergocalciferol	2.17
Tocopherol	2.46
Retinol	9.46

Following identification of peaks in the HPLC chromatogram, various concentrations of Vitlipid® N Adult were analysed and a calibration curve was generated. A 100% concentration for the purpose of these experiments is 10 ml of Vitlipid® N Adult added to 40 ml of ultra-filtered deionised water. 40%, 60%, 80%, 100% and 120% concentrations were made, analysed and plotted to produce a calibration curve. The calibration curves for ergocalciferol, tocopherol and retinol can be seen in Figure 5.2.

(a)



(b)



(c)

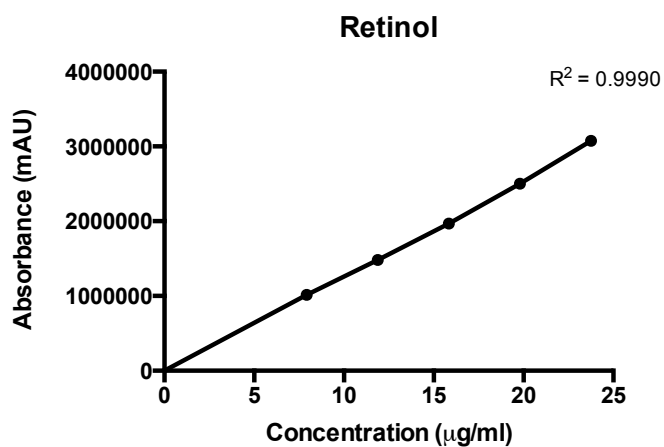


Figure 5.2. Calibration curves for (a) ergocalciferol, (b) tocopherol and (c) retinol with trendlines and R^2 values, $n=3$

RSD and R^2 values for the vitamins identified can be seen in Table 5.3. This assay has small RSD values suggesting high precision in the generation of results. The R^2 values are in excess of 0.99 demonstrating that the linearity of the method is appropriate.

Table 5.3. R^2 values and mean RSD of ergocalciferol, tocopherol and retinol analysed by a stability-indicating HPLC assay.

Vitamin	R^2	Mean RSD (%)
Ergocalciferol	0.9986	1.54
Tocopherol	0.9990	1.19
Retinol	0.9990	1.12

5.2.1.3. Types of lipid emulsion used

The contents for each of the lipid emulsions used in these experiments can be seen in Table 5.4. IL10 and IL20 are very similar with the exception of IL20 having 100 g more of purified soybean oil (SBO) in 1000 ml. SMOF has a very different composition compared to IL10 and IL20 with the inclusion of medium-chain triglycerides (MCT), refined olive oil, fish oils and vitamin E. The protective effects of the various lipid compositions will be investigated and quantified.

Table 5.4. Contents of IL10, IL20 and SMOF in 1000 ml (As per container labels)

Lipid	Contents in 1000 ml
IL10	Purified soybean oil 100 g, purified egg phospholipids, glycerol anhydrous, sodium hydroxide, water for injections pH approx. 8
IL20	Purified soybean oil 200 g, purified egg phospholipids, glycerol anhydrous, sodium hydroxide, water for injections pH approx. 8
SMOF	Refined soya-bean oil 60 g, medium-chain triglycerides 60 g, refined olive oil 50 g, fish oil (rich in omega-3 fatty acids) 30 g, glycerol, egg lecithin, all-rac- α -tocopherol (vitamin E), water for injections, sodium hydroxide (pH adjustment) and sodium oleate

5.2.2. Sample preparation

Two 50 ml Becton Dickinson® syringes, a control and a test, were filled with 12 ml of Vitlipid® N Adult and made up to 60 ml with either water, IL10, IL20 or SMOF (Fresenius Kabi, UK). Another test syringe was filled with 10 ml of Vitlipid® N Adult and made up to 50 ml with either ultra-filtered deionised water, IL10, IL20 or SMOF. All syringes were end-capped removing all excess air and inverted 20 times to ensure adequate mixing had taken place.

5.2.3. Experiment design and physico-chemical testing

Following sample preparation, 10 ml of each syringe containing 60 ml (one test, one control) was taken and used for physical testing at 0 hours leaving 50 ml in each syringe. The two test syringes were then exposed to either cool white, warm white or UVA light at 25 °C for a period of 24 hours. The remaining syringe was used as a control and protected from light. In warm and cool white light the illuminance was set at approximately 5,000 lux. In UVA, the light intensity was set at 1.2 Wm^{-2} . Chemical testing was performed using the previously described validated stability-indicating HPLC assay at 0, 8, 16 and 24 hours. Substantial degradation is defined as a loss of greater than 10% over the 24-hour period. Physical testing, as described in Chapter Three, was performed at 0 and 24 hours. All syringes were stored in a SANYO® stability chamber due to variability in room temperature from day to day in the laboratory over the test period. Each experiment was repeated to allow statistical analysis.

5.2.4. Statistical analysis of vitamin degradation in ultra-filtered deionised water, IL10, IL20 and SMOF

Statistical analysis was conducted using IBM® SPSS® Version 20. Independent sample t-tests were conducted and comparisons were made between all syringes examined.

To perform such tests three main assumptions have to be made:

- (1) The sampling distribution is normally distributed.
- (2) Samples are randomly selected from the unknown population
- (3) The variances of the known population are the same as the unknown population.

The t-test is often stated to be a robust test and can provide good statistical information even if the distributions are only vaguely normally distributed (Hinton 1996).

5.3. Results

5.3.1. Visual examination

All the syringes maintained their colour throughout the test period when exposed to cool white, warm white and UVA light. There was no sign of creaming or any free oil in any of the syringes suggesting that the emulsion integrity had not been compromised. There were no other visible signs of degradation in any of the syringes examined.

5.3.2. Microscopy

Most of the syringes that were exposed to cool white light did not exhibit any microscopic changes when compared with the control. However, in one of the test syringes, containing IL20 and Vitlipid® N Adult, there was a slight increase in maximum globule size (from 10 to 20 μm) and an increase in globules sized between 7.5 to 10 μm from 2 to 12 globules. Nevertheless, all the syringes examined exhibited separate spherical globules with no evidence of flocculation occurring.

Similar results were seen with the microscope analysis of syringes exposed to warm white light. When compared with the controls, for the most part, the emulsions that were exposed to warm white light remained relatively stable with no noticeable increases in maximum globule size or amounts of globules over 7.5 μm except for one test syringe that contained SMOF and Vitlipid® N Adult. In this case there was a substantial increase in maximum globule size increasing from 10 μm to 40 μm over the 24 hour period. In addition, some flocculation was observed in this syringe. Despite some increases in size, the globules in all the syringes maintained their spherical morphology.

The integrity of the emulsions in the syringes exposed to UVA light were maintained in all the syringes apart from one syringe containing Vitlipid® N Adult and IL20. In this sample the maximum globule size increased from 10 μm

to 30 μm and there was also an increase in the amount of globules sized above 7.5 μm following exposure from 14 to 31 globules.

5.3.3. Laser diffraction

The following tables (Table 5.5, 5.6 and 5.7) show the mean and standard deviation values of D[4,3] and 'size under' (see Chapter Three) for control and test syringes at 0 and 24 hours.

Table 5.5 Control and test sample means and SD of D[4,3] (μm) and 'Size under' (μm) when exposed to cool white light over 24 hours

		0 Hours				24 Hours			
		D[4,3] (μm)		'Size under' (μm)		D[4,3] (μm)		'Size under' (μm)	
		Control	Test	Control	Test	Control	Test	Control	Test
Water	Mean	0.259	0.258	1.259	1.259	0.258	0.258	1.259	1.259
	SD	0.007	0.008	0.000	0.000	0.009	0.009	0.000	0.000
IL10	Mean	0.242	0.237	2.512	2.512	0.239	0.236	2.512	2.512
	SD	0.010	0.002	0.000	0.000	0.002	0.001	0.000	0.000
IL20	Mean	0.282	0.281	1.150	1.096	0.281	0.280	1.332	1.096
	SD	0.002	0.001	0.084	0.000	0.004	0.002	0.578	0.000
SMOF	Mean	0.319	0.299	1.096	1.096	0.316	0.298	1.096	1.096
	SD	0.003	0.021	0.000	0.000	0.005	0.023	0.000	0.000

Table 5.6 Control and test sample means and SD of D[4,3] (μm) and ‘Size under’ (μm) when exposed to warm white light over 24 hours

		0 Hours				24 Hours			
		D[4,3] (μm)		‘Size under’ (μm)		D[4,3] (μm)		‘Size under’ (μm)	
		Control	Test	Control	Test	Control	Test	Control	Test
Water	Mean	0.257	0.258	1.259	1.259	0.255	0.254	1.259	1.259
	SD	0.008	0.009	0.000	0.000	0.005	0.007	0.000	0.000
IL10	Mean	0.243	0.237	2.512	2.512	0.238	0.238	2.512	2.512
	SD	0.010	0.002	0.000	0.000	0.002	0.002	0.000	0.000
IL20	Mean	0.283	0.282	1.096	1.096	0.281	0.284	1.123	1.123
	SD	0.004	0.002	0.000	0.000	0.003	0.002	0.067	0.067
SMOF	Mean	0.311	0.306	1.096	1.096	0.310	0.303	1.096	1.096
	SD	0.016	0.019	0.000	0.000	0.008	0.015	0.000	0.000

Table 5.7 Control and test sample means and SD of D[4,3] (μm) and ‘Size under’ (μm) when exposed to UVA light over 24 hours

		0 Hours				24 Hours			
		D[4,3] (μm)		‘Size under’ (μm)		D[4,3] (μm)		‘Size under’ (μm)	
		Control	Test	Control	Test	Control	Test	Control	Test
Water	Mean	0.253	0.252	1.259	1.259	0.251	0.251	1.259	1.259
	SD	0.002	0.002	0.000	0.000	0.002	0.002	0.000	0.000
IL10	Mean	0.218	0.219	1.878	2.033	0.218	0.218	1.878	1.878
	SD	0.004	0.002	0.480	0.379	0.004	0.002	0.480	0.480
IL20	Mean	0.285	0.284	1.096	1.096	0.281	0.281	1.178	1.123
	SD	0.002	0.004	0.000	0.000	0.004	0.003	0.089	0.067
SMOF	Mean	0.323	0.331	1.096	1.096	0.323	0.331	1.096	1.096
	SD	0.002	0.006	0.000	0.000	0.005	0.008	0.000	0.000

From these data, it is evident that the maximum globule size and D[4,3] remain small regardless of storage conditions. The maximum globule size recorded was

2.512 μm in syringes exposed to each of the lights described, which is far below the 5 μm threshold that may cause capillary occlusion in patients (Driscoll, 2006). The recorded D[4,3] values indicate a small mean volume diameter of globules in syringes that were exposed to light. In addition, the standard deviation values for all the syringes remain very small indicating good precision with these results.

5.3.4. Osmolality

The tables below (Table 5.8, 5.9 and 5.10) give the mean osmolality values for control and test syringes in different types of lipid emulsion. The osmolality should remain constant throughout the testing period and was measured at 24 hours.

Table 5.8 Mean osmolality values for syringes exposed to cool white light

	Water		IL10		IL20		SMOF	
	Control	Test	Control	Test	Control	Test	Control	Test
Osmolality (mOsm/kg)	53.5	54.5	319.0	319.0	324.0	333.0	367.0	365.0

Table 5.9 Mean osmolality values for syringes exposed to warm white light

	Water		IL10		IL20		SMOF	
	Control	Test	Control	Test	Control	Test	Control	Test
Osmolality (mOsm/kg)	55.0	54.5	321.5	320.0	322.0	325.5	362.5	365.5

Table 5.10 Mean osmolality values for syringes exposed to UVA light

	Water		IL10		IL20		SMOF	
	Control	Test	Control	Test	Control	Test	Control	Test
Osmolality (mOsm/kg)	54.0	54.0	299.5	300.0	322.5	326.5	369.5	370.0

The highest mean osmolality value for all syringes was 370.0 mOsm/kg. This is below 1000 mOsm/kg, therefore, unlikely to cause thrombophlebitis (Madan et al., 1992).

5.3.5. pH

Tables 5.11, 5.12 and 5.13 show the pH values for control and test syringes exposed to the three types of artificial light. pH differences in red indicate decreases in pH and those in green represent increases in pH over the time period. Differences in pH values at 0 and 24 hours are calculated, with pH differences in excess of 0.5 units indicative of instability.

Table 5.11. pH of syringes at 0 and 24 hours exposed to cool white light

Time (Hours)	Water				IL 10				IL 20				SMOF			
	Control		Test		Control		Test		Control		Test		Control		Test	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
0	6.80	6.98	6.80	7.01	5.62	4.92	5.37	5.05	7.30	7.32	7.39	7.41	6.97	7.06	6.95	6.99
24	7.06	7.21	7.06	7.32	5.60	5.37	5.30	5.09	7.35	7.32	7.35	7.41	6.95	7.04	6.85	6.95
pH change	0.26	0.23	0.16	0.31	0.02	0.45	0.07	0.04	0.05	0.00	0.04	0.00	0.02	0.02	0.10	0.04

Table 5.12. pH of syringes at 0 and 24 hours exposed to warm white light

Time (Hours)	Water				IL 10				IL 20				SMOF			
	Control		Test		Control		Test		Control		Test		Control		Test	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
0	7.19	6.90	7.15	6.80	6.07	4.79	5.89	4.40	7.36	7.33	7.39	7.33	7.06	6.97	7.18	6.88
24	7.17	7.23	7.03	7.13	6.01	4.61	5.80	4.40	7.33	7.37	7.36	7.39	7.09	7.09	6.98	6.89
pH Change	0.02	0.33	0.12	0.33	0.06	0.18	0.09	0.00	0.03	0.04	0.03	0.06	0.03	0.12	0.20	0.01

Table 5.13. pH of syringes at 0 and 24 hours exposed to UVA light

Time (Hours)	Water				IL 10				IL 20				SMOF			
	Control		Test		Control		Test		Control		Test		Control		Test	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
0	6.95	6.9	7.02	6.91	6.86	6.87	6.92	7.05	7.00	7.00	7.04	7.03	6.81	6.76	6.77	6.71
24	7.03	6.87	7.07	7.00	6.91	6.99	7.01	7.05	7.05	7.04	7.07	7.05	6.84	6.76	6.79	6.72
pH Change	0.08	0.03	0.05	0.09	0.05	0.12	0.09	0.00	0.05	0.04	0.03	0.02	0.03	0.00	0.02	0.01

The pH of all the syringes were within 0.5 pH units following exposure to either cool white, warm white or UVA light indicating stable admixtures.

5.3.6. HPLC analysis

5.3.6.1. Tocopherol

These graphs (Figures 5.3-5.5) show the protective effects of different lipid emulsions on tocopherol degradation when exposed to warm white, cool white and UVA light. These graphs also show the mean degradation of all the controls (protected from light) examined in each of the experiments.

In this experiment, tocopherol was exposed to cool white, warm white and UVA light and there was no substantial degradation observed over 24 hours. Nevertheless, an interesting pattern emerged with tocopherol. The greatest loss was seen in syringes containing ultra-filtered deionised water and Vitlipid® N adult following exposure, then IL10, IL20 and lastly SMOF. There is a trend of an increase in lipid concentration and a decrease in tocopherol loss, with stability greatest in syringes containing SMOF. Similar degradation profiles for tocopherol can be seen in all three types of light.

