Formulation, *in vitro* release and transdermal diffusion of Vitamin B_3 for treatment of acne

Telanie Venter

(B. Pharm)

Dissertation submitted in the partial fulfilment of the requirements for the degree

MAGISTER SCIENTIAE

(PHARMACEUTICS)

in the

School of Pharmacy

at the

North-West University (Potchefstroom Campus)

Supervisor: Prof. J. du Plessis

Co-supervisor: Prof. J. du Preez

Assistant-supervisor: Dr. M.M. Malan

Potchefstroom

2009

TABLE OF CONTENTS

List of figures		**xiii**
List of tables		**xvi**
Acknowledgements		**xix**
Abstract		**xxi**
Uittreksel		**xxiv**
Foreword		**xxvii**
CHAPTER 1: INTRODUCTION AND STATEMENT OF THE PROBLEM		**1**
CHAPTER 2: TRANSDERMAL DRUG DELIVERY THROUGH HUMAN SKIN		**5**
2.1	**INTRODUCTION**	**5**
2.2	**ACNE**	**5**
2.2.1	**Factors causing acne**	**6**
2.2.1.1	Increase in sebum production	6
2.2.1.2	Abnormal follicular desquamation	6
2.2.1.3	Bacterial proliferation	6
2.2.1.4	Inflammation	7
2.2.2	**Physical properties of acne**	**7**
2.2.3	**Treatment of acne**	**8**
2.2.3.1	Topical treatments	9
2.2.3.1.1	Topical antibiotics	9
2.2.3.1.2	Topical retinoids	10
2.2.3.1.3	Azelaic acid	10
2.2.3.1.4	Benzoyl peroxide	10
2.2.3.2	Oral treatments	11
2.2.3.2.1	Antibiotics	11
2.2.3.2.2	Isotretinoin	12
2.2.3.2.3	Hormonal therapy	12

2.2.4 Patient education 13

2.3 NICOTINAMIDE 13

2.3.1 Physicochemical characteristics 14

2.3.2 Mechanism of action 14

2.3.3 Pharmacokinetics 15

2.3.4 Function in the human body 15

2.3.5 Clinical uses 15

2.3.6 Adverse reactions 17

2.4 ANATOMY AND FUNCTIONS OF HUMAN SKIN 17

2.4.1 Structure of the skin 18

2.4.1.1 The epidermis 18

2.4.1.1.1 Stratum basale 19

2.4.1.1.2 Stratum spinosum 19

2.4.1.1.3 Stratum granulosum 19

2.4.1.1.4 Stratum corneum 19

2.4.1.2 The dermis 20

2.4.2 Functions of the skin 21

2.5 TRANSDERMAL DRUG DELIVERY 21

2.5.1 Advantages and limitations of transdermal drug delivery 21

2.5.1.1 Advantages 22

2.5.1.2 Limitations 22

2.5.2 Pathways of transdermal penetration 23

2.5.2.1 Intercellular diffusion through the lipid lamellae 24

2.5.2.2 Transcellular diffusion through both the keratinocytes and lipid lamellae 24

2.5.2.3 Diffusion through appendages 25

2.5.3 Factors influencing permeation across the skin 25

2.5.3.1 Biological factors 25

2.5.3.1.1 Skin age 25

2.5.3.1.2 Skin condition 25
2.5.3.1.3 Regional skin sites 26
2.5.3.1.4 Skin metabolism 26
2.5.3.1.5 Circulatory effects 26
2.5.3.1.6 Species differences 26
2.5.3.2 Physicochemical factors 27
2.5.3.2.1 Skin hydration 27
2.5.3.2.2 Temperature 27
2.5.3.2.3 pH 27
2.5.3.2.4 Penetration enhancers 28
2.5.3.2.5 Molecular size 28
2.5.3.2.6 Partition coefficient 28
2.5.3.2.7 Diffusion coefficient 29
2.5.3.3 Mathematical approach to drug permeation 29
2.5.3.3.1 Fick's law of diffusion 29
2.5.4 Penetration enhancement 30
2.5.4.1 Chemical penetration enhancement 31
2.5.4.1.1 Water 31
2.5.4.1.2 Isopropyl myristate and Isopropyl alcohol 31
2.5.4.1.3 Oleic acid 32
2.5.4.1.4 Azone 32
2.5.4.1.5 Vesicular structures 32
2.5.4.2 Physical penetration enhancement 32
2.5.4.2.1 Ultrasound 32
2.5.4.2.2 Iontophoresis 33
2.5.4.2.3 Electroporation 33
2.5.4.2.4 Magnetophoresis 34
2.5.4.2.5 Photomechanical wave 34

2.5.4.3 Penetration enhancement by bypassing or removing the stratum corneum 34
2.5.4.3.1 Micro-needle array 34
2.5.4.3.2 Stratum corneum ablated 34
2.6 PHEROID™ TECHNOLOGY 35
2.6.1 Pheroid™ technology for transdermal delivery 35
2.6.1.1 Structural characteristics 35
2.6.1.2 Functional characteristics 36
2.6.1.2.1 Pliable system design and versatility 36
2.6.1.2.2 Entrapment efficiency 36
2.6.1.2.3 Penetration efficiency 36
2.6.1.2.4 Uptake of Pheroid™ and entrapped compounds by cells 36
2.6.1.2.5 Metabolism, targeting and distribution 37
2.6.1.3 Therapeutic efficacy 37
2.6.1.4 Possible applications of Pheroid™ technology in cosmetics 37
2.6.1.5 Advantages of Pheroid™ technology as a transdermal drug delivery system 38
2.6.1.6 Conclusion 38
2.7 SUMMARY 39
2.8 REFERENCES 41
CHAPTER 3: ARTICLE FOR PUBLICATION IN THE INTERNATIONAL JOURNAL OF PHARMACEUTICS 46
3 ABSTRACT 47
3.1 INTRODUCTION 48
3.2 MATERIALS AND METHODS 50
3.2.1 Materials 50
3.2.2 Methods 50
3.2.2.1 Formulation of semi-solid products 50
3.2.2.2 Standard preparation 50
3.2.2.3 Sample preparation 50

3.2.2.4 Stability testing 51

3.2.2.4.1 Concentration assay 51

3.2.2.4.2 pH 52

3.2.2.4.3 Viscosity 52

3.2.2.4.4 Confocal laser scanning micorscopy 52

3.2.2.4.5 Visual appearance 52

3.2.2.4.6 Mass loss 52

3.2.2.5 Physicochemical properties 53

3.2.2.5.1 Aqueous solubility 53

3.2.2.5.2 Partition coefficient 53

3.2.2.6 Diffusion experiments 53

3.2.2.6.1 Skin preparation 53

3.2.2.6.2 Preparation of PBS buffer 54

3.2.2.6.3 Diffusion studies 54

3.2.2.6.4 Skin diffusion 54

3.2.2.6.5 Membrane permeation 55

3.2.2.6.6 Tape stripping 55

3.2.2.6.7 HPLC analysis of nicotinamide 56

3.2.2.6.8 Data analysis 56

3.2.2.6.9 Statistical analysis 56

3.3 RESULTS AND DISCUSSION 57

3.3.1 Formulation of semi-solid products 57

3.3.2 Stability tests 57

3.3.2.1 Concentration assay 57

3.3.2.2 pH 58

3.3.2.3 Viscosity 58

3.3.2.4 Confocal laser scanning microscopy 59

3.3.2.5 Visual appearance 59

3.3.2.6	Mass loss	59
3.3.3	**Physicochemical properties**	**59**
3.3.3.1	Aqueous solubility and Log D	59
3.3.4	**Diffusion experiments**	**60**
3.3.4.1	Membrane release studies	60
3.3.4.2	Diffusion studies	60
3.3.4.3	Tape stripping	61
3.3.4.4	Statistical analysis	62
3.4	**CONCLUSION**	**65**
3.5	**ACKNOWLEDGEMENTS**	**67**
3.6	**REFERENCES**	**68**
CHAPTER 4: FINAL CONCLUSIONS AND FUTURE PROSPECTS		**75**
APPENDIX A: VALIDATION OF THE HPLC ANALYTICAL METHOD FOR ASSAY ANALYSIS		**78**
A.1	**PURPOSE OF THE VALIDATION**	**78**
A.2	**CHROMATOGRAPHIC CONDITIONS**	**78**
A.3	**PREPARATION OF STANDARD AND SAMPLES**	**80**
A.3.1	**Standard preparation**	**80**
A.3.2	**Placebo preparation**	**80**
A.3.3	**Sample preparation**	**80**
A.4	**VALIDATION PARAMETERS**	**81**
A.4.1	**Linearity**	**81**
A.4.1.1	Linear regression analysis	81
A.4.2	**Accuracy**	**87**
A.4.2.1	Accuracy analysis	88
A.4.3	**Precision**	**92**
A.4.3.1	Intraday precision (Repeatability)	93
A.4.3.2	Interday precision (Reproducibility)	98