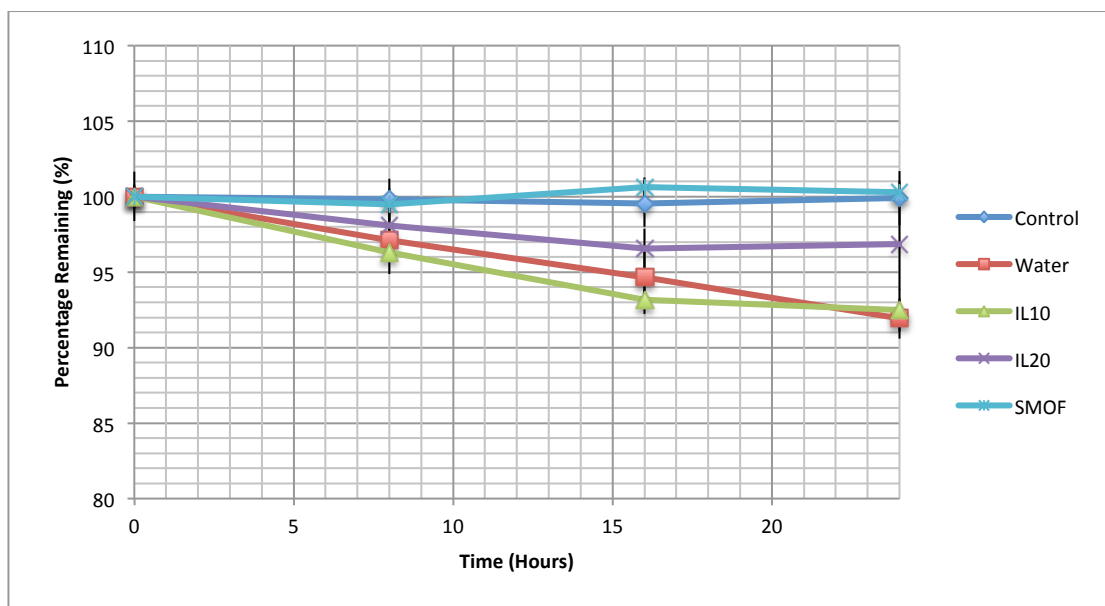


Figure 5.3. Tocopherol degradation in 50 ml Becton Dickinson® syringes when exposed to cool white light. Syringes contained Vitlipid® N Adult and either (a) ultra-filtered deionised water, (b) IL10, (c) IL20 or (d) SMOF

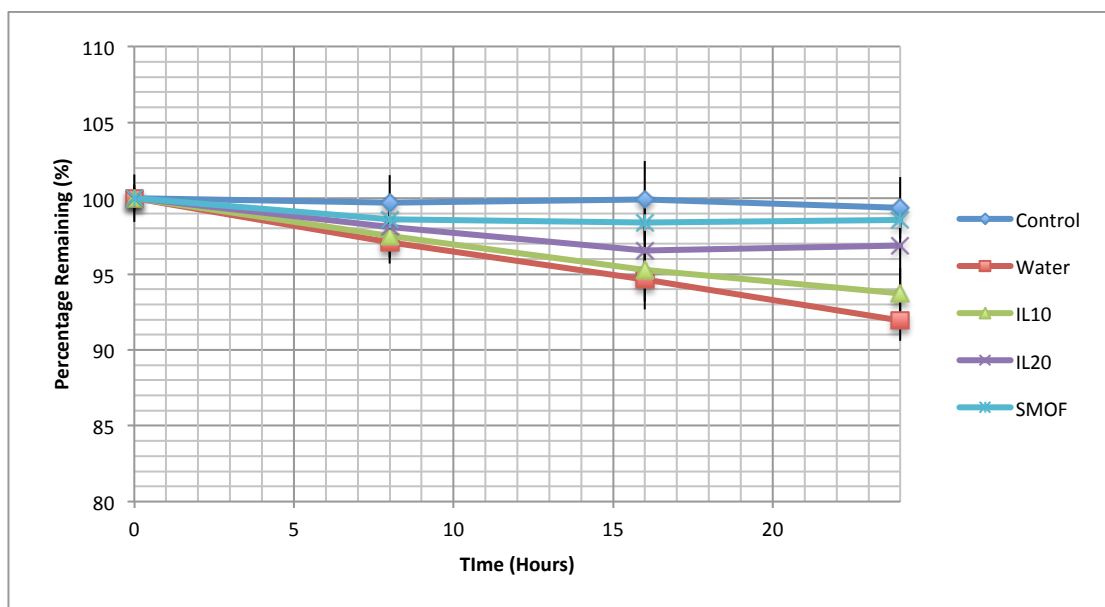


Figure 5.4. Tocopherol degradation in 50 ml Becton Dickinson® syringes when exposed to warm white light. Syringes contained Vitlipid® N Adult and either (a) ultra-filtered deionised water, (b) IL10, (c) IL20 or (d) SMOF

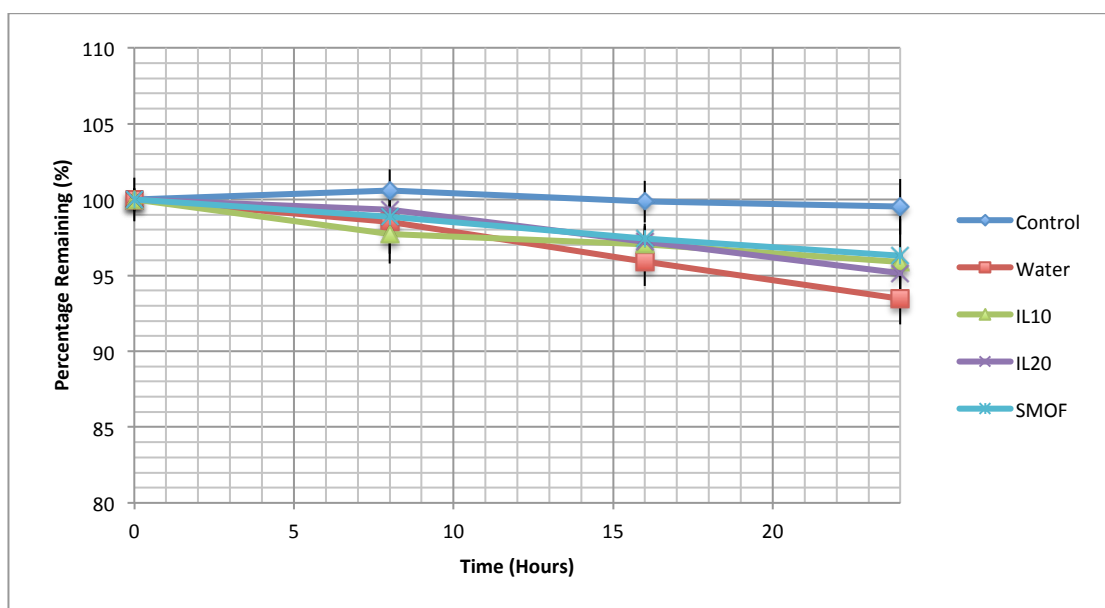


Figure 5.5. Tocopherol degradation in 50 ml Becton Dickinson® syringes when exposed to UVA light. Syringes contained Vitlipid® N Adult and either (a) ultra-filtered deionised water, (b) IL10, (c) IL20 or (d) SMOF.

5.3.6.2. Retinol

These graphs (Figures 5.6-5.8) illustrate the protective effects of lipid emulsions on retinol degradation when exposed to different types of artificial light. These graphs also show the mean degradation of all the controls (protected from light) examined in each of the experiments.

Following 24 hours of exposure in cool white light there was approximately a 48% loss of retinol in the test syringes containing water. Retinol losses in syringes containing IL10, IL20 and SMOF exposed to cool white light were

similar to each other. SMOF provided the most protection, averaging approximately a 19% loss after 24 hours of exposure. IL20 averaged approximately a 20% loss after the 24 hour-period, whereas IL10 averaged approximately a 22% loss over the same time period when exposed to cool white light.

A similar degradation profile was recorded when the syringes were exposed to warm white light. Approximately a 43% loss in retinol concentration was recorded in syringes containing Vitlipid® N Adult and ultra-filtered deionised water. In comparison syringes containing IL10 and IL20 had losses of 22% and 18% respectively. SMOF provided very similar protection to IL20 with approximately an 18% loss over the 24 hour period.

This pattern of degradation was repeated with retinol in the presence of UVA light, however, the extent of degradation was notably greater. In this type of light syringes containing ultra-filtered deionised water had approximately a 74% loss over the 24-hour period. The degradation in IL10, IL20 and SMOF was also more substantial with approximately 47%, 40% and 36% losses respectively over the 24 hours when exposed to UVA light.

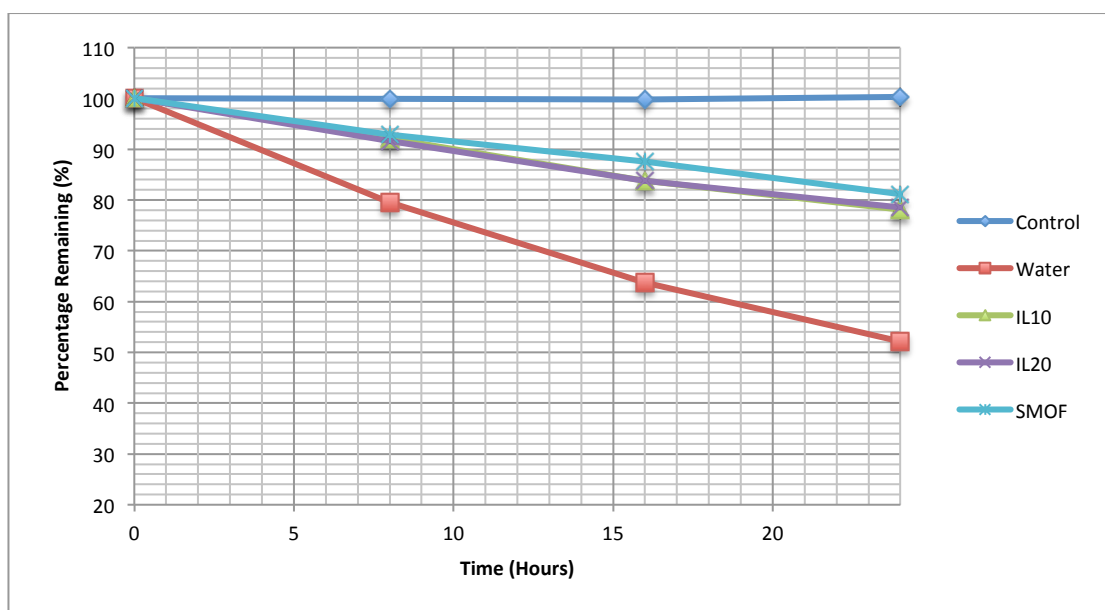


Figure 5.6. Retinol degradation in 50 ml Becton Dickinson® syringes exposed to cool white light. Syringes contained Vitlipid® N Adult and either (a) ultra-filtered deionised water (b) IL10 (c) IL20 and (d) SMOF

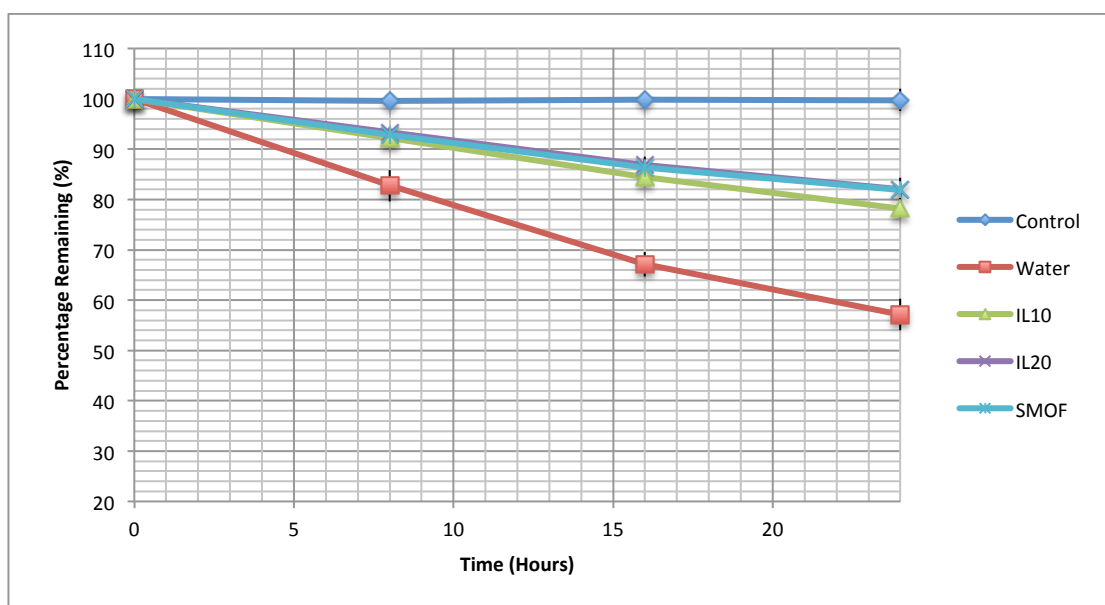


Figure 5.7. Retinol degradation in 50 ml Becton Dickinson® syringes exposed to warm white light. Syringes contained Vitlipid® N Adult and either (a) ultra-filtered deionised water (b) IL10 (c) IL20 and (d) SMOF

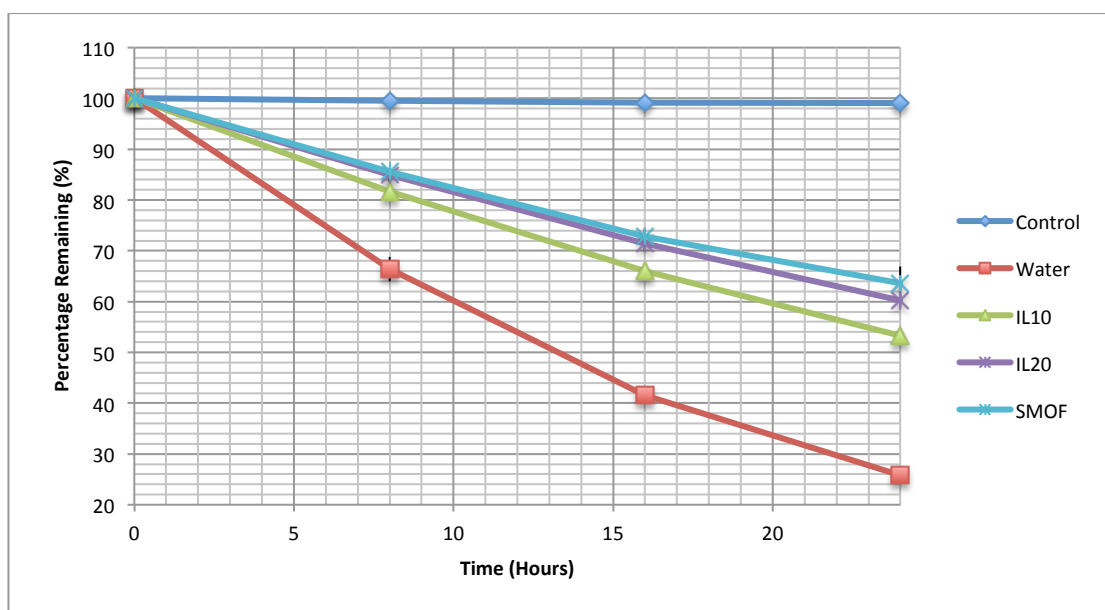


Figure 5.8. Retinol degradation in 50 ml Becton Dickinson® syringes exposed to UVA light. Syringes contained Vitlipid® N Adult and either (a) ultra-filtered deionised water (b) IL10 (c) IL20 and (d) SMOF

5.3.7. Statistical analysis

Statistical analysis was conducted on retinol results. Tocopherol results were not tested as the losses seen with this vitamin were not substantial therefore unlikely to have any clinical impact. Figures 5.9, 5.10 and 5.11 summarise the results found in this statistical analysis.

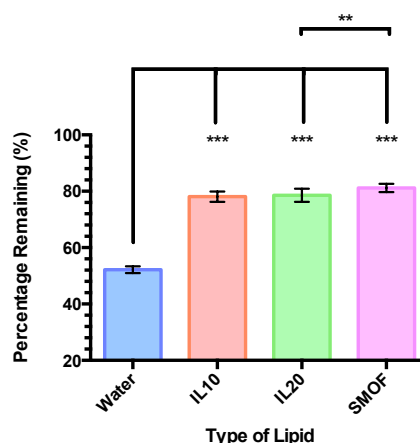


Figure 5.9. Retinol degradation following 24 hours of exposure to cool white light. Data are mean \pm SD from n = 12 (Four test syringes analysed in triplicate). Statistical analysis was calculated using independent t-tests, *P < 0.05, **P < 0.01 and ***P < 0.001. Note, brackets indicate samples that had significant differences.

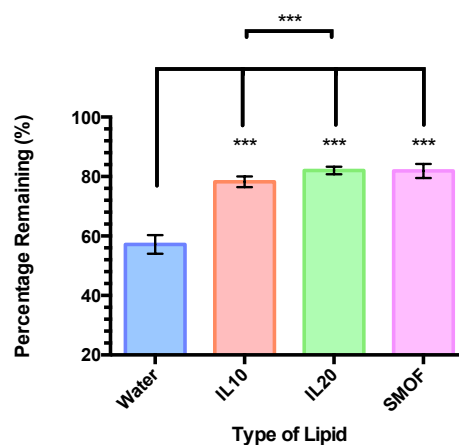


Figure 5.10. Retinol degradation following 24 hours of exposure to warm white light. Data are mean \pm SD from n = 12 (Four test syringes analysed in triplicate). Statistical analysis was calculated using independent t-tests, *P < 0.05, **P < 0.01 and ***P < 0.001. Note, brackets indicate samples that had significant differences.

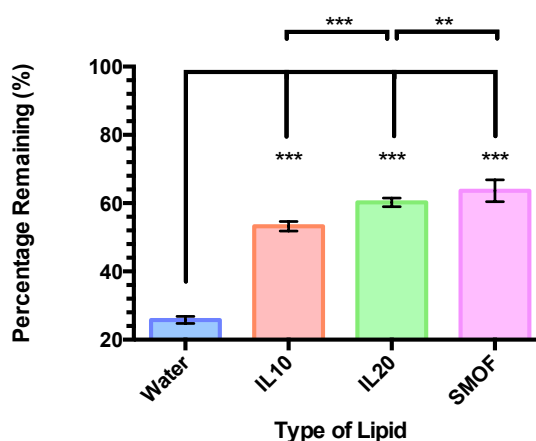


Figure 5.11. Retinol degradation following 24 hours of exposure to UVA light. Data are mean \pm SD from $n = 12$ (Four test syringes analysed in triplicate). Statistical analysis was calculated using independent t-tests, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. Note, brackets indicate samples that had significant differences.

From these data, it is evident that lipid emulsion presence has a protective effect on retinol degradation. When comparing syringes containing ultra-filtered deionised water with syringes containing lipid emulsion there is a significant difference in all three types of light tested. The concentration of the lipid also seems to have an effect on retinol degradation. Increasing the concentration of the lipid in the emulsion, from IL10 to IL20, decreases the amount of retinol loss following exposure in all three types of light. However, only when exposed to warm white and UVA light was there a statistically significant difference between these groups. The types of lipid used in the emulsion may also have an impact with a statistically significant increase in vitamin degradation when syringes containing IL20 were exposed to cool white and UVA light in comparison to those containing SMOF.

5.4. Discussion

All the samples, irrespective of exposure to artificial light sources, showed good physical stability over the 24-hour period. There were only some isolated incidences of increases in globule size when examined under the microscope, but these results were not reproduced in repeats. The visual analysis, pH and laser diffraction gave no indication of emulsion instability.

HPLC analysis of tocopherol reveals similar degradation profiles when syringes are exposed to either cool white, warm white or UVA light with no substantial degradation. As excess oxygen was removed from the syringe prior to testing, degradation did not occur to a large extent as tocopherol is oxidised in a reaction catalysed by light (Allwood & Martin 2000). Even though small losses were observed with tocopherol, an interesting trend emerged, which was subsequently repeated with retinol. This pattern showed syringes containing ultra-filtered deionised water had the greatest vitamin losses and stability was improved in the presence of lipid, with tocopherol seeming the most stable in SMOF closely followed by IL20 and IL10. However, this may be misleading as SMOF contains higher levels of tocopherol (the exact concentration is not known) to help maintain the integrity of the emulsion and so any losses may have been seen to a greater extent in the other preparations with smaller concentrations of the vitamin (i.e. IL10 and IL20).

The HPLC analysis of retinol degradation, when syringes were exposed to cool white light, shows protective effect of IL10, IL20 and SMOF in comparison to syringes containing ultra-filtered deionised water. There is approximately a 29% difference in retinol loss between syringes containing Vitlipid® N Adult and SMOF and those containing Vitlipid® N Adult and ultra-filtered deionised water. It is evident that lipid emulsions have a protective effect on this vitamin and this is supported by statistical analysis, which showed a significant difference between the syringes containing water and the syringes containing lipid emulsions. A higher degree of protection was seen when increasing the concentration of lipid in the emulsion from IL10 to IL20, however, this was not statistically significant. Protection was increased further still when SMOF was used in the syringe.

The patterns in retinol degradation in cool white light can also be seen in warm white light, which is expected as they have a similar spectra. However, statistically significant differences could be seen between IL10 and IL20 but not between IL20 and SMOF.

As with exposure to previous types of light a similar pattern of lipid protection was seen in UVA light. Although, there were higher rates of degradation in comparison to cool and warm white light as retinol is more sensitive to light in UV spectrum and statistically significant differences were also seen between all samples examined (Allwood & Plane 1986).

The data gathered in Chapters Three and Four show a maximum degradation of 10% when exposed to cool white and warm white light, whereas these results show a more pronounced degradation. This could be due to a yellow tinge from the addition of Solivito® N to the syringes in the previous chapters. This yellow coloration is likely to repel light in the blue and red sections of the spectrum, possibly explaining a decrease in degradation rates in these syringes.

How lipid emulsions provide protection to retinol is not known and there could be several possible explanations. It may be due to the more opaque nature of the syringes containing Intralipid® and SMOF that prevent lights from interacting with retinol. Or perhaps the lipid emulsions interact with the vitamin thereby providing a protective layer absorbing some of the damaging rays of light. As described earlier in this chapter, SMOF contains MCTs, refined olive oil, fish oils and vitamin E that are not found in IL10 or IL20. A possible reason for the increased protection seen with SMOF is that it contains larger quantities of vitamin E, which may prevent the production of peroxides and thereby preventing the degradation of retinol. Further investigations into this will be conducted in Chapter Seven.

5.5. Conclusion

From these data it is apparent that the presence of lipid emulsion has an important role in vitamin protection from light. Losses are significantly decreased following the addition of IL10, IL20 and SMOF when exposed to artificial light sources. It is likely that lipid ingredients and concentration influence the rate of degradation. Nevertheless, the retinol loss in syringes containing lipid is still approximately 20% over the 24-hour period, emphasising the importance of light protection with all PN containing vitamins.

CHAPTER SIX

Investigating the Photo-Protective Effects of
Lipid Emulsions on Light Sensitive Vitamins in
Administration Sets During Simulated Delivery

6.1. Introduction

Thus far investigations have primarily been concerned with stability and incompatibility issues in containers during storage. Similar problems can also occur in administration sets with sorption of vitamins to administration set materials being the main reported route of incompatibility or instability. Retinol is the most well known for this, however, sorption has been greatly reduced with use of a palmitate rather than an acetate ester form (Allwood & Kearney 1998; Koletzko et al. 2005d). Other vitamins that have reported losses following administration include folic acid and ergocalciferol (Gillis et al. 1983; Lee et al. 1980).

Another possible route of degradation that has not been extensively investigated is the effect of light on vitamin degradation in administration sets during PN delivery. Administration sets rarely have light protective covers and are a potential source of vitamin loss. It is evident from data generated in previous chapters that artificial light sources can cause vitamin degradation and lipid can influence the extent of light degradation of some vitamins in PN during storage. However, there have been no reports in the literature on the protective effects of lipid emulsions in administration sets during simulated infusion. The purpose of this experiment is to determine the extent of retinol and tocopherol degradation in administration sets when exposed to either cool white, warm white or UVA light during simulated delivery.

6.2. Materials and Methods

6.2.1. Stability indicating HPLC assay used for quantification of retinol and tocopherol degradation

Stability of retinol and tocopherol was conducted using the validated stability-indicating HPLC assay described in Chapter Five. This assay was used as the sampling was conducted every two hours, therefore, a short assay was required to allow timely analysis of the samples.

6.2.2. Sample preparation

Three 50 ml Becton Dickinson syringes, a control and two tests, were filled with 10 ml of Vitlipid® N Adult and made up to volume with either ultra-filtered deionised water, IL10, IL20 or SMOF. The syringes were inverted 20 times to ensure that they were adequately mixed. Due to restrictions in the experimental set-up only one syringe could be examined at a time. While awaiting testing the other syringes were protected from light and stored between 2 and 8 °C.

6.2.3. Experimental design

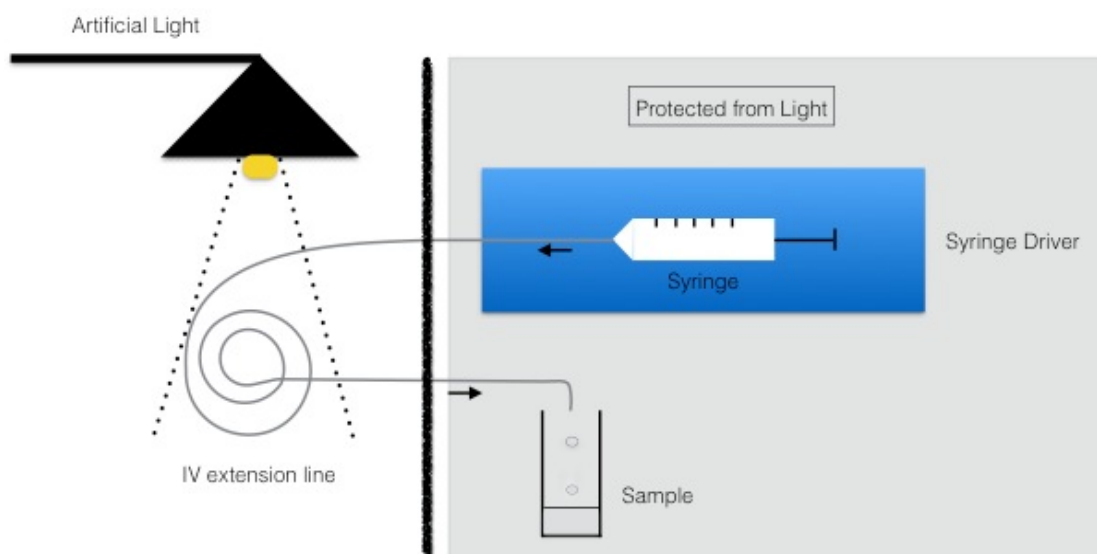


Figure 6.1. Experimental set-up of simulated delivery of Vitlipid® N Adult and either ultra-filtered deionised water, IL10, IL20 or SMOF. Samples were exposed to either, cool white, warm white or UVA light. IV extension line controls were protected from light.

The experimental set-up can be seen in Figure 6.1. When testing, each syringe was attached to a Protect-A-Line I P.E. lined I.V. Extension line, length 200 cm, 2.0 ml volume (Vygon Ltd, Swindon). The syringes were then placed in an ALARIS® ASENSA CC syringe pump (ALARIS Medical systems, Basingstoke) and primed at 200 ml/hr so that the entire length of the extension line contained sample. The syringe pump then pushed the contents of the syringes through the IV extension lines at a rate of 2.5 ml/hr for a period of 2 hours collecting 5 ml of sample. All test and control syringes were protected from light. Test samples were exposed to either cool white, warm white or UVA light and these were compared with control samples that were protected from light. In warm and

cool white light the illuminance was set at approximately 5,000 lux. In UVA, the light intensity was set at 1.2 Wm^{-2} . Physical and chemical tests were performed before and after the simulated administration. Substantial degradation is defined as a loss of greater than 10% over the administration period of 2 hours.

6.3. Results

6.3.1. Visual examination

All samples maintained a white coloration following simulated delivery. There was no evidence of creaming and there was no free oil present suggesting that the integrity of the emulsion was maintained. There were no other visible signs of instability.

6.3.2. Microscopy

Simulated delivery in cool white light had little influence on globule size and no influence on globule morphology. The majority of test samples displayed no increases in maximum globule size or globules sized between 7.5 and 10 μm . However, in one test sample containing Vitlipid® N Adult and IL10 the maximum globule size slightly increased from 7.5 to 10 μm . All globules remained spherical and singular irrespective of exposure to artificial light sources.

In warm white light there were similar results. All globules measured maintained their singular and spherical morphology through out the testing period. For the most part, the globule size did not change following simulated delivery. The only exception was one test syringe containing Vitlipid® N Adult and IL10 where the maximum globule size increased from 7.5 to 30 μm . The amount of globules sized over 10 μm in this sample also increased from 0 to 3 globules.

All lipid emulsions remained stable during simulated delivery in UVA light. There were no increases in lipid globule size in any of the samples tested. In each of the lipid emulsions, globules remained singular and retained their spherical morphology.

6.3.3. Laser diffraction

Laser diffraction values for the controls and test syringes can be seen in Table 6.1, 6.2 and 6.3. The values recorded are the 'size under' and D[4,3] as measured in previous chapters.

Table 6.1. Mean and SD of D[4,3] (μm) and 'Size under' (μm) of control and test samples before (0 Hours) and after (2 Hours) exposure to cool white light

		0 Hours				2 Hours			
		D[4,3] (μm)		'Size Under' (μm)		D[4,3] (μm)		'Size Under' (μm)	
		Control	Test	Control	Test	Control	Test	Control	Test
Water	Mean	0.253	0.253	1.445	1.445	0.252	0.251	1.445	1.352
	SD	0.006	0.002	0.000	0.000	0.002	0.001	0.000	0.102
IL10	Mean	0.220	0.215	2.156	1.445	0.215	0.213	1.445	1.352
	SD	0.003	0.001	0.001	0.000	0.003	0.001	0.000	0.102
IL20	Mean	0.280	0.279	1.096	1.178	0.279	0.278	1.096	1.096
	SD	0.001	0.002	0.000	0.089	0.002	0.002	0.000	0.000
SMOF	Mean	0.317	0.310	1.259	1.259	0.315	0.309	1.259	1.259
	SD	0.001	0.003	0.000	0.000	0.660	0.003	0.000	0.000

Table 6.2. Mean and SD of D[4,3] (μm) and 'Size under' (μm) of control and test samples before (0 Hours) and after (2 Hours) exposure to warm white light

		0 Hours				2 Hours			
		D[4,3] (μm)		'Size Under' (μm)		D[4,3] (μm)		'Size Under' (μm)	
		Control	Test	Control	Test	Control	Test	Control	Test
Water	Mean	0.251	0.248	1.259	1.259	0.251	0.250	1.259	1.259
	SD	0.003	0.002	0.000	0.000	0.001	0.001	0.000	0.000
IL10	Mean	0.215	0.214	1.445	1.445	0.214	0.214	1.383	1.321
	SD	0.001	0.001	0.000	0.000	0.001	0.001	0.107	0.096
IL20	Mean	0.279	0.282	1.096	1.096	0.278	0.279	1.096	1.096
	SD	0.002	0.002	0.000	0.000	0.001	0.003	0.000	0.000
SMOF	Mean	0.319	0.326	1.259	1.259	0.319	0.326	1.259	1.259
	SD	0.001	0.004	0.000	0.000	0.002	0.003	0.000	0.000

Table 6.3. Mean and SD of D[4,3] (μm) and 'Size under' (μm) of control and test samples before (0 Hours) and after (2 Hours) exposure to UVA light

		0 Hours				2 Hours			
		D[4,3] (μm)		'Size Under' (μm)		D[4,3] (μm)		'Size Under' (μm)	
		Control	Test	Control	Test	Control	Test	Control	Test
Water	Mean	0.250	0.252	1.259	1.259	0.253	0.251	1.259	1.259
	SD	0.001	0.002	0.000	0.000	0.002	0.003	0.000	0.000
IL10	Mean	0.218	0.215	1.878	1.414	0.213	0.214	1.205	1.259
	SD	0.002	0.003	0.536	0.379	0.002	0.002	0.0941	0.000
IL20	Mean	0.280	0.281	1.096	1.096	0.283	0.280	1.096	1.096
	SD	0.002	0.003	0.000	0.000	0.003	0.003	0.000	0.000
SMOF	Mean	0.265	0.284	0.955	1.096	0.263	0.283	0.955	1.096
	SD	0.001	0.006	0.000	0.000	0.002	0.004	0.000	0.000

These laser diffraction values show no noticeable changes between initial and final readings in both test and control experiments. Mean volumes and maximum particle sizes are small regardless of whether they were exposed to any of the artificial light sources. The standard deviations are also very small demonstrating the precision of the results.

6.3.4. Osmolality

The tables below (Table 6.4, 6.5 and 6.6) describe the average osmolality values for control and test samples in different types of lipid following exposure to artificial light sources.

Table 6.4. Average osmolality values for samples exposed to cool white light

	Water		IL10		IL20		SMOF	
	Control	Test	Control	Test	Control	Test	Control	Test
Mean Osmolality (mOsm/kg)	52.0	54.0	300.0	300.0	325.0	321.0	352.0	351.5

Table 6.5. Average osmolality values for samples exposed to warm white light

	Water		IL10		IL20		SMOF	
	Control	Test	Control	Test	Control	Test	Control	Test
Mean Osmolality (mOsm/kg)	55.0	54.5	300.0	300.0	328.0	330.0	354.0	352.0

Table 6.6. Average osmolality values for samples exposed to UVA light

	Water		IL10		IL20		SMOF	
	Control	Test	Control	Test	Control	Test	Control	Test
Mean Osmolality (mOsm/kg)	54.0	53.0	300.0	299.0	339.0	331.0	356.0	350.0

The highest mean osmolality value was 356.0 mOsm/kg in these samples. This is below 1000 mOsm/kg, therefore, unlikely to cause thrombophlebitis (Madan et al. 1992).

6.3.5. pH

The pH was recorded at the start and end of simulated delivery (2 hours later).

pH changes in excess of 0.5 units are deemed indicative of instability.

The results for changes in pH following simulated delivery can be seen in Table 6.7, 6.8 and 6.9. pH differences in red indicate decreases in pH and those in green represent increases in pH over the time period. None of the differences in pH exceeded 0.5 units indicating a stable admixture.