A.4.4 Ruggedness 100

A.4.4.1 Sample stability 100

A.4.4.2 System repeatability 106

A.5 CONCLUSION 109

APPENDIX B: FORMULATION OF A COSMECEUTICAL CREAM AND EMULGEL WITH NICOTINAMIDE AS THE ACTIVE INGREDIENT 110

B.1 INTRODUCTION 110

B.2 DEVELOPMENT PROGRAM FOR THE FORMULATION OF COSMECEUTICAL PRODUCTS 110

B.2.1 Formulation of cosmeceutical products 110

B.2.2 Pre-formulation 111

B.2.3 Early formulation 111

B.2.4 Final formulation 111

B.2.5 Preservation of cosmeceutical products 112

B.3 FORMULATION OF A CREAM 112

B.3.1 Purpose and function of a cream 113

B.3.2 Main ingredients of a cream 113

B.3.3 General method for manufacturing a cream 113

B.4 FORMULATION OF A GEL 114

B.4.1 Purpose and function of a gel 114

B.4.2 Main ingredients of a gel 115

B.4.3 General method for manufacturing a gel 115

B.5 FORMULATION OF A NICTOINAMIDE CONTAINING CREAM, PHEROID™ CREAM, EMULGEL AND PHEROID™ EMULGEL DURING THIS STUDY 116

B.6 FORMULATION OF A NICOTINAMIDE CONTAINING CREAM DURING THIS STUDY 117

B.6.1 Formula of nicotinamide cream 117

B.6.2 Procedure to prepare the nicotinamide cream 117

B.6.3 Procedure to prepare the nicotinamide Pheroid™ cream 117

B.6.4 Outcomes 118

B.7 FORMULATION OF A NICOTINAMIDE CONTAINING EMULGEL DURING THIS STUDY 118

B.7.1 Formula of nicotinamide emulgel 118

B.7.2 Procedure to prepare the nicotinamide emulgel 118

B.7.3 Procedure to prepare the nicotinamide Pheroid™ emulgel 119

B.7.4 Outcomes 119

B.8 CONCLUSION 119

B.9 REFERENCES 120

APPENDIX C: STABILITY TESTING OF SEMI-SOLID FORMULATIONS 121

C.1 INTRODUCTION 121

C.2 METHODS 122

C.2.1 Concentration assay 122

C.2.1.1 Chromatographic conditions 122

C.2.1.2 Standard preparation 123

C.2.1.3 Sample preparation 123

C.2.2 pH 124

C.2.3 Viscosity 124

C.2.4 Confocal laser scanning microscopy 125

C.2.5 Visual appearance assessment 125

C.2.6 Mass loss 125

C.3 RESULTS AND DISCUSSION 126

C.3.1 Concentration assay 126

C.3.1.1 Cream 126

C.3.1.2 Pheroid™ cream 127

C.3.1.3 Emulgel 128

C.3.1.4 Pheroid™ emulgel 129

C.3.2 pH 130

C.3.2.1 Cream 131

C.3.2.2 Pheroid™ cream 132

C.3.2.3 Emulgel 134

C.3.2.4 Pheroid™ emulgel 135

C.3.3 Viscosity 137

C.3.4 Confocal laser scanning microscopy 138

C.3.5 Visual appearance assessment 140

C.3.5.1 Cream 140

C.3.5.2 Pheroid™ cream 140

C.3.5.3 Emulgel 141

C.3.5.4 Pheroid™ emulgel 141

C.3.6 Mass loss 141

C.3.6.1 Cream 142

C.3.6.2 Pheroid™ cream 143

C.3.6.3 Emulgel 144

C.3.6.4 Pheroid™ emulgel 145

C.4 CONCLUSIONS 146

C.5 REFERENCES 147

APPENDIX D: FRANZ CELL DIFFUSION STUDIES 148

146D.1 INTRODUCTION 148

D.2 METHODS 148

D.2.1 Aqueous solubility 148

D.2.2 Log D 148

D.2.3 Skin preparation 149

D.2.4 Preparation of PBS (pH 7.4) 149

D.2.5 Diffusion studies 149

D.2.6 Skin diffusion 150

D.2.7 Membrane diffusion 150

D.2.8 Tape stripping 150

D.2.9	**HPLC analysis of nicotinamide**	**151**
D.2.10	**Data anlysis**	**151**
D.3	**RESULTS AND DISCUSSION**	**152**
D.3.1	**Aqueous solubility**	**152**
D.3.2	**Octanol-buffer partition coefficient (log D)**	**152**
D.3.3	**Membrane release studies**	**152**
D.3.4	**Diffusion studies**	**153**
D.3.5	**Tape stripping**	**158**
D.4	**CONCLUSION**	**160**
D.5	**PHOTOS OF APPARATUS USED DURING FRANZ CELL DIFFUSION STUDIES**	**162**
D.6	**REFERENCES**	**164**
APPENDIX E: INTERNATIONAL JOURNAL OF PHARMACEUTICS: GUIDE FOR AUTHORS		**166**
E.1	**SCOPE OF THE JOURNAL**	**166**
E.2	**EDITORIAL POLICY**	**166**
E.3	**SUBMISSION OF MANUSCRIPTS**	**167**
E.3.1	**Europe, Africa, Near East**	**167**
E.3.2	**The Americas, Australia and New Zealand**	**168**
E.3.3	**Japan and Far East**	**168**
E.4	**MANUSCRIPT TYPES**	**168**
E.4.1	**Full length manuscripts**	**168**
E.4.1.1	Title	168
E.4.1.2	List of authors	168
E.4.1.3	Affiliations	168
E.4.1.4	Abstract	168
E.4.1.5	Keywords	168
E.4.1.6	Corresponding author	169
E.4.1.7	Text	169

E.4.1.8	Nomenclature	169
E.4.1.9	Figure legends, table legends, footnotes	169
E.4.1.10	References	169
E.4.2	**Rapid communications**	**169**
E.4.3	**Notes**	**170**
E.4.4	**Reviews and mini-reviews**	**170**
E.5	**REFERENCES**	**170**
E.5.1	**Text citation**	**170**
E.5.2	***Reference list***	**170**
E.5.3	**Use of digital object identifier**	**172**
E.6	**ARTICLES IN SPECIAL ISSUES**	**172**
E.7	**FIGURES AND TABLES**	**172**
E.7.1	**Figures**	**172**
E.7.2	**Tables**	**173**
E.7.3	**DNA sequences and GENBANK accession numbers**	**173**
E.8	**COPYRIGHT GUIDELINES FOR AUTHORS**	**173**
E.9	**AUTHORS' RIGHTS**	**174**
E.10	**PROOFS, OFFPRINTS AND PAGE CHARGES**	**174**
E.11	**LANGUAGE SERVICES**	**175**
E.12	**FUNDING BODY AGREEMENTS AND POLICIES**	**175**
E.13	**AUTHOR ENQUIRIES**	**175**

LIST OF FIGURES

CHAPTER 2:

Figure 2.1: Development of acne 7

Figure 2.2: Different forms of acne 8

Figure 2.3: Overview of acne medications and mechanisms of action 11

Figure 2.4: Structural formula of nicotinamide 14

Figure 2.5: Anatomy of the skin 18

Figure 2.6: The different layers of the epidermis 20

Figure 2.7: Skin permeation routes 24

CHAPTER 3:

Figure 1: Average cumulative amount that penetrated through the skin as a function of time to illustrate the average flux (0-2 h) value in Pheroid™ cream, cream, Pheroid™ emulgel and emulgel 72

Figure 2: Box-plots and red lines of the flux values in Pheroid™ emulgel, Pheroid™ cream, emulgel and cream after application to teh skin to illustrate median and average flux 73

Figure 3: Comparison of the concentration (µg/ml) nicotinamide in the dermis an epidermis between the different formulations 74

APPENDIX A:

Figure A.1: Chromatogram of ingredients 79

Figure A.2: Linear regression curve of nicotinamide standards 82

Figure A.3: Linear regression curve of methyl paraben standards 83

Figure A.4: Linear regression curve of propyl paraben standards 84

Figure A.5: Linear regression curve of BHT standards 85

Figure A.6: Linear regression curve of tocopherol standards 86

APPENDIX C:

Figure C.1: Agilent® 1100 Series HPLC 124

Figure C.2: Mettler Toledo pH meter 124

Figure C.3: Brookfield Viscometer 125

Figure C.4: Shimadzu scale 126

Figure C.5:	The change in pH between months 0 and 6 for cream at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)	131
Figure C.6:	The change in pH between months 0 and 6 for Pheroid™ cream at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)	133
Figure C.7:	The change in pH between months 0 and 6 for emulgel at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)	134
Figure C.8:	The change in pH between months 0 and 6 for Pheroid™ emulgel at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)	136
Figure C.9:	The change in viscosity between month 0 and 6 for cream (1), Pheroid™ cream (2), emulgel (3) and Pheroid™ emulgel (4) at 25°C/60% RH	137
Figure C.10:	Confocal image of cream on month 0 (A) and month 6 (B), Pheroid™ cream on month 0 (C) and month 6 (D), emulgel on month 0 (E) and month 6 (F) and Pheroid™ emulgel on month 0 (G) and month 6 (H)	139
Figure C.11:	Change in colour of cream from month 0 (A) to month 6 at 25°C/60% RH (B), 30°C/60% RH (C) and 40°C/70% RH (D)	140
Figure C.12:	Change in colour of Pheroid™ cream from month 0 (A) to month 6 at 25°C/60% RH (B), 30°C/60% RH (C) and 40°C/70% RH (D)	140
Figure C.13:	Change in colour of emulgel from month 0 (A) to month 6 at 25°C/60% RH (B), 30°C/60% RH (C) and 40°C/70% RH (D)	141
Figure C.14:	Change in colour of Pheroid™ emulgel from month 0 (A) to month 6 at 25°C/60% RH (B), 30°C/60% RH (C) and 40°C/70% RH (D)	141
Figure C.15:	The change in mass between months 0 and 6 for cream at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)	142
Figure C.16:	The change in mass between months 0 and 6 for Pheroid™ cream at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)	143
Figure C.17:	The change in mass between months 0 and 6 for emulgel at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)	144
Figure C.18:	The change in mass between months 0 and 6 for Pheroid™ emulgel at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)	145
APPENDIX D:		
Figure D.1:	Box-plots and red lines of the flux values in Pheroid™ emulgel, Pheroid™ cream, emulgel and cream after application to the skin to illustrate median and average flux, respectively	155
Figure D.2:	Average cumulative amount that penetrated through the skin as a function of time to illustrate the average flux (0-2 h) value in cream	156
Figure D.3:	Average cumulative amount that penetrated through the skin as a function of time to illustrate the average flux (0-2 h) value in Pheroid™	156

cream

Figure D.4: Average cumulative amount that penetrated through the skin as a function of time to illustrate the average flux (0-2 h) value in emulgel 157