Table 6.7. pH of samples before (0 hours) and after (2 hours) exposure to cool white light

	Water			IL10			IL20			SMOF		
	Control	Test		Control	Test		Control	Test		Control	Test	
	1	1	2	1	1	2	1	1	2	1	1	2
0 Hours	6.63	6.78	6.70	6.87	6.83	6.87	7.26	7.20	7.49	6.64	6.78	6.76
2 Hours	6.42	6.43	6.35	6.59	6.51	6.51	7.29	7.29	7.40	6.62	6.63	6.57
pH Difference	0.21	0.35	0.35	0.28	0.32	0.36	0.03	0.09	0.09	0.02	0.14	0.19

Table 6.8. pH of samples before (0 hours) and after (2 hours) exposure to warm white light

	Water			IL10			IL20			SMOF		
	Control	Test		Control	Test		Control	Test		Control	Test	
	1	1	2	1	1	2	1	1	2	1	1	2
0 Hours	6.82	6.81	6.97	6.87	6.83	6.87	7.19	7.23	7.29	6.87	6.87	6.88
2 Hours	6.48	6.43	6.62	6.59	6.51	6.51	7.26	7.28	7.19	6.78	6.73	6.73
pH Difference	0.34	0.38	0.35	0.28	0.32	0.36	0.07	0.05	0.10	0.09	0.14	0.15

Table 6.9. pH of samples before (0 hours) and after (2 hours) exposure to UVA light

	Water			IL10			IL20			SMOF		
	Control	Test		Control	Test		Control	Test		Control	Test	
	1	1	2	1	1	2	1	1	2	1	1	2
0 Hours	6.81	6.87	6.65	6.93	7.10	7.01	7.38	7.57	7.36	6.68	6.85	6.67
2 Hours	6.58	6.43	6.84	6.77	6.79	6.79	7.36	7.10	7.22	6.60	6.79	6.65
pH Difference	0.23	0.44	0.19	0.16	0.31	0.22	0.02	0.47	0.14	0.08	0.06	0.02

6.3.6. HPLC

HPLC analysis of tocopherol (Figures 6.2-6.7) reveals no substantial degradation following exposure to artificial light sources during simulated delivery. There is no pattern to any of the losses seen with tocopherol in these experiments with regards to lipid emulsion type.

Analysis of retinol degradation (Figures 6.8-6.13) reveals substantial losses in all three types of light following simulated delivery. Largest losses were observed when samples are exposed to UVA light. There were slightly larger losses seen when samples were exposed to cool white light in comparison with warm white light.

There is a correlation of increases in lipid concentration and increases in retinol stability in all three types of artificial light. Retinol degradation is smallest in SMOF and IL20 with both lipid emulsions preparations having similar protective profiles.

6.3.6.1. Tocopherol

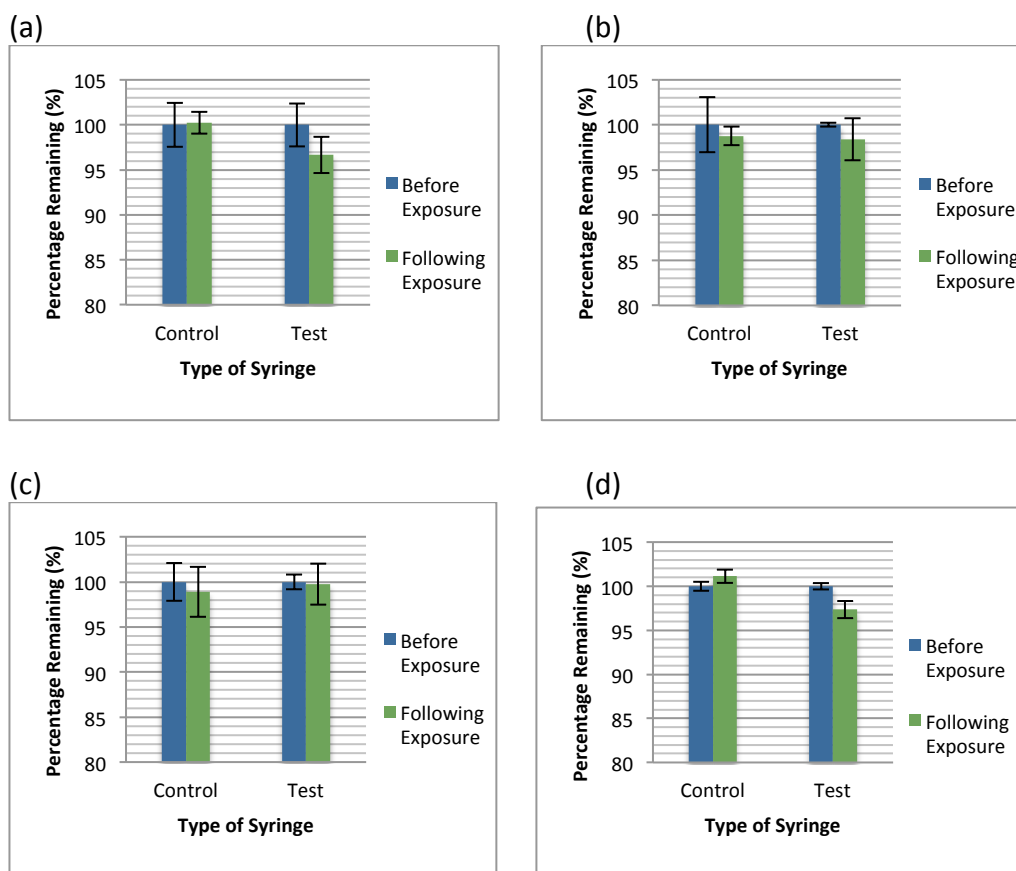


Figure 6.2. Tocopherol degradation following simulated delivery when exposed to cool white light. Syringes contained Vitlipid® N Adult and either (a) ultra-filtered deionised water, (b) IL10, (c) IL20 or (d) SMOF.

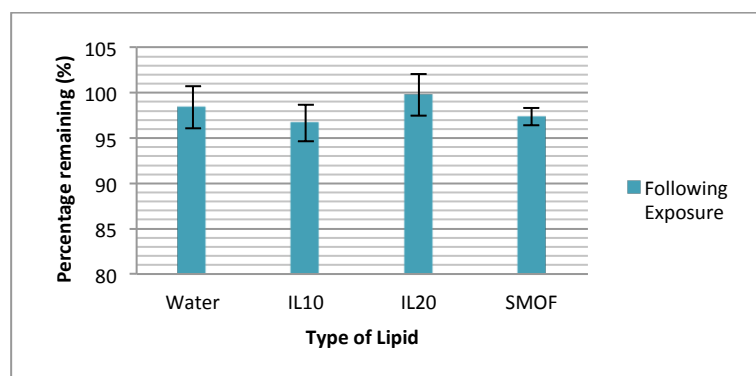


Figure 6.3. Combined tocopherol degradation following simulated delivery in cool white light

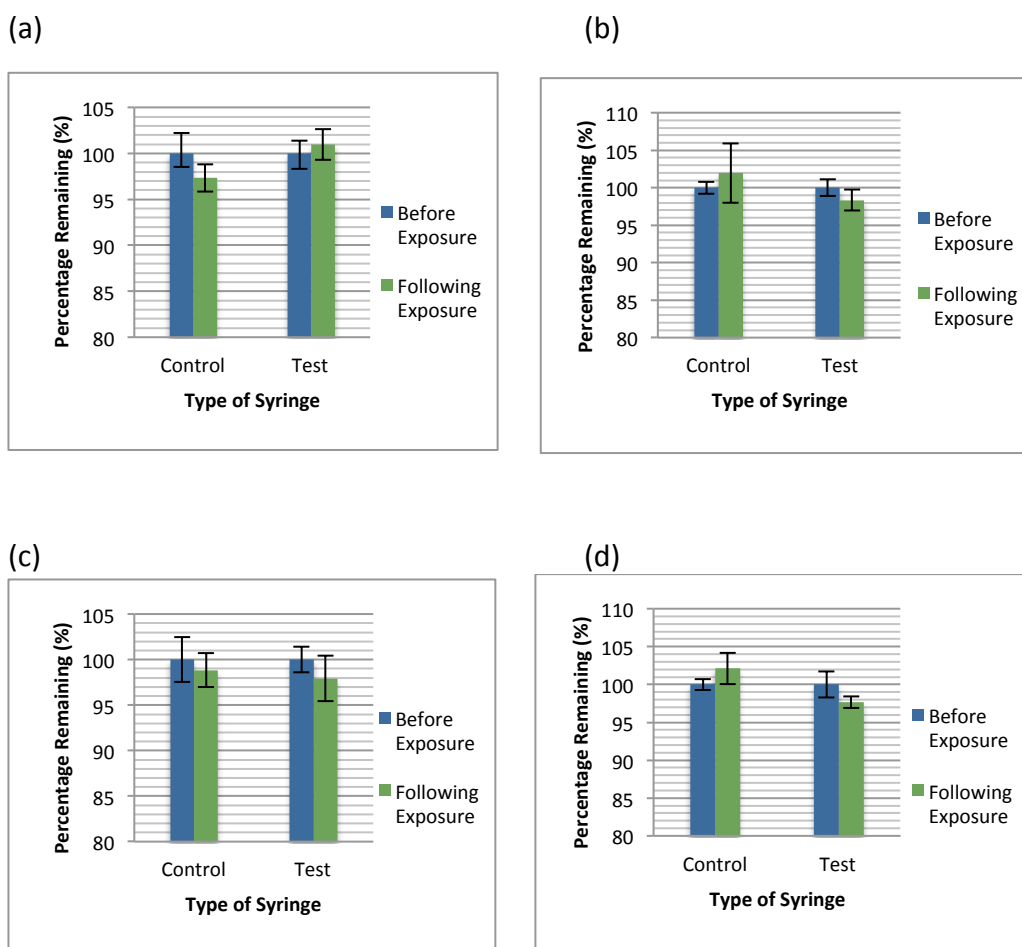


Figure 6.4. Tocopherol degradation following simulated delivery when exposed to warm white light. Syringes contained Vitlipid® N Adult and either (a) ultra-filtered deionised water, (b) IL10, (c) IL20 or (d) SMOF.

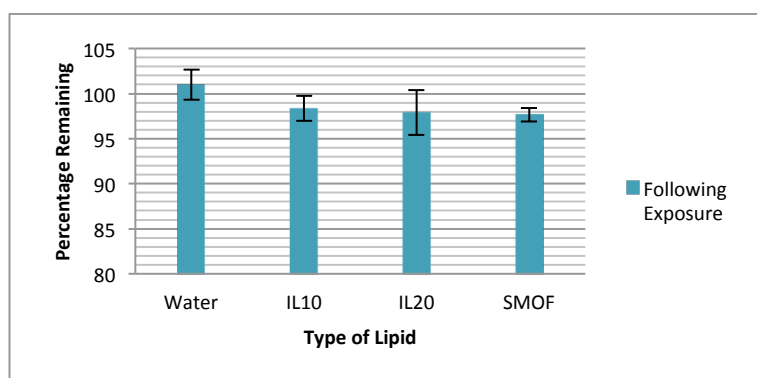


Figure 6.5. Combined tocoopherol degradation following simulated delivery in warm white light.

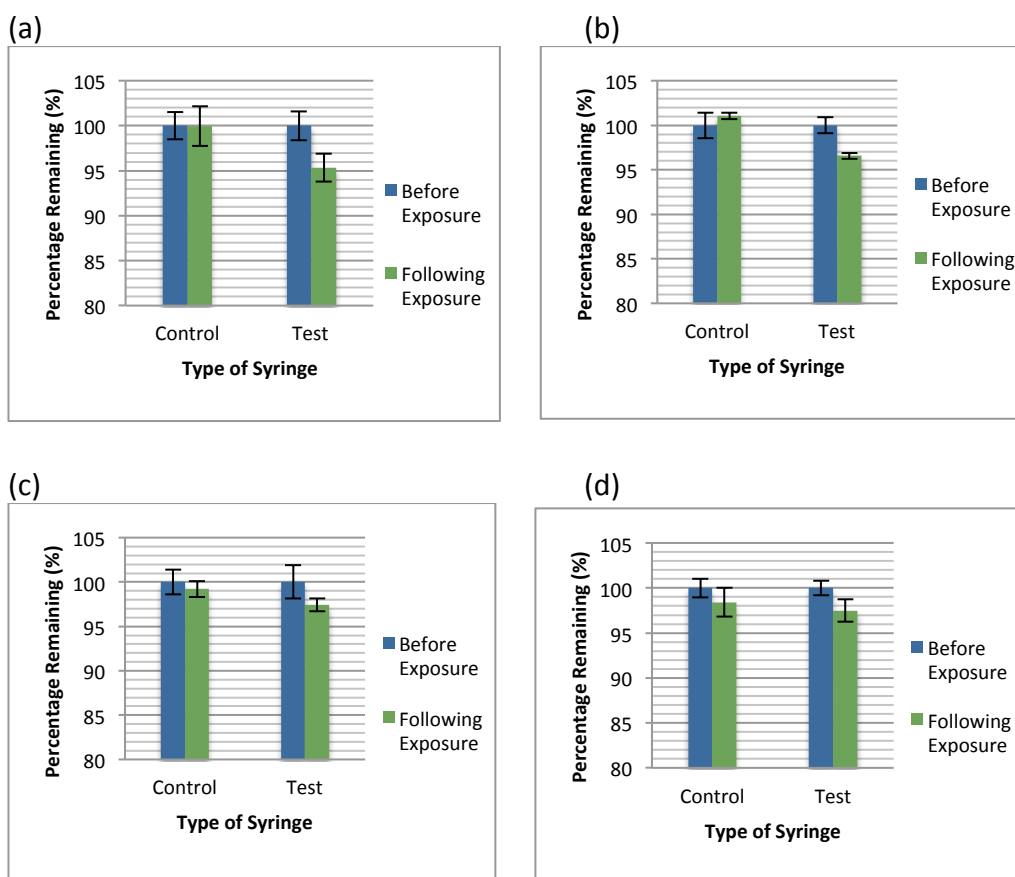


Figure 6.6. Tocopherol degradation following simulated delivery when exposed to UVA light. Syringes contained Vitlipid® N Adult and either (a) ultra-filtered deionised water, (b) IL10, (c) IL20 or (d) SMOF.

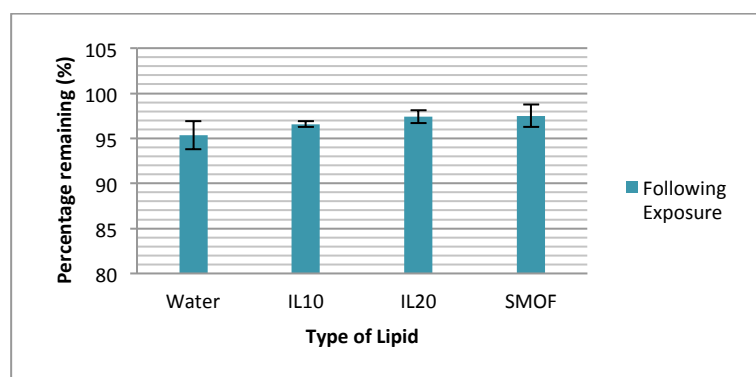


Figure 6.7. Combined tocoopherol degradation following simulated delivery in UVA light

6.3.6.2. Retinol

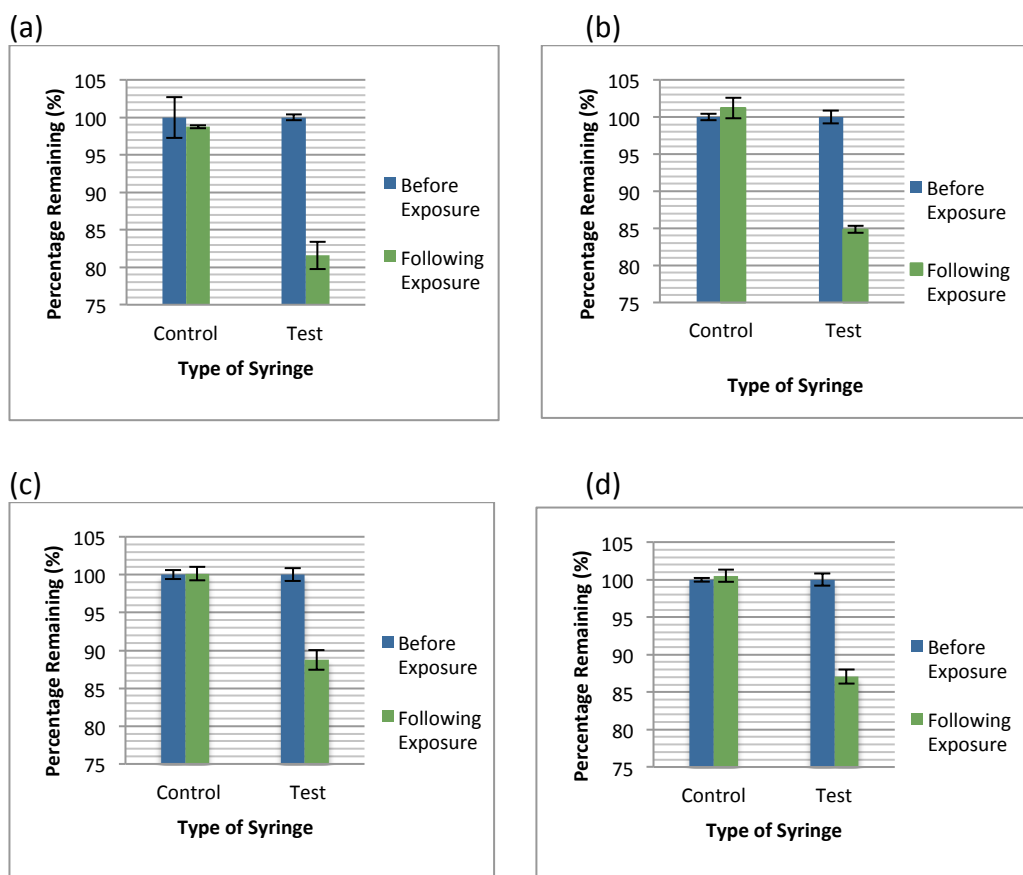


Figure 6.8. Retinol degradation following simulated delivery when exposed to cool white light. Syringes contained Vitlipid® N Adult and either (a) ultra-filtered deionised water, (b) IL10, (c) IL20 or (d) SMOF.