Figure D.5: Average cumulative amount that penetrated through the skin as a function of time to illustrate the average flux (0-2 h) value in Pheroid™ emulgel 157

Figure D.6: A) Vertical Franz diffusion cell with donor and receptor compartments and 162

B) Horseshoe clamps

Figure D.7: A) Dow Corning vacuum grease and 162

B) Assembled Franz diffusion cells

Figure D.8: A) Grant water bath and 162

B) Syringes used to withdraw samples from receptor compartments

Figure D.9: A) HPLC vials where samples were placed in for analyses and 163

B) Eppendorf Centrifuge system

Figure D.10: A) Milli-Q water purifying system and 163

B) Agilent® 1200 Series HPLC

Figure D.11: A) Reversed phase Phenomenex® Luna C18 (2) column and 163

B) Variomag® magnetic stirring plate

LIST OF TABLES

CHAPTER 2:

Table 2.1	A structured approach for the treatment of acne	9
Table 2.2	Survey of the most important systemic uses of nicotinamide	16

CHAPTER 3:

Table 1:	Indication of which formulations were stable during the concentration assay over the whole stability testing period	71

APPENDIX A:

Table A.1:	Peak area ratio values of nicotinamide standards	82
Table A.2:	Peak area ratio values of methyl paraben standards	83
Table A.3:	Peak area ratio values of propyl paraben standards	84
Table A.4:	Peak area ratio values of BHT standards	85
Table A.5:	Peak area ratio values of tocopherol standards	86
Table A.6:	Accuracy parameters of nicotinamide	88
Table A.7:	Accuracy parameters of methyl paraben	89
Table A.8:	Accuracy parameters of propyl paraben	90
Table A.9:	Accuracy parameters of BHT	91
Table A.10:	Accuracy parameters of tocopherol	92
Table A.11:	Intraday precision parameters of nicotinamide	93
Table A.12:	Intraday precision parameters of methyl paraben	94
Table A.13:	Intraday precision parameters of propyl paraben	95
Table A.14:	Intraday precision parameters of BHT	96
Table A.15:	Intraday precision parameters of tocopherol	97
Table A.16:	Interday precision parameters of nicotinamide	98
Table A.17:	Interday precision parameters of methyl paraben	98
Table A.18:	Interday precision parameters of propyl paraben	99
Table A.19:	Interday precision parameters of BHT	99
Table A.20:	Interday precision parameters of tocopherol	100

Table A.21:	Sample stability parameters for nicotinamide	101
Table A.22:	Sample stability parameters for methyl paraben	102
Table A.23:	Sample stability parameters for propyl paraben	103
Table A.24:	Sample stability parameters for BHT	104
Table A.25:	Sample stability parameters for tocopherol	105
Table A.26:	Variations in response (% RSD) of the detection system regarding peak area and retention time of nicotinamide	106
Table A.27:	Variations in response (% RSD) of the detection system regarding peak area and retention time of methyl paraben	107
Table A.28:	Variations in response (% RSD) of the detection system regarding peak area and retention time of propylparaben	107
Table A.29:	Variations in response (% RSD) of the detection system regarding peak area and retention time of BHT	108
Table A.30:	Variations in response (% RSD) of the detection system regarding peak area and retention time of tocopherol	109
APPENDIX B:		
Table B.1:	Ingredients used in the selected formulations	116
Table B.2:	Formula of cream	117
Table B.3:	Formula of emulgel	118
APPENDIX C:		
Table C.1:	Percentage of each active ingredient in cream at the different conditions after each time interval	126
Table C.2:	Percentage of each active ingredient in Pheroid™ cream at the different condition after each time interval	127
Table C.3:	Percentage of each active ingredient in emulgel at the different conditions after each time interval	128
Table C.4:	Percentage of each active ingredient in Pheroid™ emulgel at the different conditions after each time interval	129
Table C.5:	pH of cream at the different conditions after each time interval	131
Table C.6:	pH of Pheroid™ cream at different conditions after each time interval	132
Table C.7:	pH of emulgel at different conditions after each time interval	134
Table C.8:	pH of Pheroid™ emulgel at different conditions after each time interval	135

Table C.9: Viscosity of cream, Pheroid^{TM} cream, emulgel and Pheroid^{TM} emulgel at 25°C/60% RH after each time interval 137

Table C.10: Mass of cream at different conditions after each time interval 142

Table C.11: Mass of Pheroid^{TM} cream at different conditions after each time interval 143

Table C.12: Mass of emulgel at different conditions after each time interval 144

Table C.13: Mass of Pheroid^{TM} emulgel at different conditions after each time interval 145

APPENDIX D:

Table D.1: Data obtained from membrane release studies 152

Table D.2: Data obtained from skin diffusion studies 153

Table D.3: Data obtained from tape stripping studies 158

ACKNOWLEDGEMENTS

I give all my praise to the Lord my God. Without the talents, opportunities and strength He gave me, completion of this study wouldn't have been possible.

I would like to thank the following people for their guidance, love, understanding and motivation. Without them, this dissertation would never have seen the light:

- Jurie, my husband and love of my life. Thank you for your support, love and motivation. I love you very much!

- My parents. Thank you for your prayers, understanding, love and support, not just through this study, but throughout my entire life. I couldn't have asked for better parents.

- My sisters, Marelie and Colette. Thank you for your laughter and love for life. I appreciate all your messages and phone calls. I can't imagine finishing my studies without your support.

- My friends with whom I have spent fun as well as tough times. Gerda, thank you for the wonderful past two years of friendship, support, hard work and laughter! It's a blessing to have you as a friend. Janus & Corli, you'll never know how much I appreciate you. Thank you for your friendship and love. Jannes, Marisa, Stephan, Corne and Pierre, thank you for the visits at the office, the motivational speeches, and fun times we had. You are all indeed friends I'll treasure forever!

- Prof Jeanetta du Plessis. Thank you for the opportunity I was given to undertake this study. Thank you for your guidance, help and support not only with this study, but also for the opportunity I had to be exposed to world class research.

- Prof Jan du Preez. Thank you for the much needed assistance with my HPLC method and analyses. Thank you that you were always willing to help.

- Dr Maides Malan. Thank you for all your help, assistance and knowledge you shared with me. It was a great privilige to have had you as part of my study.

- Dr Minja Gerber. Thank you for all your help and guidance. Without your knowledge and support this dissertation would never have seen the light.

- Ms. Hester de Beer. Thank you for your help with the administrative part of this study. Thank you for always helping and listening!

- Liezl-Marie Nieuwoudt. Thank you for your help with the formulation of the Pheroid™ containing creams and emulgels.

- Prof Faans Steyn. Thank you for the statistical analysis of my data.

- The National Research Foundation (NRF) and the Unit for drug Research and Development, North-West University, Potchefstroom for the funding of this project.

- My colleagues. Arina, Reinette, Tizane, Gerda, Sarita, Heidi, Juanita, Monique, Nadia and Kristen. Thank you for the sacrifices and support, laughing and talking in the office and your precious friendship.

ABSTRACT

Acne is an extremely common condition, affecting almost 80% of adolescents and young adults. It is an inflammatory disease, characterised by comedones, papules, pustules and sometimes cysts. Factors causing acne include enhanced sebum excretion, hypercornification of the sebaceous duct, ductal coloniazation with *Propionibacterium acnes* and production of inflammation (Gollnick & Cunliffe, 2003:1).

Because of the widespread use of topically applied antimicrobial agents in the treatment of inflammatory acne, resistance of disease-related micro-organisms developed. Therefore new strategies for the treatment of moderate inflammatory acne are necessary. Nicotinamide is a new approach to topical treatment of moderate inflammatory acne without the development of resistant micro-organisms (Otte *et al.*, 2005:257).

Using the skin as an alternative route for the administration of nicotinamide for the treatment of acne, may be beneficial. When nicotinamide permeates through the skin, it is directly delivered to the dermis, the place where action is needed and better results can thus be expected after the treatment has started. Another benefit is that smaller amounts of the drug are absorbed systemically with decreased adverse reactions. Unfortunately, using the skin as an alternative route for administering drugs (transdermal drug delivery), has numerous limitations. One of these limitations is the barrier function of the skin (Naik *et al.*, 2000:319). Because of the skin's outstanding ability to protect the body against unwanted substances from its surroundings, it is necessary to use methods to enhance drug penetration through the skin.

A new technology, named Pheroid™ technology, was used in this study to enhance penetration through the skin. This technology is based on the use of vesicular structures with no phospholipids or cholesterol to enhance penetration (Grobler *et al.*, 2008:283). The aim of this study was to formulate four different semi-solid formulations with nicotinamide as the active ingredient, and to determine which of the formulations deliver nicotinamide best to the target site. Stability tests over a six months period were also performed on the different formulations.

A 3% nicotinamide cream, with and without Pheroid™ vesicles, and a 3% nicotinamide gel, with and without Pheroid™ vesicles, were formulated.

The aqueous solubility and log D partition coefficient of nicotinamide were determined. A log D value of -0.3, indicated that the compound was unfavourable to penetrate the skin (Roberts & Walters, 1998:34), whereas the aqueous solubility of 212.92 mg/ml in PBS (pH 7.4) at a temperature of 32 °C, indicated favourable penetration (Naik *et al.*, 2000:321).

Franz cell diffusion studies were performed over a 12 hour period, followed by tape stripping experiments to determine which semi-solid formulation delivered nicotinamide best to the target site. The results of the different formulations were compared. The emulgel had the highest percentage nicotinamide (0.069%) that diffused transdermally after 12 hours. It also had the highest flux value of 3.166 µg/ml ± 0.079. The Pheroid™ emulgel released the highest percentage nicotinamide (2.938%) after 6 hours. It also had the highest concentration nicotinamide of 6.16 µg/ml in the epidermis. In the dermis nicotinamide in the Pheroid™ cream had the highest concentration of 16.047 µg/ml.

To determine the stability of the different semi-solid formulations, the formulated products were stored at 25°C + 60% RH, 30°C + 60% RH and 40°C + 70% RH. HPLC analysis was used to determine the concentrations of the ingredients in all the formulated products. Other stability tests included appearance, pH, viscosity, mass loss and confocal laser scanning microscopy (CLSM).

Unfortunately a change in colour, viscosity and concentration of the ingredients in the formulations, indicated that the products were unstable over the six months period.

Keywords: Nicotinamide, acne, transdermal diffusion, formulation, stability testing

References

GOLLNICK, H. & CUNLIFFE, W. 2003. Management of acne – A report from a global alliance to improve outcomes in acne. *Journal of the American academy of dermatology,* 2003(49):1-38.

GROBLER, A., KOTZE, A. & DU PLESSIS, J. 2008. The design of a skin-friendly carrier for cosmetic compounds using Pheroid™ technology. (*In* Wiechers, J. *ed.* Science and applications of skin delivery systems. Wheaton, IL: Allured Publishing. p. 283-311.)

NAIK, A., KALIA, Y.N. & GUY, R.H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical science & technology today,* 3(9):318-326.

OTTE, N., BORELLI, C. & KORTING, H.C. 2005. Nicotinamide – biologic action of an emerging cosmetic ingredient. *International journal of cosmetic science,* 2005(27):255-261.

ROBERTS, M.S. & WALTERS, K.A. 1998. The relationship between structure and barrier function of skin. (*In* Roberts, M.S. & Walters, K.A. *eds.* Dermal absorption and toxicity assessment. New York: Marcel Dekker. p. 1-42.)

UITTREKSEL

Aknee is 'n baie algemene veltoestand en beïnvloed sowat 80% adolessente en jong volwassenes. Dit is 'n inflammatoriese siekte wat gepaard gaan met komedo's, puisies en soms ook siste. Faktore wat aknee veroorsaak sluit verhoogde sebum sekresie, verharding van die vetkanaal, kolonisasie van *Propionibacterium acnes* en produksie van inflammasie in (Gollnick & Cunliffe, 2003:1).

As gevolg van die algemene gebruik van topikale antimikrobiese produkte in die behandeling van inflammatoriese aknee, ontwikkel mikro-organismes weerstand teen die behandeling. Daarom is nuwe strategieë vir die behandeling van matige inflammatoriese aknee noodsaaklik. Nikotienamied is 'n nuwe benadering in die topikale behandeling van matige inflammatoriese aknee, sonder die moontlikheid dat mikro-organismes weerstandig kan raak teen die behandeling (Otte *et al.*, 2005:257).

Deur die vel as alternatiewe roete vir die toediening van nikotienamied vir die behandeling van aknee te gebruik, hou verskeie voordele in. Wanneer nikotienamied die vel deurdring, word dit direk in die dermis waar die behandeling nodig is, afgelewer. Daarom kan beter resultate verwag word nadat die behandeling begin het. Nog 'n voordeel is dat kleiner konsentrasies geneesmiddel die sistemiese absorpsie bereik, wat dus newe-effekte sal verminder. Ongelukkig het transdermale aflewering van geneesmiddels verskeie beperkings. Een daarvan is die beskermende funksie van die vel (Naik *et al.*, 2000:319). Omdat die vel die liggaam uitstekend beskerm teen ongewenste substansies in die omgewing, is dit nodig dat metodes gebruik moet word om transdermale aflewering van geneesmiddels te bevorder.

'n Nuwe tegnologie nl. Pheroid™ tegnologie, is in hierdie studie gebruik om transdermale aflewering te bevorder. Hierdie tegnologie is gebaseer op blaasagtige strukture met geen fosfolipiede of cholesterol om transdermale aflewering te bevorder nie (Grobler *et al.*, 2008:283). Die doel van die studie was om vier verskillende semi-soliede formulerings met nikotienamied as die aktiewe bestanddeel te vervaardig, en om te bepaal watter formulering nikotienamied die beste in die teikenarea aflewer. Stabiliteitstoetse op die verskillende formulerings is oor 'n tydperk van ses maande uitgevoer.

'n 3% Nikotienamied room, met - en sonder Pheroid™ tegnologie, en 'n 3% emulgel, met - en sonder Pheroid™ tegnologie, is geformuleer.

Die wateroplosbaarheid en log D koëffisiënt van nikotienamied is bepaal. 'n Log D waarde van -0.3, het gewys dat transdermal diffusie ongunstig is vir die verbinding (Roberts & Walters,

1998:34). Aan die ander kant het nikotienamied se wateroplosbaarheid van 212.92 mg/ml in PBS (pH 7.4) by 'n temperatuur van 32 °C, aangedui dat transdermale diffusie gunstig is (Naik *et al.*, 2000:321).

Om te bepaal watter semi-soliede formulering nikotienamied die beste in die teikenarea aflewer, is Franz-sel diffusiestudies oor 'n 12-uur periode uitgevoer, gevolg deur "tape stripping" eksperimente. Die resultate van die verskillende formulerings is vergelyk. Die emulgel het die hoogste persentasie nikotienamied (0.069%) na 12 ure gelewer. Dit het ook die hoogste fluksie waarde van 3.166 µg/ml ± 0.079 getoon. Die Pheroid™ emulgel het die hoogste persentasie nikotienamied (2.938%) na 6 ure vrygestel. Dit het ook die hoogste konsentrasie nikotienamied (6.16 µg/ml) in die epidermis getoon. In die dermis is nikotienamied in die Pheroid™ room die beste afgelewer met 'n konsentrasie van 16.047 µg/ml.

Om die stabiliteit van die verskillende semi-soliede formulerings te bepaal, is die geformuleerde produkte by 25°C + 60% RH, 30°C + 60% RH en 40°C + 70% RH gestoor. HPLC analise is gebruik om die konsentrasies van die bestanddele in die formulerings te bepaal. Ander stabiliteitstoetse het voorkomsbepaling, pH, viskositeit, massaverlies, en konfokaal laser mikroskopie ingesluit.

Ongelukkig het 'n verandering in kleur, viskositeit en konsentrasie van die bestanddele, onstabiliteit oor die ses maande aangedui.

Sleutelwoorde: Nikotienamied, aknee, transdermale aflewering, formulering, stabiliteitstoetse

Bronnelys

GOLLNICK, H. & CUNLIFFE, W. 2003. Management of acne – A report from a global alliance to improve outcomes in acne. *Journal of the American academy of dermatology,* 2003(49):1-38.

GROBLER, A., KOTZE, A. & DU PLESSIS, J. 2008. The design of a skin-friendly carrier for cosmetic compounds using Pheroid™ technology. (*In* Wiechers, J. *ed.* Science and applications of skin delivery systems. Wheaton, IL: Allured Publishing. p. 283-311.)

NAIK, A., KALIA, Y.N. & GUY, R.H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical science & technology today,* 3(9):318-326.

OTTE, N., BORELLI, C. & KORTING, H.C. 2005. Nicotinamide – biologic action of an emerging cosmetic ingredient. *International journal of cosmetic science,* 2005(27):255-261.

ROBERTS, M.S. & WALTERS, K.A. 1998. The relationship between structure and barrier function of skin. (*In* Roberts, M.S. & Walters, K.A. *eds.* Dermal absorption and toxicity assessment. New York: Marcel Dekker. p. 1-42.)

FOREWORD

In this study we aimed at investigating the transdermal delivery of nicotinamide - a relatively new drug in the treatment of acne. Nicotinamide within a cream and emulgel, with and without the use of Pheroid^{TM} technology, was formulated. The formulations were stored under different conditions, and stability tests were performed over a six months period.

This dissertation is presented in the so-called article format, which includes introductory chapters and a full length article for publication in a pharmaceutical journal. The data procured during the studies are attached in the appendices. The article in this dissertation is to be submitted for publication in The International Journal of Pharmaceutics of which the complete guide for authors is included in the Appendix E.

The past two years, while doing my Masters degree study, have definitely been part of the best years of my life. I've gained countless experience, not only in my field of study, but also in other aspects of my life. I've learned self-discipline, endurance, patience and an absolute love for my work. I'm looking forward to a future filled with opportunities, challenges and achievements!

CHAPTER 1

INTRODUCTION AND STATEMENT OF THE PROBLEM

Acne vulgaris, more commonly known as acne, is the most common skin disease treated by physicians (Krowchuck, 2000:841). According to Gollnick and Cunliffe (2003:1) acne is affecting almost 80% of adolescents and young adults aged 11 to 30. Acne is a chronic condition that may last for years and cause emotional distress and permanent scarring (Krowchuck, 2000:841). It is characterised by comedones, papules, pustules and sometimes cysts, and commonly affects the face, chest and upper back (Long, 2002:48). The four etiological factors of acne are enhanced sebum excretion, hypercornification of the sebaceous duct, ductal colonization with *Propionibacterium acnes* and production of inflammation (Brajac, *et al.*, 2004:21).

The assessment of acne depends on the severity thereof and can be divided into four main groups, namely mild acne which requires topical therapy, moderate acne which requires topical and oral antibiotic therapy, severe acne which requires topical and oral therapy with regular review and very severe acne which requires urgent referral to a specialist for prescription of large doses of antibiotics, steroids or oral isotretinoin. The type of treatment needed, thus depends on the severity of the case (Shaw & Kennedy, 2007:387):

The widespread use of antimicrobial drugs used for the treatment of acne led to resistance of micro-organisms and therefore new treatment strategies had to be investigated. Nicotinamide can be used for the treatment of moderate inflammatory acne, without development of resistant micro-organisms (Otte *et al.*, 2005:257). Nicotinamide is a form of vitamin B_3, and has shown great and exciting benefits when applied to the skin (Lupo, 2001:468). It essentially acts as an antioxidant, thus increasingly gaining interest in the prevention and treatment of skin diseases like acne (Otte *et al.*, 2005:255).