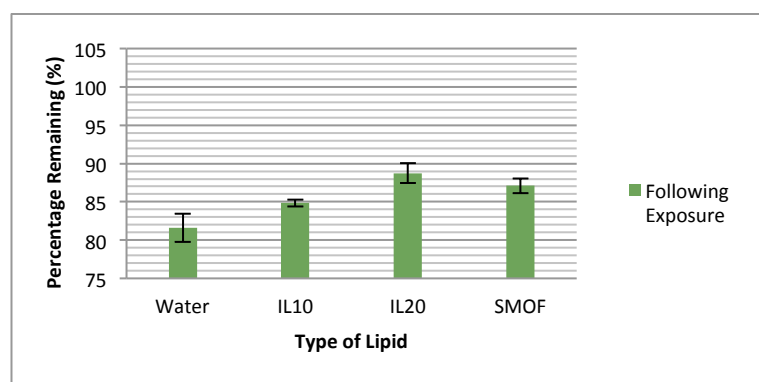


Figure 6.9. Combined retinol degradation following simulated delivery in cool white light.

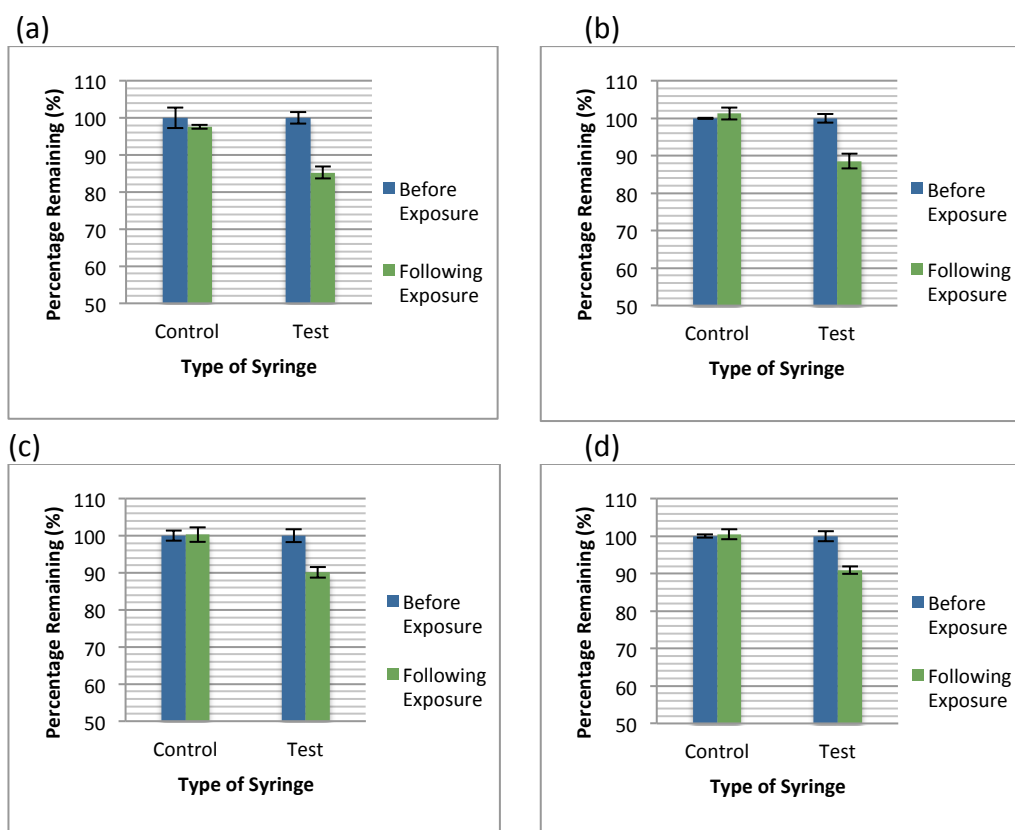


Figure 6.10. Retinol degradation following simulated delivery when exposed to warm white light. Syringes contained Vitlipid® N Adult and either (a) ultra-filtered deionised water, (b) IL10, (c) IL20 or (d) SMOF.

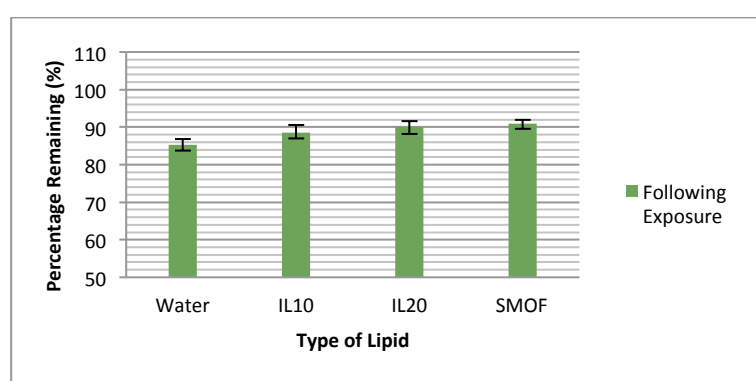


Figure 6.11. Combined retinol degradation following simulated delivery in warm white light.

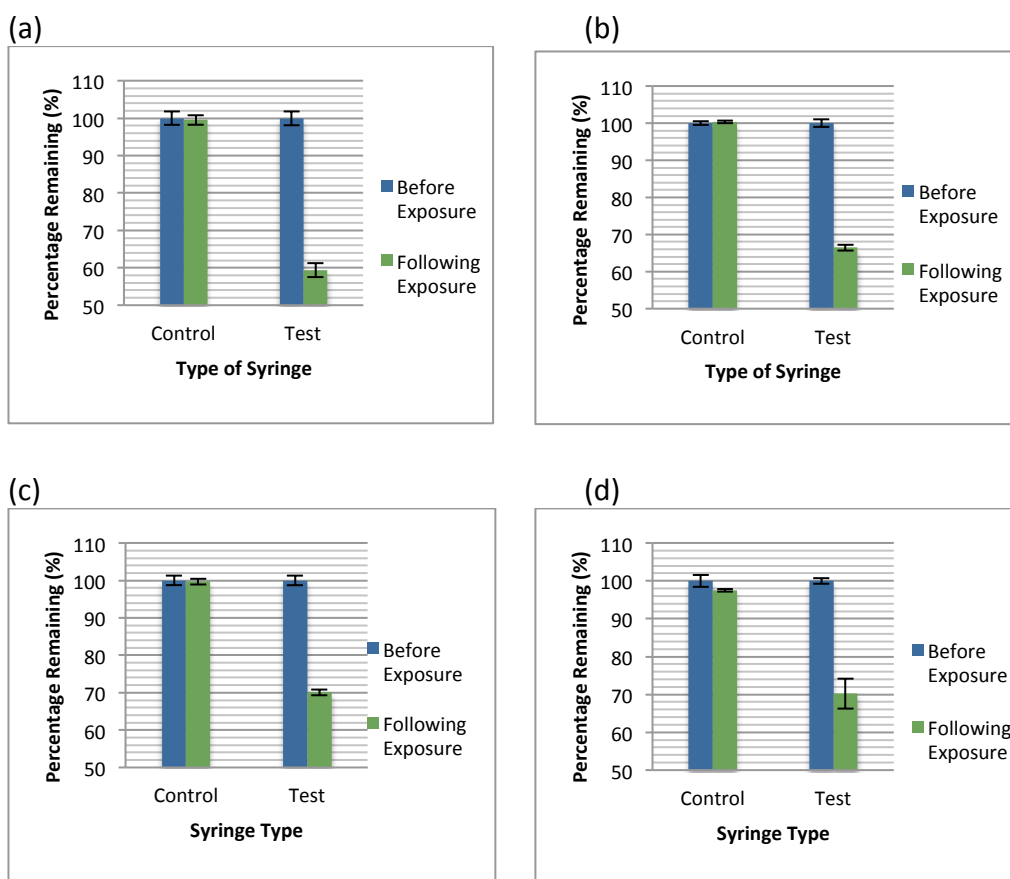


Figure 6.12. Retinol degradation following simulated delivery when exposed to UVA light. Syringes contained Vitlipid® N Adult and either (a) ultra-filtered deionised water, (b) IL10, (c) IL20 or (d) SMOF.

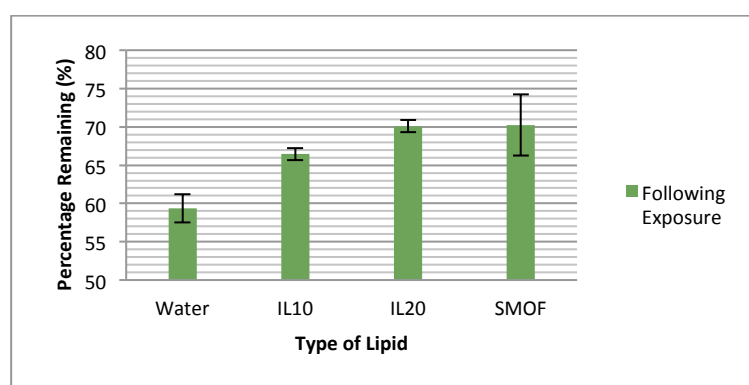


Figure 6.13. Combined retinol degradation following simulated delivery in UVA light

6.4. Discussion

The physical integrity of the admixtures remained stable throughout the experiments regardless of the type of lipid emulsion preparation used. There were no noticeable visible changes with the admixtures. There were also no changes in globule size or morphology, apart from an isolated incident in a sample containing Vitlipid N Adult and IL10 exposed to warm white light. pH values were all less than 0.5 units apart from each other indicating that emulsion was physically stable.

The chemical stability of tocopherol in different storage conditions has been explored in Chapters Three to Five and also in the literature, however, there is no information with regards to its sensitivity to light when in administration sets during simulated delivery. It is important to improve our understanding of this vitamin, as administration sets are routinely exposed to light sources in a clinical setting. In this experiment, HPLC analysis of tocopherol revealed that it was stable following simulated administration regardless of lipid emulsion type. Tocopherol is oxidised in a reaction catalysed by light and as excess oxygen was removed from the administration system prior to simulation, degradation could not occur (Allwood & Martin 2000).

Retinol had substantial amounts of degradation recorded in all three types of artificial light. Retinol is known to be most sensitive to UVA emissions so as expected losses were far greater in UVA light when compared with cool or

warm white light. Losses were also slightly increased in cool white light when compared with warm white light. This is understandable as cool white light has a higher proportion of light in the lower end of the visible spectrum closer to the UVA wavelengths at which retinol is most sensitive.

As the concentration of lipid was increased the stability of retinol improved in all artificial light sources. Samples containing IL20 and SMOF had very similar degradation rates in cool white, warm white and UVA light. Future work would increase the number of samples analysed thereby allowing statistical analysis to determine the significance between the different groups. Even though the stability of retinol is improved in the presence of lipid emulsion there is still substantial degradation in all three types of light. Therefore, light protection is still an important consideration during administration, as in practice the storage container will also be exposed to light by some degree increasing degradation rates.

6.5. Conclusion

These investigations demonstrate the extent of vitamin degradation in IV extension sets following simulated delivery in the presence of artificial light sources. Tocopherol is stable following exposure to cool white, warm white and UVA light during simulated delivery, providing all excess oxygen is removed. However, a substantial amount of retinol is degraded in all three types of light

with the most degradation occurring in UVA light. Lipid has a protective effect on retinol degradation with increases in lipid concentration resulting in decreasing degradation rates. In practice, these administration tubes are rarely covered and these investigations support the use of light protection, via coloured tubing or tube coverings, at all times to maximise stability.

CHAPTER SEVEN

Investigating the Light Protective Effects of Lipid Emulsions

7.1. Introduction

As described in Chapter Five and Six, there are some conflicting opinions regarding the influence of lipid emulsions on the photo-degradation of vitamins. These chapters also provide some data showing a correlation between increases in lipid concentration and decreases in degradation of light sensitive vitamins, especially retinol. The reason for the improved stability of light sensitive vitamins is unknown. It may be due to an increase in opacity or perhaps the lipid provides a protective layer around the light sensitive vitamins. The aim of this chapter is to investigate how lipid emulsions exert their protective effects on light sensitive vitamins such as retinol.

7.2. Materials and Methods

7.2.1. Experimental designs

There are two main experiments in this chapter. The first investigates the obscuration of light caused by the plastic material in syringe containers through comparison with glass multi-dose vials (MDVs). It also examines the extent of obscuration caused by different types of lipid emulsion. A better understanding of how much light is absorbed or reflected gives an insight into whether obscuration is the main route of light protection of vitamins. The second experiment was aimed at investigating whether lipid has a light protective

effect on vitamins independent of light obscuration. To do this retinol palmitate was to be added to soybean oil (SBO), a major component of PN lipid products, to see if SBO alone provided protection to light sensitive vitamins. SBO is a clear liquid, therefore, it was hypothesised that it would not obscure light to the same extent as a visually opaque emulsion and would give an indication on how lipid protects light sensitive vitamins.

7.2.1.1. Investigating light obscuration of containers and different types of lipid emulsion

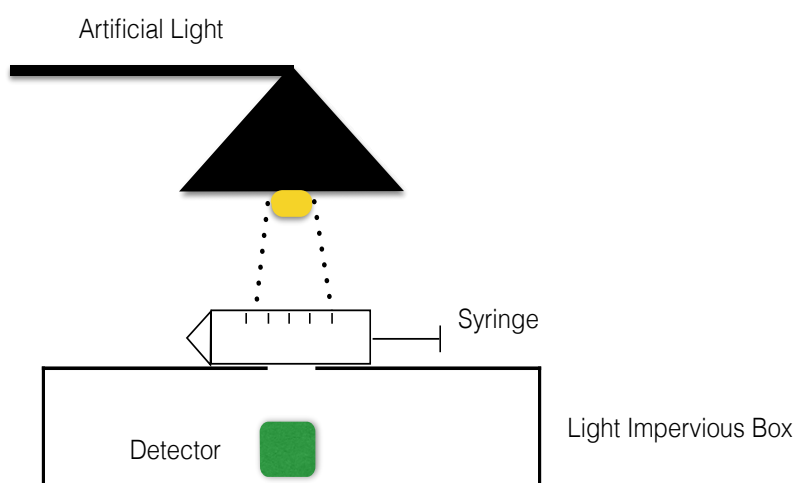


Figure 7.1. Set-up for an experiment investigating the obscuration of cool white, warm white and UVA light.

Three 50 ml Becton Dickinson syringes and three MDVs were filled to 50 ml with either ultra-filtered deionised water, Vitlipid® N Adult and ultra-filtered deionised water (10 ml + 40 ml), IL10, IL20 or SMOF. Care was taken to ensure that excess air was removed from the syringes. An initial reading was taken

using a lux (cool and warm white light) or Wm^{-2} meter (UVA light) before placing the syringes or MDVs on their sides over the detector as shown in Figure 7.1. and exposing them in triplicate to either cool white, warm white or UVA light. The amount of light passing through the containers was then measured using the appropriate light meter and the extent of obscuration was calculated. Results were then analysed using independent sample t-tests via IBM SPSS® version 20.

7.2.1.2. Investigating the light protective effects of SBO on vitamins

Using the HPLC assay used in Chapter Five, the aim of this experiment was to quantify the amount of retinol palmitate in SBO following exposure to cool white, warm white and UVA light and compare these with results generated in Chapter Five.

Initially, it was important to ascertain the amount of obscuration SBO caused following exposure to the different artificial light sources. Using the same set-up as described in Figure 7.1, three 50 ml Becton Dickinson® syringes containing SBO (Sigma-Aldrich Corp, Louis, Missouri, USA) were exposed in triplicate to either cool white, warm white or UVA light. Initial readings were compared with readings in the presence of the syringe and the obscuration was calculated.

Following this, an experiment was designed with the aim of understanding the protective effects of SBO on vitamins when exposed to warm white, cool white and UVA light. In this experiment, three 50 ml Becton Dickinson® syringes containing 1 mg of retinol palmitate (Sigma-Aldrich Corp, Louis, Missouri, USA) were to be made up to volume with SBO. Syringes were to be exposed to either cool white, warm white or UVA light over a period of 24 hours and chemical analysis was to be performed every six hours using the validated HPLC method described in Chapter Five. Test syringes were then to be compared with controls thereby indicating the extent of photo-protection exerted by SBO on light-sensitive vitamins. However, this experiment was unsuccessful as the HPLC assay designed in Chapter Five was not appropriate for chemical analysis of retinol in SBO. This is discussed further in 7.4.