Using the skin as an alternative route for the administration of drugs has become very popular over the last few decades. Although most drugs are administered orally, for some drugs this route of administration is not always possible. Therefore administration through the skin, also known as transdermal drug delivery, is an excellent alternative, with several advantages (Bouwstra *et al.*, 2003:2). Unfortunately, using the skin as an alternative route for administering drugs also has numerous limitations. One of these limitations is the barrier function of the skin (Naik *et al.*, 2000:319).

Although the stratum corneum gives the body outstanding protection against unwanted substances from its surroundings, it is possible for drugs to be administered transdermally.

Three possible pathways for transport of drugs through the skin exist, namely intercellular diffusion through the lipid lamellae, transcellular diffusion through the keratinocytes and lipid lamellae and diffusion through hair follicles and sweat ducts (Ho, 2003:50). There are however several factors that may affect permeation of drugs through the skin and a few of these factors are skin age, skin condition, skin site, skin metabolism, skin hydration, temperature, pH and the presence of penetration enhancers (Dayan, 2007:31).

Penetration enhancement can be divided into chemical- and physical penetration enhancers. Chemical penetration enhancement involves the use of chemicals that can reversibly compromise the skin's barrier function and allow the entry of otherwise poorly penetrating molecules, into the membrane and finally to the systemic circulation (Naik *et al.*, 2000:321). Physical penetration enhancement relies on providing a drug reservoir of drug on the skin's surface, from which the required levels of drug delivery can be achieved (Thomas & Finnin, 2004:699). A new technology named Pheroid ™ technology is also a method to enhance penetration across the skin. This technology uses vesicular structures with no phospholipds or cholesterol to enhance penetration (Grobler *et al.*, 2008:283).

The aims and objectives of this study include the following:

- Formulation of a cream and gel with nicotinamide as the active ingredient used for the treatment of acne;
- Formulation of a cream and gel containing Pheroid™ vesicles with nicotinamide as the active ingredient used for the treatment of acne;
- Stability tests on the different formulations;
- Developing and validating a HPLC (High Performance Liquid Chromatograph) method to quantitatively determine concentrations of the different ingredients in the formulations;
- Determination of the aqueous solubility and partition coefficient of nicotinamide;
- Determining whether nicotinamide diffuses through the skin or into the target site of delivery (tape stripping) when the different formulations are applied to the skin.

References

BOUWSTRA, J.A., HONEYWELL-NGUYEN, P.L., GOORIS, G.S. & PONEC, M. 2003. Structure of the skin barrier and its modulation by vesicular formulations. *Progress in Lipid Research,* 42(1):1-36.

BRAJAC, I., ZULLE-BILIC, L., TKALCIC, K.L. & GRUBER, F. 2004. Acne vulgaris: myths and misconceptions among patients and family physicians. *Patient education and counseling,* 2004(54):21-25.

DAYAN, N. 2007. Pathways for Skin Penetration. (*In* Kozlowski, A. Biotechnology in Cosmetics: Concepts, Tools and Techniques. USA : Allured Publishing Corporation. p. 29-42.)

GOLLNICK, H. & CUNLIFFE, W. 2003. Management of acne – A report from a global alliance to improve outcomes in acne. *Journal of the American academy of dermatology,* 2003(49):1-38.

GROBLER, A., KOTZE, A. & DU PLESSIS, J. 2008. The design of a skin-friendly carrier for cosmetic compounds using Pheroid™ technology. (*In* Wiechers, J., *ed.* Science and applications of skin delivery systems. Wheaton, IL: Allured Publishing. p. 283-311.)

HO, C.K. 2003. Probabilistic modeling of percutaneous absorption for risk-based exposure assessments and transdermal drug delivery. *Statistical Methodology,* 1(2004): 47-69.

KROWCHUK, D.P. 2000. Managing acne in adolescents. *Pediatric clinics of North America,* 47(4):841-857.

LONG, C.C. 2002. Common skin disorders and their topical treatment. (*In* WALTER, K.A. *ed.* Dermatological and Transdermal formulation. Vol.119. New York : Marcel Dekker. p.41-60.)

LUPO, M.P. 2001. Antioxidants and Vitamins in cosmetics. *Clinics in dermatology,* 2001(19):467-473.

NAIK, A., KALIA, Y.N. & GUY, R.H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical Science and Technology Today,* 3(9): 318-326.

OTTE, N., BORELLI, C. & KORTING, H.C. 2005. Nicotinamide – biologic action of an emerging cosmetic ingredient. *International Journal of cosmetic science,* 2005(27):255-261.

SHAW, L. & KENNEDY, C. 2007. The treatment of acne. *Pediatrics and child health,* 17(10):385-389.

THOMAS, B.J. & FINNIN, B.C. 2004. The transdermal revolution. *Drug discovery today*, 9(16):697-703.

CHAPTER 2

TRANSDERMAL DELIVERY OF NICOTINAMIDE FOR THE TREATMENT OF ACNE

2.1 Introduction

Acne vulgaris, more commonly known as acne, is the most common skin disease treated by physicians and an estimated 17 million people in the United States are affected. Acne is a chronic condition that may last for years and cause emotional distress and permanent scarring (Krowchuk, 2000:841a). The most common form of acne is acne vulgaris. It is common in teenagers, but by their mid-20's the majority of cases have resolved, although a few people still require treatment in their 30's and 40's. Treatment aims to reduce the bacterial population and remove the keratinised layer blocking the follicles (Sweetman, 2008).

One of the major research topics in the pharmaceutical field is the controlled delivery of drugs. Although most drugs are administered orally, this route of administration is not possible for a variety of drugs. This is due to the high metabolic activity in the gastro-intestinal tract and in the liver (Bouwstra *et al.*, 2003:2). The potential of using the skin, as an alternative route for administering systemically active drugs, has attracted considerable interest in recent years. However, the stratum corneum, which is responsible for the skin's impermeability, is well known for its function as a protective barrier against the loss of physiologically essential substances and to the diffusion of potentially toxic chemicals from the external environment into the body. Generally, the stratum corneum is only permeable to small, lipophilic molecules. Thus, in order to expand the range of molecules available for transdermal drug delivery (TDD), it is necessary to employ enhancement technologies, such as chemical penetration enhancers, iontophoresis, sonophoresis and Pheroid™ Technology (Turner & Nonato, 1997:1).

2.2 Acne

According to Gollnick and Cunliffe (2003:1), acne is an extremely common condition, affecting almost 80% of adolescents and young adults aged 11 to 30. Acne is a disorder of the pilosebaceous follicle and common features include increased sebum production, follicular keratinisation, colonisation by *Propionibacterium acnes*, and localised inflammation. Barry (1983:38) defines acne as a chronic inflammatory disease, characterised by comedones, papules, pustules and sometimes cysts, involving the sebaceous glands and follicles. Several variants of acne are recognised, including infantile acne, which occurs on the face during the first few months and usually settles spontaneously, and occupational acne, resulting from

exposure to oil, coal tar, chlorinated hydrocarbons or insecticides. Acne vulgaris commonly affects the face, chest and upper back, and usually presents during puberty (Long, 2002:48).

2.2.1 FACTORS CAUSING ACNE

According to Gollnick and Cunliffe (2003:2), the most notable pathophysiological factors that influence the development of acne are an increase in sebum production, abnormal follicular desquamation, bacterial proliferation and inflammation.

2.2.1.1 INCREASE IN SEBUM PRODUCTION

According to Wertz and Michniak (2000:52) there is a positive correlation between the occurrence and severity of acne and the sebum secretion rate. Acne formation requires the presence of relatively large sebaceous follicles, coupled with increased sebum release by enlarged sebaceous glands and insignificant hair shafts. In individuals with acne, the process of keratinisation fails to proceed normally just below the surface of the skin. This leads to the production of "sticky" cells, which occlude the follicle and produce micro-comedones invisible to the naked eye. With time, these may enlarge into closed comedones, which subsequently generate open comedones (Landow, 1997:94).

2.2.1.2 ABNORMAL FOLLICULAR DESQUAMATION

In the normal follicle, the keratinocytes are shed as single cells to the lumen and are then excreted. In acne, keratinocytes hyperproliferate and are not shed as they would normally do. They become densely packed along with monofilaments and lipid droplets (Gollnick & Cunliffe, 2003:3).

2.2.1.3 BACTERIAL PROLIFERATION

One of the causes of acne is the colonization of the comedo by *Propionibacterium acnes* (Cordain, 2005:84). *Propionibacterium acnes*, a gram-positive bacterium, proliferates within the obstructed hair follicle and may break down the lipid esters of sebum to liberate potentially irritating fatty acids (Long, 2002:49).

2.2.1.4 INFLAMMATION

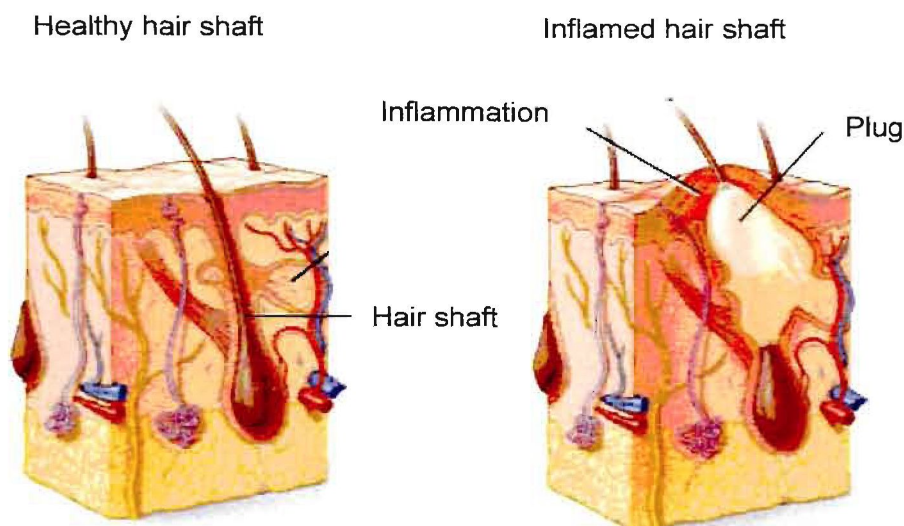
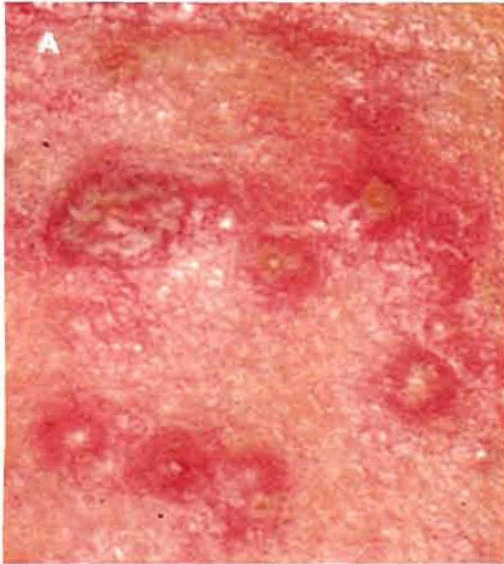


Figure 2.1: Development of acne (Adapted from Skinspots, 2009)

The sebaceous glands and pilosebaceous canal enlarge under the influence of androgen and some hair follicles, become sebaceous follicles. In some of these follicles in patients with acne, keratinised cells accumulate within the infundibulum of the pilosebaceous canal, that part of the follicle above the sebaceous ducts. As this impaction increases, either the follicles remain intact and develop into mature comedones, or the epithelial wall disrupts. The resulting tissue reactions produce the inflammatory lesions of acne (Knutson, 1974:288). Figure 2.1 illustrates how acne develops. Patients often seek treatment for acne once inflammatory lesions occur. These lesions may be macules, papules or nodules. An inflamed area may progress from one type of lesion to another. In severe cases, scarring may occur (Gollnick & Cunliffe, 2003:4).

2.2.2 PHYSICAL PROPERTIES OF ACNE

Acne vulgaris usually resents during puberty and commonly affects the face, chest and upper back. The clinical features include an increased rate of sebum secretion, comedones, papules and pustules. Severe acne may be aggravated by atrophic or nodular keloid-type scars, or by the formation of chronic nodules and cysts (Long, 2002:48). Figure 2.2 shows the different forms of acne.



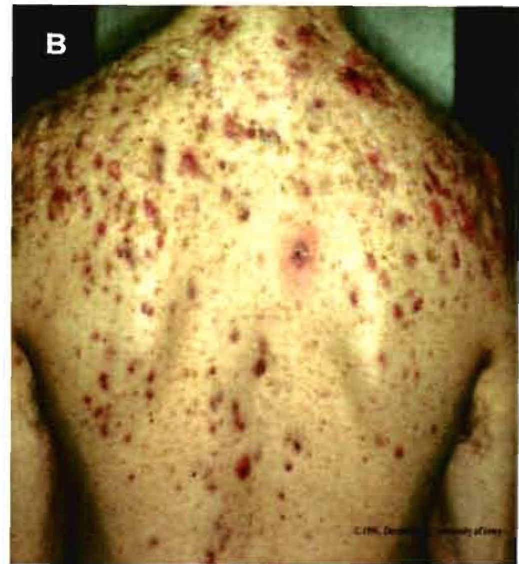


Figure 2.2: Different forms of acne:
A) Comedones and pustules on the face (Health tips review, 2009)
B) Scarring on the back (American Academy of Dermatology, 2009)

2.2.3 TREATMENT OF ACNE

According to Long (2002:49), the aims for treatment of acne are to reduce the bacterial population of the hair follicles, to encourage the shedding of comedones, to reduce the rate of sebum production and to reduce the degree of inflammation.

According to Shaw and Kennedy (2007:387) a structured approach for the treatment of acne is summarised in Table 2.1.

Table 2.1: A structured approach for the treatment of acne

Mild acne	Moderate acne	Severe acne
Topical treatment Start with a topical comedolytic (retinoid, benzoyl peroxide or azelaic acid) If inflammatory lesions are a feature, add an anti-inflammatory	Oral plus topical treatment indicated Topical comedolytic treatment Oral antibiotics (tetracycline if over 12 years, erythromycin if younger)	Oral plus topical treatment indicated Topical comedolytic treatment Oral antibiotic (tetracycline if over 12 years, erythromycin if younger) PLUS hormone therapy in suitable girls Isotretinoin if the above gives no significant improvement in 3 to 4 months Have a low threshold for starting isotretinoin early with nodulo-cystic acne
NON-RESPONDERS OR RELAPSE ON TREATMENT: Check compliance and manage side-effects Try alternative or combined topical treatments Consider treatment as for moderate acne if there is sufficient patient motivation	NON-RESPONDERS OR RELAPSE ON TREATMENT: Check compliance and manage side-effects Try alternative or combined topical treatments Change oral antibiotics Gram negative folliculitis and consider oral trimethoprim treatment should be considered Consider hormone therapy in girls Consider treatment as for severe acne	

2.2.3.1 TOPICAL TREATMENTS

Topical treatments aim at preventing the formation of new comedones and can take several months to deliver maximal benefit (Shaw & Kennedy, 2007:386).

2.2.3.1.1 Topical antibiotics

Topical erythromycin, clindamycin and tetracycline are all effective in the treatment of acne. These antibiotics reduce the population of *P. acnes* and may have a separate anti-inflammatory action. The advantage of topical antibiotics is the reduction in the risk of potential systemic side effects (Long, 2002:49). According to Shaw and Kennedy (2007:387), erythromycin and clindamycin are the most common, but there is a problem with increasing antibiotic resistance. Topical antibiotics should not usually be used as monotherapy. If monotherapy is necessary, it

should be maintained for only short periods. Combination with benzoyl peroxide will also reduce the risk of *P. acnes* resistance (Krautheim & Gollnick, 2004:401).

2.2.3.1.2 Topical retinoids

According to Gollnick and Cunliffe (2003:5) topical retinoids should be used as first-line therapy for mild to moderate inflammatory acne. Gollnick and Cunliffe (2003:6) also indicate that topical retinoids are the preferred agents for maintenance therapy, because the goal of acne treatment is to minimise antibiotic use. Topical retinoids act by decreasing epidermal proliferation and reducing the abnormal keratinisation process in the hair follicle. This prevents that new comedones are being formed and softens and removes existing comedones. There is also a reduction in the level of *P. acnes* within the hair follicle (Long, 2002:49). Examples of topical retinoids include tretinoin, isotretinoin and adapalene.

According to Krautheim and Gollnick (2004:400), tretinoin has shown to significantly reduce non-inflammatory and inflammatory acne lesions, and its main side effects are skin irritation. Topically applied isotretinoin has similar effectiveness as tretinoin in clearing acne lesions, although it causes less skin irritation (Krautheim & Gollnick, 2004:400). Adapalene is a third-generation naphtoic acid derivative of reninoic acid and has a significantly better cutaneous tolerance than tretinoin (Krautheim & Gollnick, 2004:400).

2.2.3.1.3 Azelaic acid

Azelaic acid is a dicarboxylic acid available in a 20% cream formulation. It is bacteriostatic against *P. acnes* and *Staphylococcus epidermidis* and normalises keratinisation. Although it would seem a logical choice for patients with mild to moderate inflammatory and comedonal acne, experience with azelaic acid is limited, and therefore its exact role in acne treatment remains to be defined (Krowchuk, 2000b:214).

2.2.3.1.4 Benzoyl peroxide

According to White (1999:311), benzoyl peroxide remains one of the best topical antimicrobial agents for acne. Its ability to kill *P. acnes* is unsurpassed, resistance cannot develop, and it is very inexpensive. Benzoyl peroxide has strong antimicrobial, slight anti-inflammatory and anti-comedogenic effects. Combination therapies of benzoyl peroxide with topical antibiotics have shown to be more effective and better tolerated than benzoyl peroxide alone (Krautheim & Gollnick, 2004:402).

2.2.3.2 ORAL TREATMENTS

Various oral products can be used for the treatment of acne and include antibiotics, isotretinoin and hormonal therapy. An overview of acne medications and mechanisms of action are shown in Figure 2.3 (Gollnick & Cunliffe, 2003:12).