7.3. Results

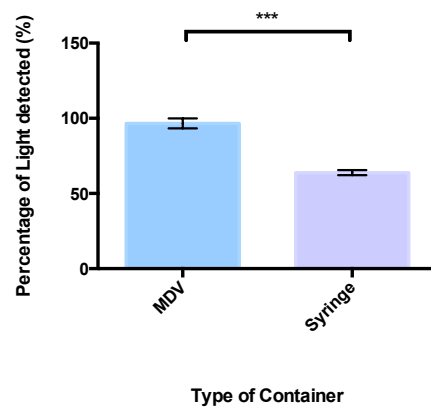
7.3.1. Light obscuration comparison of MDVs and syringes

This experiment investigated the effect of container type on light obscuration. Syringes and glass MDVs were filled with water and the extent of their light obscuration was compared in cool white, warm white and UVA light. Statistical analysis was performed to show any statistically significant difference between container types.

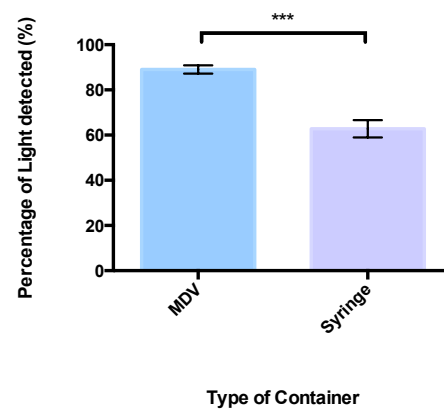
Table 7.1. Summary table of mean \pm SD light obscuration of glass MDVs and BD plastic syringes containing ultra-filtered deionised water

	Percentage of Light passing through (%)	
	MDV	Syringe
Cool White	96.59 \pm 3.30	63.87 \pm 1.63
Warm White	89.05 \pm 1.85	62.80 \pm 3.83
UVA	60.19 \pm 3.67	46.30 \pm 4.39

(a)



(b)



(c)

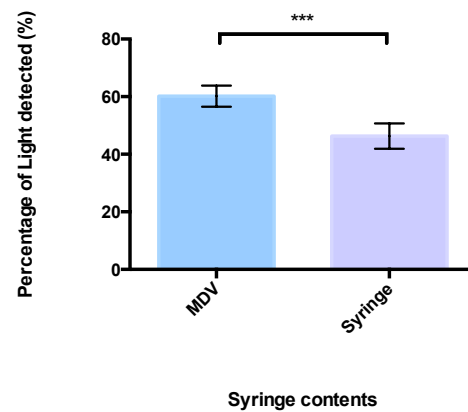


Figure 7.2. Comparison of container material on obscuration of (a) cool white, (b) warm white and (c) UVA light. Note, * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$. Note, brackets indicate containers that had significant differences

There is a large, statistically significant difference between glass MDVs and syringes containing water in all three types of light ($p < 0.001$). In cool white, warm white and UVA light the difference between MDVs and syringes is approximately 33%, 26% and 14% respectively.

7.3.2. Light obscuration comparison between different lipid types in syringes

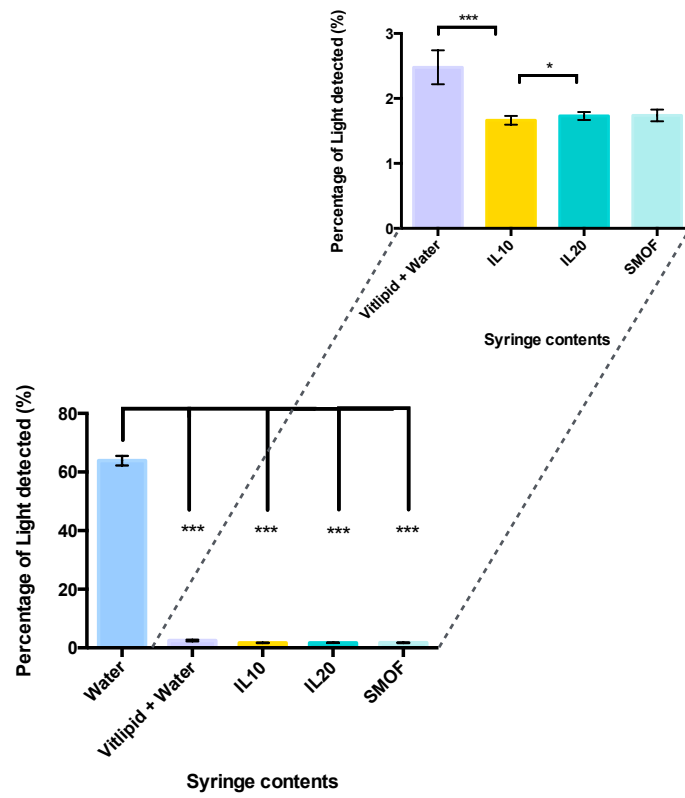
This experiment examined the impact of different lipid preparations on light obscuration in syringes. Ultra-filtered deionised water, Vitlipid® Adult N and ultra-filtered deionised water, IL10, IL20 and SMOF were all compared in cool white, warm white and UVA light. Statistical analysis was used to determine if there were any statistically significant differences between groups.

Table 7.2. Summary of mean \pm SD light obscuration of syringes containing ultra-filtered deionised water, ultra-filtered deionised water and Vitlipid® N adult, IL10, IL20 and SMOF.

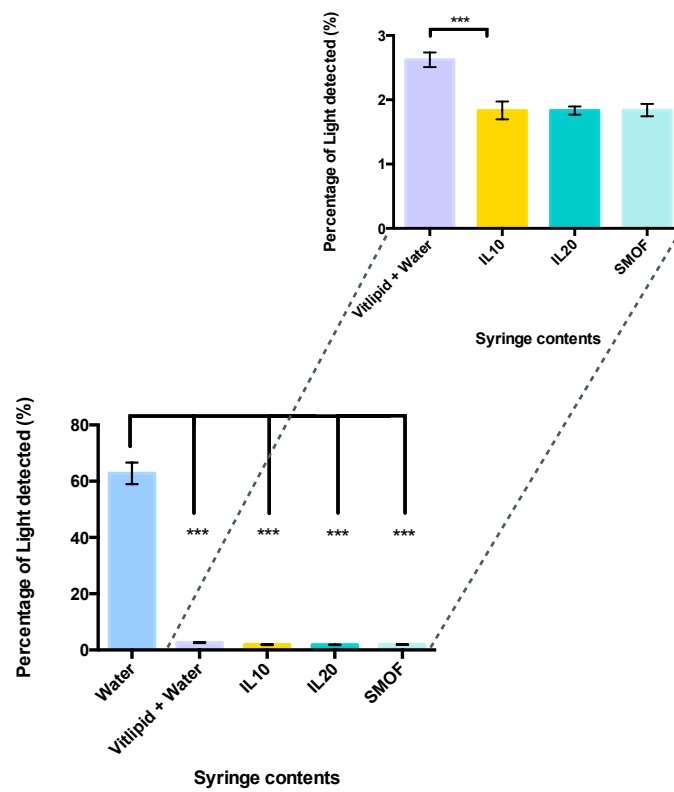
Type of Light	Percentage of Light passing through (%)				
	Water	Water + Vitolipid® N Adult	IL10	IL20	SMOF
Cool White	63.87 \pm 1.63	2.48 \pm 0.26	1.66 \pm 0.07	1.73 \pm 0.06	1.74 \pm 0.09
Warm White	62.80 \pm 3.83	2.62 \pm 0.11	1.83 \pm 0.14	1.83 \pm 0.06	1.84 \pm 0.10
UVA	46.30 \pm 4.39	8.33 \pm 0.00	8.33 \pm 0.00	8.33 \pm 0.00	8.33 \pm 0.00

- CHAPTER SEVEN -

(a)



(b)



(c)

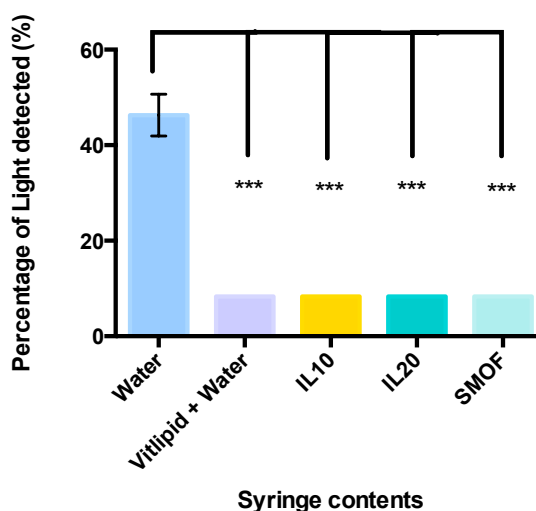


Figure 7.3. Comparison of contents on light obscuration in (a) cool white, (b) warm white and (c) UVA light. Note, * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$. Note, brackets indicate samples that had significant differences.

The inclusion of all types of lipid emulsion into the contents of syringes in cool and warm white light increased the obscuration in comparison with water by a statistically significant amount ($p < 0.001$). Increases in lipid concentration from vitlipid and water to IL10 in cool white and warm white light is also statistically significant ($p > 0.001$). However, further increases in concentration from IL10 to IL20 and changes in lipid emulsion preparation to SMOF did not cause large increases in obscuration in cool and warm white light.

In UVA light, obscuration is significantly increased in the presence of lipid emulsions. When there is a change in lipid concentration or lipid type no significant changes are seen. Although, the Wm^{-2} meter is not as precise as lux meter used, therefore, differences are more difficult to measure.

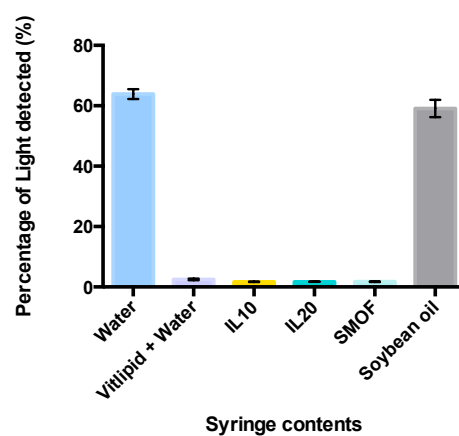
7.3.3. Light obscuration of SBO in cool white, warm white and UVA light

The data described below show the light obscuration of SBO in syringes. Comparison with syringes containing ultra-filtered deionised water, ultra-filtered deionised water and Vitlipid® N Adult, IL10, IL20 and SMOF described earlier reveals some interesting patterns.

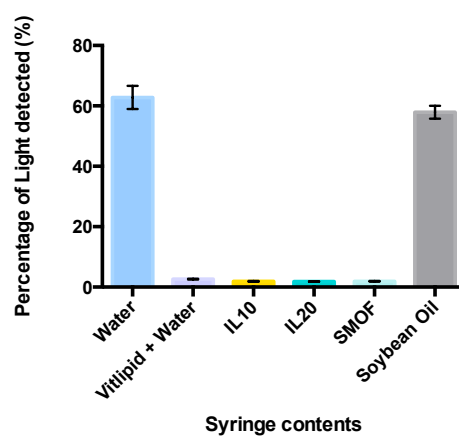
Table 7.3. Summary of mean \pm SD light obscuration in syringes containing ultra-filtered deionised water, ultra-filtered deionised water and Vitlipid® N Adult, IL10, IL20, SMOF and SBO

Type of Light	Percentage of Light passing through (%)					
	Water	Water + Vitlipid® N Adult	IL10	IL20	SMOF	Soybean
Cool White	63.87 \pm 1.63	2.48 \pm 0.26	1.66 \pm 0.07	1.73 \pm 0.06	1.74 \pm 0.09	59.02 \pm 2.85
Warm White	62.80 \pm 3.83	2.62 \pm 0.11	1.83 \pm 0.14	1.83 \pm 0.06	1.84 \pm 0.10	57.88 \pm 2.13
UVA	46.30 \pm 4.39	8.33 \pm 0.00	8.33 \pm 0.00	8.33 \pm 0.00	8.33 \pm 0.00	0.00 \pm 0.00

(a)



(b)



(c)

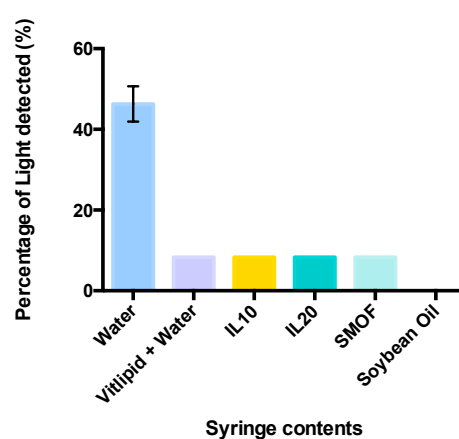


Figure 7.4. Comparing light obscuration of syringes containing SBO with those containing different types of lipid in (a) cool white and (b) warm white light and (c) UVA light.

In both cool and warm white light, obscuration data of syringes containing SBO was similar to those containing water with approximately a 5% difference. Yet in UVA light, the syringes containing SBO when compared with those containing water have a 46% difference in obscuration. Furthermore, these syringes had a higher light obscuration than syringes containing lipid emulsions.

7.4. Discussion

These investigations although not entirely successful provide some interesting insights into the protective effects lipid emulsions may have on vitamins in the presence of different artificial light sources. The first results generated compared containers and their impact on light obscuration. Syringes and glass MDVs were compared in this experiment in cool white, warm white and UVA light. There is a statistically significant difference ($p > 0.001$) when comparing these two container types, with syringes causing more obscuration than glass MDVs in the three types of artificial light examined. Syringes are visibly more opaque than glass and this obscuration is likely to be a result of either absorbing or reflecting these light sources. Both MDVs and syringes seem to absorb or reflect a higher percentage of emitted UVA light than cool or warm white light. However, this is difficult to compare, as the measuring units on the detectors are different and also the UVA detector is less precise than the lux meter used for cool or warm white light. Future work would examine the effect PN bags have on light obscuration and whether multi-layered bags can cause increased

obscuration. It would also be interesting to investigate the protective effect of these different container materials on the chemical stability of light sensitive vitamins such as retinol.

When comparing lipid emulsions in syringes it became evident immediately that even small amounts of lipid caused significant obscuration ($p > 0.001$) with large differences between control syringes containing ultra-filtered deionised water and those containing lipid emulsions. When examining obscuration in different lipid emulsion groups there were very few differences. This is intriguing as work in Chapter Five and Six showed far more degradation in syringes containing ultra-filtered deionised water and Vitlipid® N Adult, yet, the obscuration in more lipid concentrated groups are similar. This suggests there may be another route of protection exhibited by the lipid emulsions.

There were a number of limitations with these investigations. Firstly, there was only one light detector in each experiment meaning that light reflection could not be measured. Therefore, we cannot be sure whether the lipid emulsions absorb or reflect the light. Secondly, there was a lack of precision in the UVA detector (Wm^{-2} meter) as it recorded levels to one decimal place only. This made it very difficult to distinguish between groups. Future work would use more detectors with increased precision.

The second part of these experiments was investigating the protective effects of SBO on vitamins in various light sources as it is a major component of IL10, IL20

and SMOF (see Table 5.4). The idea was to investigate the degradation of retinol palmitate in SBO using the validated stability-indicating HPLC assay described in Chapter Five. Unfortunately, due to the high lipophilicity of SBO, it is not miscible with the methanol mobile phase used in this assay. To overcome this, it was decided to use a co-solvent to help dissolve the SBO, thereby allowing the identification and quantification of retinol. Acetonitrile, tetrahydrofuran and propan-2-ol were all tried initially but the SBO was not sufficiently soluble in any of these. Dichloromethane was then tried which was miscible with the SBO, although, when analysing retinol using the HPLC assay there were no identifiable retinol peaks. The peaks were small and had poor shape on the chromatogram, therefore HPLC assay could not be used in this experiment. Other assay methods were identified, although, due to time restrictions these could not be pursued (Andrés et al. 2014; Gundersen & Blomhoff 2001). These assays may allow quantification of retinol in SBO and would provide an interesting insight into SBOs protective effects.

An interesting pattern was discovered when comparing the obscuration of syringes containing SBO to those containing ultra-filtered deionised water, ultra-filtered deionised water and Vitlipid® N Adult, IL10, IL20 and SMOF. SBO is a straw coloured clear liquid that, as hypothesised, behaved in a similar way to syringes containing water in cool and warm white light. However, when exposed to UVA light, SBO showed more prominent obscuration than any lipid-containing syringe previously tested. This suggests that the SBO either absorbs or reflects UVA wavelengths. Such characteristics may help to explain protective

effects seen with retinol in previous chapters as it predominantly sensitive to UVA emissions (Allwood & Plane 1986). The concentration and viscosity of the liquid may influence the extent of obscuration of UVA light seen. It seems that SBO is an important component in lipid preparations providing protection to light sensitive vitamins even though it is a clear liquid.

7.5 Conclusion

These experiments show that even a small amount of lipid emulsion can cause significant obscuration. However, when comparing to previous data generated in Chapter Five and Six obscuration is unlikely to be the only reason why lipid emulsions cause protection. SBO is excellent at either repelling or absorbing UVA rays and this may help protect light sensitive vitamins, such as tocopherol and retinol, which are sensitive to wavelengths in the UVA range.