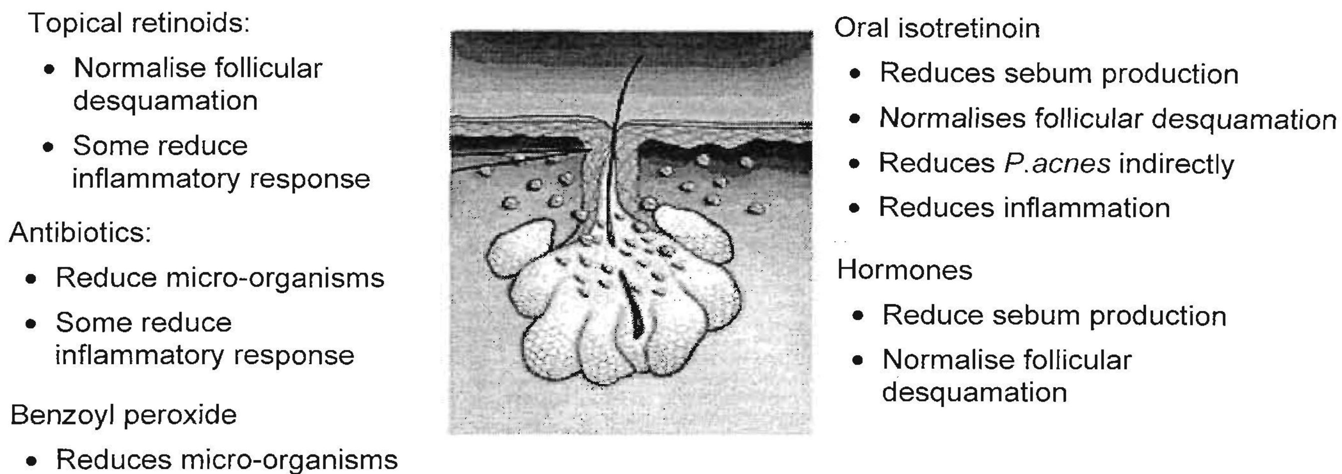


Figure 2.3: Overview of acne medications and mechanisms of action (Adapted from Gollnick *et al.*, 2003:12)

2.2.3.2.1 Antibiotics

For moderate to severe, or for mild but treatment-resistant acne, oral antibiotics should be given in conjunction with topical therapy. The choice of oral antibiotics is often based on cost, degree of sun exposure, and whether the patient prefers to take the medication on a full or empty stomach (White, 1999:316). Due to lack of efficacy or safety considerations, cephalosporins, fluoroquinolones, aminoglycosides, chloramphenicol, sulphonamides and gyrase inhibitors should not routinely be used for the systemic treatment of acne (Gollnick & Cunliffe, 2003:15). The following antibiotics can however be used for the treatment of acne:

- Macrolides: Erythromycin, clindamycin and azithromycin are 3 members of the macrolide family that are used for treating acne (Webster & Graber, 2008:183). The increasing antimicrobial resistance to erythromycin and other macrolides, limit the use of these agents to cases where tetracyclines are contra-indicated or not tolerated (Gollnick & Cunliffe, 2003:16);
- Tetracyclines: Tetracycline continues to be an excellent first-line choice for moderate acne. Sun sensitivity is a concern during the summer months, although it is less than that associated with doxycycline (White, 1999:316). The tetracycline family is extremely

useful in acne because they have multiple modes of action, functioning as antibiotics that reduce bacterial populations, and as anti-inflammatory drugs that attack acne from a second front (Webster & Graber, 2008:184);

- Doxycycline: Doxycycline is an excellent alternative to tetracycline for moderate to severe acne. One of doxycycline's significant side-effects, is photosensitivity and appears clinically as redness of the dorsum of the hands, fingers and nose (White, 1999:317); and
- Minocycline: Minocycline remains the premier oral antibiotic for treatment-resistant acne.

2.2.3.2.2 Isotretinoin

Isotretinoin, an oral retinoid, is indicated for severe nodular acne and moderate or severe acne that is unresponsive to topical therapy (Gollnick *et al.*, 2003:26). According to White (1999:320), the indications for isotretinoin include severe scarring acne, acne that is resistant to oral antibiotics and acne present for many years that quickly relapses when oral antibiotic therapy is discontinued.

Isotretinoin reduces sebum production, affects follicular epithelial differentiation and has anti-inflammatory effects. It is very expensive and has significant side-effects. It is only licensed for use under the supervision of a consultant dermatologist. Skin- and mucous membrane dryness occur in almost all who are treated. Skin lesions may also worsen in the first week or two of treatment, and patients should be informed regarding it (Shaw & Kennedy, 2007:388).

2.2.3.2.3 Hormonal therapy

For many years physicians have known that oral contraceptives can reduce the incidence of acne and help diminish acne lesions (Huber & Walch, 2006:23). Women who are not responding to conventional therapy, or those who have clinical indication of androgen excess, may be evaluated for "hormone-related" acne (White, 1999:325). Hormonal therapy is an excellent option for women, especially if oral contraception is also desirable. In some circumstances hormonal therapy is used as an alternative to repeated courses of isotretinoin (Gollnick & Cunliffe, 2003:20). According to Redmond (1998:30), the initial inciting event in the development of acne, is the action of androgens on the sebaceous gland to stimulate increased sebum production. This is a normal event at the onset of puberty, when androgen production in the ovaries and adrenal glands increases. This effect can become more pronounced as androgen levels rise, and in susceptible individuals, leads to the next stage in the process namely abnormal keratinisation, in which the surface protein of the skin becomes stickier. This

leads to plugging of the pores, whereby sebum becomes trapped inside. This, in turn, provides an ideal medium in which bacteria may grow and inflammation results. The inflammation eventually resolves the infection, but the process of wound-healing results often leaves permanent, visible scars. Hormone therapy acts at the early stage of androgenic stimulation of sebum production, while other medical treatments intervene at the latter stages in the causative chain of events that comprises the development of acne.

2.2.4 PATIENT EDUCATION

Treatment of acne begins with patient education to dispel myths, establish reasonable expectations and answer questions. Patients should be told that acne is not caused by an improper diet or inadequate hygiene. They should be encouraged to wash affected areas with any standard soap once or twice a day and perhaps also after strenuous physical activities. Harsh detergents, scrubs containing granules, rubbing alcohol, and topical exfoliants should not be used (Landow, 1997:95). Skin care should be focused on gentle cleansing and moisturisers may be useful for persons with dry skin or irritation due to a topical medication. Patients should be taught to select non-comedogenic skincare products and cosmetics. In general, lotions and oil-based products are more comedogenic than gel-based products (Gollnick & Cunlliffe, 2003:30).

2.3 Nicotinamide

Vitamin B is a group of water-soluble nutrients found in many food sources, especially whole grains and green leafy vegetables. Nicotinamide is a form of vitamin B_3, and has shown to have some great and exciting benefits when applied to the skin (Lupo, 2001:468). According to Nicoli *et al.* (2008:613) it is used as a cosmetic active ingredient for its moisturising and depigmentation properties. Nicotinamide essentially acts as an antioxidant, thus increasingly gains interest in the prevention and treatment of skin diseases (Otte *et al.*, 2005:255). Nicotinamide is unpalatable when taken in solution and is usually given in capsules or tablet forms (Knip *et al.*, 2000:1341). Nicotinamide is also well tolerated when applied topically and does not induce skin irritation responses (Choi & Berson, 2006:164).

2.3.1 PHYSICOCHEMICAL CHARACTERISTICS

Nicotinamide's structure consists of a pyridine ring with an amide group in position 3 as shown in Figure 2.4 (Otte *et al.*, 2005:255).

Synonyms:	Niacinamide, Vitamin B_3
Chemical name:	Pyridine-3-carboxamide
Appearance:	White, crystalline powder or colourless crystals
Molecular formula:	$C_6H_6N_2O$
Molecular weight:	122.1 Da
CAS number:	98-92-0
Solubility:	Freely soluble in water and ethanol (Reynolds, 1993:1045).

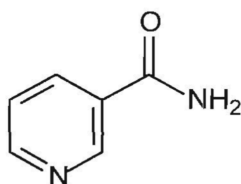


Figure 2.4: Structural formula of nicotinamide (Adapted from Otte *et al.*, 2005:255)

2.3.2 MECHANISM OF ACTION

Nicotinamide has anti-inflammatory properties that result in improvement of acne. The pyridine ring present in nicotinamide is believed to be responsible for the anti-inflammatory activity. Additional studies on the anti-inflammatory effects of nicotinamide have shown to suppress of antigen-induced lymphocyte transformation and inhibit potassium-iodide-induced inflammation (Lupo, 2001:468). Nicotinamide is a precursor of NADP (nicotinamide adenine dinucleotide phosphate) as well as its reduced form NADPH, which are potent antioxidants. Nicotinamide improves the lipid barrier component of the epidermis by increasing ceramide and free fatty acids, as well as the epidermal barrier proteins such as keratin, filaggrin and involucrin. This results in a decrease in transepidermal water loss. Nicotinamide also stimulates collagen synthesis by fibroblasts. It inhibits melanosome transfer from melanocytes to keratinocytes and subsequently reduces melanin content in the skin. Another benefit of nicotinamide is the reduction of skin yellowing or sallowness by preventing oxidative glycation of proteins (Choi & Berson, 2006:164).

2.3.3 PHARMACOKINETICS

The pharmacokinetics of nicotinamide depends on species, gender, route of administration and dosage. Nicotinamide is rapidly absorbed parenterally and orally from all parts of the gastro-intestinal tract. Peak concentrations are achieved in humans within approximately 1 h after oral ingestion of standard preparations. Nicotinamide also disappears rapidly from the circulation and is distributed into all tissues. It has a high hepatic excretion ratio. In patients with hepatic insufficiency, the plasma clearance of nicotinamide can be reduced (Knip *et al.,* 2000:1338). There is a negligible metabolism of nicotinamide to nicotinic acid, due to bacterial activity. In contrast to nicotinic acid, nicotinamide has no effect on blood pressure, pulse or body temperature (Otte *et al.*, 2005:256).

2.3.4 FUNCTION IN THE HUMAN BODY

Half of the nicotinic acid in the body is obtained from dietary sources and the rest is synthesised in the liver from the essential amino acid tryptophan. In the amide form, nicotinamide is a component of the coenzymes NAD and NADP, which are involved in oxidation/reduction reactions essential for tissue respiration reactions. Deficiency may cause pellagra which is characterised by gastro-intestinal, skin and nervous system abnormalities like diarrhoea, dermatitis and dementia. It is also often associated with other vitamin B-complex deficiency states. Skin lesion can progress to pigmentation, cracking and peeling, and often the skin of the neck is involved (Gibbon, 2005:80).