CHAPTER EIGHT

General Discussion

8.1. General Discussion

Modern PN has been used for over 50 years and still there are still many research questions that need addressing. In recent years, there has been an increasing number of patients who are being treated with HPN. This change in emphasis necessitates the generation of physico-chemical stability data to extend and validate expiry dates thereby allowing the safe administration of sufficient amounts of nutrients.

The stability of vitamins is an important consideration, as they are one of the most reactive components in PN. There is insufficient research into the effects of light sources on vitamin degradation, especially since the introduction of new artificial light sources with energy-saving bulbs. The experiments in this thesis investigate the effects of artificial light sources on vitamin photo-degradation.

A large proportion of this project was aimed at designing a validated stability-indicating HPLC assay for the analysis of fat and water-soluble vitamins. It was hoped that this assay could be used as means for pharmacists to check the quantities of vitamins in PN preparations to ensure patients are being administered the correct doses. Following the optimisation of this novel assay, eight water and fat-soluble vitamins could be identified simultaneously in one 47-minute run. This assay allowed accurate quantification of vitamins, with degradation indicated by a reduction in peak area. This assay was used for some

experiments, however, due to its long run time a shorter assay had to be used, in some instances, to allow more timely analysis of samples.

There were two experiments focusing on the extent of light degradation following exposure to artificial light sources. Both the 24-hour and 6+1 day studies revealed similar results, illustrating that additional storage in a refrigerator has little impact vitamin degradation. New data was generated for vitamins such as folic acid, nicotinamide and ergocalciferol, which have not been extensively tested previously. The only vitamins that had substantial degradation following exposure were retinol and riboflavin. Retinol was degraded to a greater extent in UVA light, whereas, riboflavin was predominantly degraded in cool and warm white light. The extensive degradation of riboflavin has not been previously identified. These results suggest that riboflavin undergoes more degradation in the visible spectrum than previously thought. Riboflavin and its degradation products have potent photosensitising potential, which may cause the degradation of other components of PN admixtures. Research on riboflavin's photosensitising potential is on-going and should be a consideration in the future.

Investigations into the effect of artificial light sources on vitamin degradation in administration sets provided some interesting insights. In practice, administration sets are routinely left exposed to light and provide a high surface area for light to interact with photo-sensitive vitamins. These studies showed substantial losses of retinol during simulated administration of vitamins. These

losses may be enhanced if containers are not covered, raising questions on how much patients are actually receiving when they are administered PN.

There have been some contrasting opinions with regards to the protective effects of lipid emulsions on light-sensitive components of PN. In this thesis there are two experiments, that investigated the protective effects of lipid emulsions on the degradation of light sensitive vitamins in both storage containers and administration sets. From the results generated, lipid emulsions seem to provide significant amounts of protection to light sensitive vitamins in both syringes and administration sets. Increasing the concentration of lipid in the emulsion also seems to improve stability, with IL20 and SMOF providing the best protection. However, there was still some substantial degradation in lipid emulsions on exposure to these light sources necessitating the use of light protection. Further investigations into the mechanism of protection exhibited by lipid emulsions has suggested that they provide significant obscuration to all three artificial light sources tested. However, there may be a secondary action of the lipid emulsions where the lipid absorbs or reflects damaging light emissions, especially UVA rays, thereby reducing their impact. This was supported by experiments investigating the obscuration of SBO, which allowed visible light but stopped UVA light from passing through. It is not certain whether UVA emissions are absorbed or reflected and further investigation is required.

8.2. Future Work

The purpose of designing a validated stability-indicating assay was so that it could be used by pharmacists as a method of quality assurance in practice. This assay can quantify eight fat and water-soluble vitamins in one run, although, the length of the run may prove impractical in a clinical setting. Therefore, designing a shorter assay that can identify the most unstable vitamins would be advantageous. The proposed assay would have to identify ascorbic acid, tocopherol, riboflavin and retinol to assure the chemical stability of the most reactive vitamins in the admixture.

Further work looking at the stability of vitamins in containers and administration sets in the presence of other components commonly found in PN would also be worthwhile. Trace elements alone are known to speed up the degradation of some vitamins. For example, ascorbic acid oxidation is increased in the presence of copper, zinc, ferric and manganese ions (DeRitter 1982).

In Chapter Seven, the mechanisms of lipid emulsions' protection are investigated. Further experiments investigating whether SBO alone provides any protection to light sensitive vitamins such as retinol would be beneficial. There are a couple of validated HPLC assays already published, which would enable retinol quantification in this medium (Andrés et al. 2014; Gundersen & Blomhoff 2001).

Finally, investigating the deficiency states of patients on long-term HPN would provide key information as to whether there are any other vitamin related stability problems that have not been previously identified. Through studies such as these stability data can be generated to ensure patients are receiving their correct doses.

8.3. Limitations

There were a number of limitations in these experiments due to the niche nature of the research area. The novel validated stability-indicating HPLC assay designed identified eight fat and water-soluble vitamins, but it took 47 minutes to run and chemical analysis of 3 samples took 7.5 hours. This meant that only two test and one control sample could be tested at each time point. Ideally, for studies such as these further repeats would be required. One solution would be to use UHPLC, which would reduce the duration of each run. Unfortunately this equipment is expensive and was not available during these experiments.

Another limitation in this study was the fluctuating temperature in the laboratory this meant that the majority of experiments had to be conducted in a stability chamber to ensure that the temperature was maintained at a constant temperature of 25 °C. Performing all the experiments in this confined space limited the types of experiments that could take place, with syringes being the only containers tested.

The UVA detector used in these experiments prevented precise measurements of light obscuration in Chapter Seven, making it difficult to distinguish between the obscuration of different lipid emulsions. Also the lack of detectors prevented the measurement of reflected light. This would be useful at determining whether light was absorbed or reflected by the lipid emulsions and SBO.

In these experiments a multi-ray lamp was used, which may not be a true representation of what patients may have in their homes. Unfortunately, there is a wide array of light sources, with different spectra, that people can buy. However, using the multi-ray lamp allows experiments to be reproduced by other researchers in the future.

8.4. Recommendations

These experiments have emphasised the need for light protection of administration sets and containers. Currently containers are light protected in most instances, but administration sets are rarely, if ever, protected. Therefore, it is conceivable that patients are being routinely administered PN with insufficient vitamins. In addition, the use of lipid emulsions in PN admixtures seems to provide extra protection of vitamins in both containers and administration sets, therefore 3in1 admixtures (containing lipid, glucose and amino acids) should be preferred if light exposure is a concern.

8.5. Concluding Remarks

This thesis provides some insights into the stability of vitamins in various artificial light sources. It also demonstrates that there are still a large number of unanswered research questions regarding the stability issues associated with vitamins in PN. It shows the importance of further research in this area and the use of quality assurance to ensure that patients are receiving their intended nutrient doses.

References

Akasah, T. 2002. *Some studies on globule size distributions in parenteral lipid emulsions*. PhD Thesis, Cardiff University.

Allwood, M. C. 1982a. Stability of vitamins in TPN solutions stored in 3L bags. *British Journal of Intravenous Therapy* 3, pp. 22–26.

Allwood, M. C. 1982b. The influence of light on vitamin A degradation during administration. *Clinical Nutrition* 1(1), pp. 63–70.

Allwood, M. C. 1983. The compatibility of four trace elements in total parenteral nutrition infusions. *International Journal of Pharmaceutics* 16(1), pp. 57–63.

Allwood, M. C. 1984a. Compatibility and stability of TPN mixtures in big bags. *Journal of Clinical and Hospital Pharmacy* 9(3), pp. 181–198.

Allwood, M. C. 1984b. Factors influencing the stability of ascorbic acid in total parenteral nutrition infusions. *Journal of Clinical and Hospital Pharmacy* 9, pp. 75–85.

Allwood, M. C. 1999. Oxalogenesis in parenteral nutrition mixtures. *Nutrition* 15(1), p. 70.

- REFERENCES -

Allwood, M. C. 2000. Pharmaceutical aspects of parenteral nutrition: from now to the future. *Nutrition* 16(7-8), pp. 615–618.

Allwood, M. C., Ball, P. A., Driscoll, D. F., & Sizer, T. 2000. Light protection during parenteral nutrition infusion: Is it really necessary? *Nutrition* 16, pp. 234–235.

Allwood, M. C., Brown, P. W., Ghedini, C., & Hardy, G. 1992. The stability of ascorbic acid in TPN mixtures stored in a multilayered bag. *Clinical Nutrition* 11, pp. 284–288.

Allwood, M. C., & Greenwood, M. 1992. Assessment of trace element compatibility in total parenteral nutrition infusions. *Pharmaceutisch Weekblad Scientific edition* 14(5), pp. 321-324.

Allwood, M. C., & Kearney, M. C. J. 1998. Compatibility and stability of additives in parenteral nutrition admixtures. *Nutrition* 14(9), pp. 697–706.

Allwood, M. C., Martin, H., Greenwood, M., & Maunder, M. 1998. Precipitation of trace elements in parenteral nutrition mixtures. *Clinical Nutrition* 17(5), pp. 223–226.

Allwood, M. C., & Martin, H. J. 2000. The photodegradation of vitamins A and E in parenteral nutrition mixtures during infusion. *Clinical Nutrition* 19, pp. 339–342.

- REFERENCES -

Allwood, M. C., & Plane, J. H. 1984. The degradation of vitamin A exposed to ultraviolet radiation. *International Journal of Pharmaceutics* 19, pp. 207–213.

Allwood, M. C., & Plane, J. H. 1986. The wavelength-dependent degradation of vitamin A exposed to ultraviolet radiation. *International Journal of Pharmaceutics* 31(1-2), pp. 1–7.

Andrés, V., Villanueva, M. J., & Tenorio, M. D. 2014. Simultaneous determination of tocopherols, retinol, ester derivatives and β -carotene in milk- and soy-juice based beverages by HPLC with diode-array detection. *LWT - Food Science and Technology* 58(2), pp. 557–562.

Anon. 1993. Malvern particle size application manual.

Anon. 1996. The Advanced Osmometer.

Anon. 1999. Malvern Operators Guide (20th ed.).

Anon. 2013. British Pharmacopoeia 2013 online. Available at <http://www.pharmacopoeia.co.uk/bp2013/ixbin/bp.cgi?a=display&r=ALbFvSDj8bZ&id=7786&tab=search> [Accessed: 22 May 2013]

- REFERENCES -

Austin, P., & Stroud, M. 2007. *Prescribing Adult Intravenous Nutrition*. London: Pharmaceutical Press, pp. 125–145.

Barker, A., Hebron, B. S., Beck, P. R., & Ellis, B. 1984. Folic Acid and total parenteral nutrition. *JPEN. Journal of Parenteral and Enteral Nutrition* 8, pp. 3–8.

Barnett, M. I., Pertkiewicz, M., Cosslett, A. G., & Mühlebach, S. 2009. Basics in clinical nutrition: Parenteral nutrition admixtures, how to prepare parenteral nutrition (PN) admixtures. *E-SPEN, the European E-Journal of Clinical Nutrition and Metabolism* 4(3), pp. e114–e116.

Baumgartner, T. G., Henderson, G. N., Fox, J., & Gondi, U. 1997. Stability of ranitidine and thiamine in parenteral nutrition solutions. *Nutrition* 13(6), pp. 547–553.

Berger, M. M. 2009. Vitamin C requirements in parenteral nutrition. *Gastroenterology* 137(5 Suppl), pp. S70–78.

Biesalski, H. K. 2009. Vitamin E requirements in parenteral nutrition. *Gastroenterology* 137(5 Suppl), pp. S92–104.

- REFERENCES -

Billany, M. 2004. Suspensions and emulsions. In: Aulton, M. E., *Pharmaceutics the science of dosage form design*. 2nd Edition. Edinburgh: Churchill Livingstone. pp. 334–359

Billion-Rey, F., Guillaumont, M., Frederich, A., & Aulagner, G. 1993. Stability of fat-soluble vitamins A (Retinol Palmitate), E (Tocopherol Acetate) and K1 (Phylloquinone) in total parenteral nutrition at home. *JPEN. Journal of Parenteral and Enteral Nutrition* 17, pp. 56–60.

Bowman, B. B., & Nguyen, P. 1983. Stability of thiamine in parenteral nutrition solutions. *JPEN. Journal of Parenteral and Enteral Nutrition* 7, pp. 567–8.

Carpentier, Y. A. 2009. Basic in clinical nutrition: Substrates used in parenteral and enteral nutrition – lipids. *E-SPEN, the European E-Journal of Clinical Nutrition and Metabolism* 4(2), pp. e66–e68.

Chen, M. F., Worth Boyce, H., & Triplett, L. 1983. Stability of B vitamins mixed in parenteral nutrition solution. *JPEN. Journal of Parenteral and Enteral Nutrition* 7, pp. 462–464.

Chen, Z., Chen, B., & Yao, S. 2006. High-performance liquid chromatography/electrospray ionization-mass spectrometry for simultaneous determination of taurine and 10 water-soluble vitamins in multivitamin tablets. *Analytica Chimica Acta* 569(1-2), pp. 169–175.

- REFERENCES -

- Ciulu, M., Solinas, S., Floris, I., Panzanelli, A., Pilo, M. I., Piu, P. C., Spano, N. Sanna, G. 2011. RP-HPLC determination of water-soluble vitamins in honey. *Talanta* 83(3), pp. 924–929.
- Combs, F. C. 1998. *The Vitamins*. 2nd ed. London: Academic Press.
- Compher, C., Kinosian, B., Stoner, N., Lentine, D., & Buzby, G. 2002. Choline and vitamin B12 deficiencies are interrelated in folate-replete long-term total parenteral nutrition patients. *JPEN. Journal of Parenteral and Enteral Nutrition* 26(1), pp. 57–62.
- Dahl, G. B., Jeppsson, R. I., & Tengborn, H. J. 1986. Vitamin stability in a TPN mixture stored in an EVA plastic bag. *Journal of Clinical and Hospital Pharmacy* 11, pp. 271–279.
- De La Rochette, A., Silva, E., Birlouez-Aragon, I., Mancini, M., Edwards, A.-M., & Morlière, P. 2000. Riboflavin photodegradation and photosensitizing effects are highly dependent on oxygen and ascorbate concentrations. *Photochemistry and Photobiology* 72(6), pp. 815–820.
- DeLuca, H. F. 2008. Evolution of our understanding of vitamin D. *Nutrition Reviews* 66(10 Suppl 2), pp. S73–87.

- REFERENCES -

DeLuca, H. F. 2009. Vitamin D and the parenteral nutrition patient. *Gastroenterology*, 137(5 Suppl), pp. S79–91.

Department of Health. 2009. *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom*. HMSO.

DeRitter, E. 1982. Vitamins in pharmaceutical formulations. *Journal of Pharmaceutical Sciences* 71(10), pp. 1073–1096.

Dionex. 2009. Simultaneous determination of water- and fat-soluble vitamins by HPLC. *Analytica Chimica Acta* 520, pp. 1–6.

Driscoll, D. F. 2005. Stability and compatibility assessment techniques for total parenteral nutrition admixtures: setting the bar according to pharmacopeial standards. *Current Opinion in Clinical Nutrition and Metabolic Care* 8(3), pp. 297–303.

Driscoll, D. F. 2006. Lipid injectable emulsions: Pharmacopeial and safety issues. *Pharmaceutical Research* 23(9), pp. 1959–1969.

Drott, P., Meurling, S., & Meurling, L. 1991. Clinical adsorption and photodegradation of the fat soluble vitamins A and E. *Clinical Nutrition* 10, pp. 348–351.

- REFERENCES -

Dupertuis, Y. M., Ramseyer, S., Fathi, M., & Pichard, C. 2005. Assessment of Ascorbic Acid stability in different multilayered parenteral nutrition bags: Critical influence of bag wall material. *JPEN. Journal of Parenteral and Enteral Nutrition* 29, pp. 125–130.

Forbes, A. 2009. Iron and parenteral nutrition. *Gastroenterology* 137(5 Suppl), pp. S47–54.

Francini-Pesenti, F., Brocadello, F., Manara, R., Santelli, L., Laroni, A., & Caregaro, L. 2009. Wernicke's syndrome during parenteral feeding: not an unusual complication. *Nutrition* 25(2), pp. 142–146.

Friel, C., & Bistran, B. 1997. Cycled total parenteral nutrition : is it more effective? *American Journal of Clinical Nutrition* 65, pp. 1078–9.

Fürst, P., Kuhn, K. S., & Stehle, P. 2001. Parenteral nutrition substrates. In: Payne-James, J., Grimble, G. & Silk, D. *Artificial nutrition support in clinical practice*. 2nd ed. London: GMM. pp. 401-434.

Gallitelli, L. 1995. Trace element and vitamin requirements in patients receiving parenteral nutrition. *Clinical Nutrition*, 14 Suppl 1, pp. 70–74.

- REFERENCES -

Gibbons, E., Allwood, M. C., Neal, T., & Hardy, G. 2001. Degradation of dehydroascorbic acid in parenteral nutrition mixtures. *Journal of Pharmaceutical and Biomedical Analysis* 25(3-4), pp. 605–611.

Gillis, J., Jones, G., & Pencharz, P. 1983. Delivery of vitamins A, D and E in total parenteral nutrition solutions. *JPEN. Journal of Parenteral and Enteral Nutrition* 7, pp. 11–14.

Grummett, M. 2012. Email to T. Ferguson 25 September 2012.

Guidetti, M., Sforzini, A., Bersani, G., Corsini, C., Zolezzi, C., Fasano, C., & Pironi, L. 2008. Vitamin A and vitamin E isoforms stability and peroxidation potential of all-in-one admixtures for parenteral nutrition. *International Journal for Vitamin and Nutrition Research*, 78(3), pp. 156–166.

Gundersen, T. E., & Blomhoff, R. 2001. Qualitative and quantitative liquid chromatographic determination of natural retinoids in biological samples. *Journal of Chromatography. A* 935(1-2), pp. 13–43.

Haas, C., Genzel-Boroviczény, O., & Koletzko, B. 2002. Losses of vitamin A and E in parenteral nutrition suitable for premature infants. *European Journal of Clinical Nutrition* 56(9), pp. 906–912.

Hardy, G. 2009. Manganese in parenteral nutrition: who, when, and why should we supplement? *Gastroenterology* 137(5 Suppl), pp. S29–35.

- REFERENCES -

Hardy, G., & Mari, G. 1988. The stability of TPN solutions in a new plastic container. *Clinical Nutrition* 7(Supplement), p. 75.

Hardy, G., Menendez, A. M., & Manzanares, W. 2009. Trace element supplementation in parenteral nutrition: pharmacy, posology, and monitoring guidance. *Nutrition* 25(11-12), pp. 1073–1084.

Hatley, J. 2012. Email to T. Ferguson 30 October 2012.

Henton, D. H., & Merritt, R. J. 1990. Vitamin A sorption to polyvinyl and polyolefin intravenous tubing. *JPEN. Journal of Parenteral and Enteral Nutrition*, 14, pp. 79–81.

Heudi, O., Kilinc, T., & Fontannaz, P. 2005. Separation of water-soluble vitamins by reversed-phase high performance liquid chromatography with ultra-violet detection: Application to polyvitaminated premixes. *Journal of Chromatography A* 1070(1-2), pp. 49–56.

Hinton, P. 1996. *Statistics Explained*. London: Routledge. pp. 65–66

Holick, M. F. 2012. The D-lightful vitamin D for child health. *JPEN. Journal of Parenteral and Enteral Nutrition*, 36(1 Suppl), pp. 9S–19S.

- REFERENCES -

- Huvaere, K., Cardoso, D. R., Homem-de-Mello, P., Westermann, S., & Skibsted, L. H. 2010. Light-induced oxidation of unsaturated lipids as sensitized by flavins. *The Journal of Physical Chemistry. B* 114(16), pp. 5583–5593.
- Jeejeebhoy, K. 2009. Zinc: an essential trace element for parenteral nutrition. *Gastroenterology* 137(5 Suppl), pp. S7–12.
- Kearney, M. C. J., Allwood, M. C., Martin, H. J., Neal, T., & Hardy, G. 1998. The influence of amino acid source on the stability of ascorbic acid in TPN mixtures. *Nutrition*, 14(2), pp. 173–178.
- Kearney, M. C. J., Allwood, M. C., Neale, T., & Hardy, G. 1995. The stability of thiamine in total parenteral nutrition mixtures stored in EVA and multi-layered bags. *Clinical Nutrition* 14(5), pp. 295–301.
- Kenyon, I. R. 2008. *The Light Fantastic*. Oxford: Oxford University Press. pp. 24.
- Khan, A., Khan, M. I., Iqbal, Z., Shah, Y., Ahmad, L., & Watson, D. G. 2010. An optimized and validated RP-HPLC/UV detection method for simultaneous determination of all-trans-retinol (vitamin A) and alpha-tocopherol (vitamin E) in human serum: comparison of different particulate reversed-phase HPLC columns. *Journal of Chromatography. B* 878(25), pp. 2339–2347.

- REFERENCES -

Kirk, B. 1985. The evaluation of a new light-protective giving set. *British Journal of Parenteral Therapy* 6, pp. 146–151.

Klejdus, B., Petrlová, J., Potešil, D., Adam, V., Mikelová, R., Vacek, J., Kizek, R. & Kubán, V. 2004. Simultaneous determination of water- and fat-soluble vitamins in pharmaceutical preparations by high-performance liquid chromatography coupled with diode array detection. *Analytica Chimica Acta* 520(1-2), pp. 57–67.

Koletzko, B., Goulet, O., Hunt, J., Krohn, K., & Shamir, R. 2005a. 3. Amino acids. *Journal of Pediatric Gastroenterology and Nutrition* 41(November), pp. S12–S18.

Koletzko, B., Goulet, O., Hunt, J., Krohn, K., & Shamir, R. 2005b. 5. Carbohydrates. *Journal of Pediatric Gastroenterology and Nutrition* 41(November), pp. S28–S32.

Koletzko, B., Goulet, O., Hunt, J., Krohn, K., & Shamir, R. 2005c. 6 . Fluid and electrolytes (Na , Cl and K). *Journal of Pediatric Gastroenterology and Nutrition*, 41(November), pp. S33–S38.

Koletzko, B., Goulet, O., Hunt, J., Krohn, K., & Shamir, R. 2005d. 8. Vitamins. *Journal of Pediatric Gastroenterology and Nutrition*, 41(November), pp. S47–S53.

- REFERENCES -

Kwong, K. W., & Tsallas, G. 1980. Dilute iron dextran formulation for addition to parenteral nutrient solutions. *American Journal of Hospital Pharmacy* 37(2), pp. 206-210.

Lavoie, J., Chessex, P., Rouleau, T., Tsopmo, A., & Friel, J. 2007. Shielding parenteral multivitamins from light increases vitamin A and E concentration in lung of newborn guinea pigs. *Clinical Nutrition* 26(3), pp. 341–347.

Lee, D. R., Ware, I., & Winsley, B. E. 1980. Survival of folic acid in TPN solutions. *British Journal of Intravenous Therapy*, 1(2), pp. 13–16.

Leung, F. Y. 1995. Trace elements in parenteral micronutrition. *Clinical Biochemistry* 28(6), pp. 561–566.

Madan, M., Alexander, D. J., & McMahon, M. J. 1992. Influence of catheter type on occurrence of thrombophlebitis during peripheral intravenous nutrition. *The Lancet* 339(8785), pp. 101–103.

Manning, R. J., & Washington, C. 1992. Chemical stability of total parenteral nutrition mixtures. *International Journal of Pharmaceutics* 81, pp. 1–20.

McGee, D. C., & Gould, M. K. 2003. Preventing complications of central venous catheterization. *The New England Journal of Medicine* 348(12), pp. 1123–1133.

- REFERENCES -

Menne, R., Adolph, M., Brock, E., Schneider, H., & Senkal, M. 2008. Cost analysis of parenteral nutrition regimens in the intensive care unit: three-compartment bag system vs multibottle system. *JPEN. Journal of Parenteral and Enteral Nutrition* 32(6), pp. 606–612.

Milla, P. 2001. Paediatric Nutrition Requirements. In: Payne-James, J., Grimble, G. & Silk, D. *Artificial nutrition support in clinical practice*. 2nd ed. London: GMM. pp. 213–224

Mirkovic, D. C., Basic, Z., & Roganovic, B. 2011. PP185-Sun Influence of type of bag, sort of admixtures and exposure to daylight upon stability of vitamins C and B2 in parenteral nutrition admixtures. *Clinical Nutrition Supplements* 6(1), pp.93–94.

Momenbeik, F., Roosta, M., & Akbar, A. 2010. Simultaneous microemulsion liquid chromatographic analysis of fat-soluble vitamins in pharmaceutical formulations : Optimization using genetic algorithm. *Journal of Chromatography A* 1217(24), pp. 3770–3773.

Moreno, P., & Salvadó, V. 2000. Determination of eight water- and fat-soluble vitamins in multi-vitamin pharmaceutical formulations by high-performance liquid chromatography. *Journal of Chromatography. A* 870(1-2), pp. 207–215.

- REFERENCES -

Moukarzel, A. 2009. Chromium in parenteral nutrition: too little or too much? *Gastroenterology* 137(5 Suppl), pp. S18–28.

Mühlebach, S., & Steger, P. J. 1998. Lipid peroxidation of intravenous fat emulsions: a pharmaceutical issue with clinical impact? *Nutrition* 14(9), pp. 720–721.

Natera, J., Massad, W. A., & García, N. A. 2011. Vitamin B1 as a scavenger of reactive oxygen species photogenerated by vitamin B2. *Photochemistry and Photobiology*, 87(2), pp. 317–323.

National Renewable Energy Laboratory. 2003. *Reference solar spectral irradiance*. Available at: <http://rredc.nrel.gov/solar/spectra/am1.5/> [Accessed: 20 May 2013]

NHS. 2010. *NHS 2010 – 2015: from good to great. preventative, people-centred, productive*. London: Department of Health.

NICE. 2006. *Nutrition support in adults: Quick reference guide*. London: NICE. Available at: <http://www.nice.org.uk/guidance/CG032> [Accessed: 4 July 2012]

Papadoyannis, I. N., Tsioni, G. K., & Samanidou, V. F. 1997. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and

- REFERENCES -

RP-HPLC analysis in pharmaceutical preparations and biological fluids. *Journal of Liquid Chromatography & Related Technologies* 20(19), pp. 3203–3231.

Pikwer, A., Åkeson, J., & Lindgren, S. 2012. Complications associated with peripheral or central routes for central venous cannulation. *Anaesthesia* 67(1), pp. 65–71.

Pittas, A. G., Laskowski, U., Kos, L., & Saltzman, E. (2010). The role of Vitamin D in adults requiring nutrition therapy. *JPEN. Journal of Parenteral and Enteral Nutrition*, 34(1), 70–78.

Pluhator-Murton, M. M., Fedorak, R. N., Audette, R. J., Marriage, B. J., Yatscoff, R. W., & Gramlich, L. M. 1999. Trace element contamination of total parenteral nutrition. *JPEN. Journal of Parenteral and Enteral Nutrition* 23, pp. 222–227.

Proot, P., De Pourcq, L., & Raymakers, A. A. 1994. Stability of ascorbic acid in a standard total parenteral nutrition mixture. *Clinical Nutrition* 13(5), pp. 273–9.

Reimund, J., Dietemann, J., Warter, J., Baumann, R., & Duclos, B. 2000. Factors associated to hypermanganesemia in patients receiving home parenteral nutrition. *Clinical Nutrition* 19(5), pp. 343–348.

Renwick, A. G., & Walker, R. 2008. Risk assessment of micronutrients. *Toxicology Letters*, 180(2), pp. 123–130.

- REFERENCES -

Ribeiro, D. O., Pinto, D. C., Lima, L. M. T. R., Volpato, N. M., Cabral, L. M., & de Sousa, V. P. 2011. Chemical stability study of vitamins thiamine, riboflavin, pyridoxine and ascorbic acid in parenteral nutrition for neonatal use. *Nutrition Journal* 10, pp. 47–56.

Rümelin, A. 2009. Ascorbic acid in postoperative intensive care patients - biochemical aspects and clinical experience. *Current Medicinal Chemistry* 16(2), pp. 184–188.

Scheiner, J. M., Araujo, M. M., & DeRitter, E. 1981. Thiamine destruction by sodium bisulfite in infusion solutions. *American Journal of Hospital Pharmacy*, 38, pp. 1911-1913.

Schmutz, C. W., Martinelli, E., & Mühlebach, S. 1992. Stability of vitamin K1 assessed by HPLC in total parenteral nutrition (TPN) admixtures. *Clinical Nutrition* 12(169), pp. 110–111.

Shearer, M. J. 2009. Vitamin K in parenteral nutrition. *Gastroenterology* 137(5 Suppl), pp. S105–118.

Shen, L., & Ji, H.-F. 2008. How α -tocopherol quenches triplet state riboflavin? Insights from theory. *Journal of Photochemistry and Photobiology A: Chemistry* 199(1), pp. 119–121.

Shenkin, A. 2006a. Micronutrients in health and disease. *Postgraduate Medical Journal* 82(971), pp. 559–567.

Shenkin, A. 2006b. The key role of micronutrients. *Clinical Nutrition* 25(1), pp. 1–13.

Sherwood, R. A. 2001. The liver and nutrient metabolism. In: Payne-James, J., Grimble, G. & Silk, D. *Artificial nutrition support in clinical practice*. 2nd ed. London: GMM. pp. 81–92

Shike, M. 2009. Copper in parenteral nutrition. *Gastroenterology* 137(5 Suppl), pp. S13–17.

Silva, E., Gonzalez, T., Edwards, A. M., & Zuloaga, F. 1998. Visible light induced lipoperoxidation of a parenteral nutrition fat emulsion sensitized by flavins. *Nutritional Biochemistry* 9, pp. 149–154.

Silva, E., Ugarte, R., Andrade, A., & Edwards, A. M. 1994. Riboflavin-sensitized photoprocesses of tryptophan. *Journal of Photochemistry and Photobiology. B, Biology*, 23(1), pp. 43–48.

Skouroliakou, M., Matthaïou, C., Chiou, A., Panagiotakos, D., Gounaris, A., Nunn, T., & Andrikopoulos, N. 2008. Physicochemical stability of parenteral nutrition

- REFERENCES -

supplied as all-in-one for neonates. *JPEN. Journal of Parenteral and Enteral Nutrition*, 32(2), pp. 201–209.

Smith, J. L., Canham, J. E., Kirkland, W. D., & Wells, P. A. 1988. Effect of intralipid, amino acids, container, temperature, and duration of storage on vitamin stability in total parenteral nutrition admixtures. *JPEN. Journal of Parenteral and Enteral Nutrition* 12, pp. 478–483.

Smith, J. L., Canham, J. E., & Wells, P. A. 1988. Effect of phototherapy light, sodium bisulfite, and pH on vitamin stability in total parenteral nutrition admixtures. *JPEN. Journal of Parenteral and Enteral Nutrition* 12, pp. 394–402.

Smith, T., Hirst, A., Jones, B., & Baxter, J. 2011. *Annual BANS report*, pp. 1–50.
Available at: http://www.bapen.org.uk/pdfs/bans_reports/bans_report_11.pdf
[Accessed 7 July 2014]

Snyder, L. R., Kirkland, J. J., & Glajch, J. L. 1997. *Practical HPLC Method Development*. 2nd Ed. New York: Wiley Interscience.

Sobotka, L. 2004. *Basics in clinical nutrition*. 3rd ed. Prague: Galen.

Sriram, K., Jayanthi, V., & Suchitra, D. 1996. Acute niacin deficiency. *Nutrition* 12(5), pp. 355–357.

- REFERENCES -

Staun, M., Pironi, L., Bozzetti, F., Baxter, J., Forbes, A., Joly, F., Jeppsen, P., Moreno, J., Hebuterne, X., Pertkiewicz, M., Muhlebach, S., Shenkin, A. & Van Gossum, A. 2009. ESPEN Guidelines on parenteral nutrition: home parenteral nutrition (HPN) in adult patients. *Clinical Nutrition* 28(4), pp. 467–479.

Supelco. 2000. *Application note 148 separating water-Soluble vitamins by reversed phase HPLC using a discovery C18 column.*

Available at: https://www.sigmaaldrich.com/content/dam/sigmaaldrich/docs/Supelco/Application_Notes/6808.pdf [Accessed 7 July 2014]

Tanumihardjo, S. A. 2011. Vitamin A : biomarkers of nutrition for development. *American Journal of Clinical Nutrition* 94(supplement), pp. s658–s665.

Waitzberg, D. L., Torrinhas, R. S., & Jacintho, T. M. 2006. New parenteral lipid emulsions for clinical Use. *JPEN. Journal of Parenteral and Enteral Nutrition*, 30(4), pp. 351–367.

Wormleighton, C. V, & Catling, T. B. 1998. Stability issues in home parenteral nutrition. *Clinical Nutrition* 17(5), pp. 199–203.

Zimmermann, M. B. 2009. Iodine: it's important in patients that require parenteral nutrition. *Gastroenterology* 137(5 Suppl), pp. S36–46.

Appendix I - Publications

Scientific papers

Ferguson, T. I., Emery, S., Price-Davies, R., & Cosslett, A. G. 2014. A review of stability issues associated with vitamins in parenteral nutrition. *E-SPEN, the European E-Journal of Clinical Nutrition and Metabolism*, 9, pp. e49-e53.

Scientific abstracts

Ferguson, T. I., Price-Davies, R., & Cosslett, A. 2013. PP259-Mon Vitamins – an Unknown Quantity. *Clinical Nutrition*, 32, pp. S219.

Conferences and meetings

Poster Presentations:-

April 2013: Cardiff School of Pharmacy and Pharmaceutical Sciences Postgraduate Day, Cardiff University, Cardiff

October 2013: ESPEN conference, Leipzig, Germany.

Oral communications: -

April 2014: Cardiff School of Pharmacy and Pharmaceutical Sciences Postgraduate Day, Cardiff University, Cardiff.

February 2014: BPNG Advance Course in Parenteral Nutrition, Regent's College, London.