2.3.5 CLINICAL USES

According to Otte *et al.* (2005:257), several reports suggest that nicotinamide may have various effects on cutaneous and internal diseases. Nicotinamide is used in several parts of medicine as a systemic drug. Table 2.2 gives a survey of reported diseases managed with systemically administered nicotinamide (Otte *et al.*, 2005:257).

Table 2.2: Survey of the most important systemic uses of nicotinamide

Disease	Effects
Psoriasis vulgaris	Anti-inflammatory Reduce hepatotoxicity of methotrexate Synergistic inhibition of arthritis in combination with TNF-∂ inhibitors
Diabetes mellitus	Prevent or delay of the onset of type 1 diabetes among high risk individuals
Stroke	Protective effects on beta-cell function Significant amelioration of necrotic and apoptotic brain injury after ischemia
Opiate addiction	Decrease of the opiate withdrawal symptoms
Schizophrenia	Stimulates gamma-amino butyric acid (GABA) receptors, without binding to the receptor site Creating a benzodiazepine-like effect

Nicotinamide is also topically used for the following skin diseases:

- Acne vulgaris: The widespread use of topically applied antimicrobial agents during treatment of inflammatory acne vulgaris, led to resistance of disease-related micro-organisms. Nicotinamide is a new approach to the management of moderate inflammatory acne, without posing the risk of the development of resistant micro-organisms (Otte *et al.*, 2005:257);
- Rosacea and contact dermatitis: Nicotinamide is a component of NADH and has a higher reduction power than well known antioxidants such as vitamin C and E. NADH acts directly as an operating antioxidant and can effectively protect the cell, and in particular its membrane, from destruction by free radicals. NADH is a safe alternative to conventional treatment of inflammatory skin diseases such as rosacea and contact dermatitis (Otte *et al.*, 2005:257);
- Atopic dermatitis: Nicotinamide may not be able to replace the common treatment but it might be a useful adjunct in the management of atopic dermatitis. Besides numerous other anti-inflammatory effects, nicotinamide is capable of stabilising mast cells and leukocytes, exerting considerable histamine-release blocking activity. Beyond disease management of acute phases a basic daily treatment is needed in eczema-prone individuals to prevent xerosis. Nicotinamide should be capable of stabilising the epidermal barrier homeostasis by increasing the level of ceramides and free fatty acids in the stratum corneum and thus decreasing transepidermal water loss (Otte *et al.*, 2005:257);

- Benign hyper pigmentation: According to Otte *et al.* (2005:258) the topical use of nicotinamide may effectively reduce cutaneous pigmentation. Nicotinamide may also aid in the prevention of UV-induced deleterious molecular and immunological events; and
- Skin ageing: During ageing of the skin, the amount of NADH and NADPH is reduced. These two coenzymes are viewed as fundamental energy "currency" units within cells, driving the metabolism of cells involved in both anabolic- and catabolic processes. The elevation of NADPH level in human skin by nicotinamide has been reported to have an anti-glycation effect and thus reduce normal ageing-induced changes in skin appearance in the sense of yellowing. It has also been found that collagen synthesis and protein secretion derived from aged donors in the presence of nicotinamide thus improving skin wrinkles. A further potential effect of the topical use of nicotinamide is the ability to upregulate the synthesis of ceramides as well as other stratum corneum intercellular lipids. They are known to play a central role in the structural and functional integrity of the epidermal permeability barrier function. Thus topically applied nicotinamide might play an important part in the maintenance of the epidermal permeability barrier via regulating stratum corneum intercellular lipid synthesis, in particular relevant with aged skin (Otte *et al.*, 2005:259).

Overall, the use of nicotinamide results in improving skin tone and texture, decreasing of fine lines and wrinkles and diminishing hyperpigmentation. Topical nicotinamide is well tolerated and does not induce skin irritation responses (Choi & Berson, 2006:164).

2.3.6 ADVERSE REACTIONS

According to Knip *et al.* (2000:1341), studies have reported an overall frequency of side effects of less than 5%. The first side effect noted with systemic nicotinamide is mostly nausea. With higher dosages, vomiting, flatulence and diarrhoea can occur. After parenteral use, mild headaches and dizziness have been reported. Topical nicotinamide is well tolerated by the skin (Otte *et al.*, 2005:260). The other side-effects that can occur when high doses of nicotinamide are used include: flushing, facial erythema, hives, sore mouth, dull headache, heartburn, inability to focus the eyes, dry hair and fatigue (Knip *et al.*, 2000:1341).

2.4 Anatomy and functions of human skin

According to Bouwstra and Honeywell-Nguyen (2002:41), the natural function of the skin is to protect the body for unwanted influences from the environment. The main barrier of the skin is located in the outermost layer of the skin, the stratum corneum. In the following paragraphs, the structure as well as the functions of the skin will be discussed.

2.4.1 STRUCTURE OF THE SKIN

The skin is the largest organ in the human body and covers approximately 1.5 to 2.0 m^2 of the average human's body surface (Menon, 2002:3; DeBenedictis *et al.*, 2001:573). It is the heaviest and most versatile organ of the body, and represents almost 16% of a human's total body weight (Sanders *et al.*, 1999:168). The anatomy of the human skin is shown in Figure 2.5. The skin consists of two distinct layers, namely the epidermis and dermis.

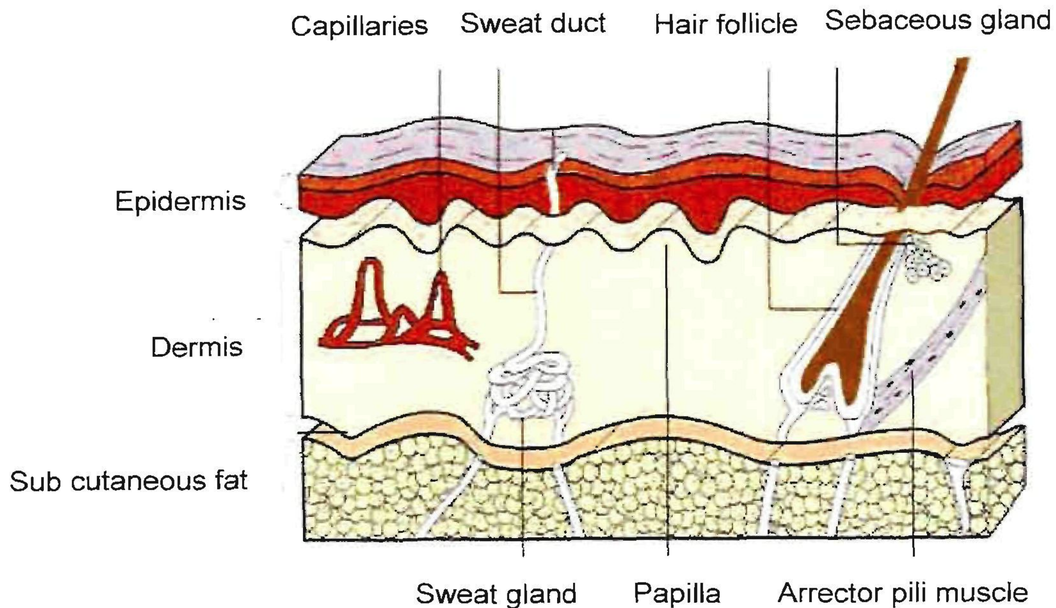


Figure 2.5: Anatomy of the skin (Adapted from Talkacne.com, 2009)

2.4.1.1 THE EPIDERMIS

The epidermis is the thin protective outer layer, typically less than 150 µm thick on the general body surface and up to 600 µm thick on the palms and soles (Sanders *et al.*, 1999:168). It is a self-renewing, stratified epithelium that functions as the interface between the human body and outer environment. The epidermis protects against mechanical, chemical and microbial attacks and functions as a permeability barrier by preventing water loss from the dermis. The epidermis also has immunological functions and provides some protection to the skin from ultraviolet light via the pigment system (Wickett & Visscher, 2006:98). The stratified epidermis is divided into four distinct layers namely, the stratum basale, stratum spinosum, stratum granulosum and stratum corneum (Menon, 2002:4).

2.4.1.1.1 Stratum Basale

The stratum basale is a single layer of columnar basal cells, which remain attached to the basement membrane via hemi-desmosomes. The cells show a high nucleo-cytoplasmic ratio with cell organelles such as mitochondria, and keratin filaments that are inserted into the hemi-desmosomes. The desmosomes connect adjacent and overlying cells (Menon, 2002:5).

2.4.1.1.2 Stratum Spinosum

Due to the abundance of desmosomes, this layer has a spiny appearance of its cells in histological sections. In addition to the typical cell organelles seen in the basal layer, the stratum spinosum also shows presence of lipid-enriched lamellar bodies. Ultra-structurally, lamellar bodies are 0.2 to 0.5 µm in diameter, with parallel stacks of lipid-enriched disks enclosed by a trilaminar membrane (Menon, 2002:5).

2.4.1.1.3 Stratum Granulosum

Distinct, dark stained keratohyaline granules are characterised by thin layer in histological preparations. Keratohyaline granules are composed of profillaggrin, loricrin and a cysteine-rich protein and they become progressively larger in the upper granulocytes, reflecting a quantitative increase in keratin synthesis (Menon, 2002:5).

2.4.1.1.4 Stratum Corneum

The thick (10 to 20 µm) surface layer is highly hydrophobic and contains 10 to 15 layers of interdigitated corneocytes, which are constantly shed and renewed. Its organisation can be described by the brick and mortar model, in which extracellular lipid accounts for 10% of the dry weight of this layer, and 90% is intracellular protein (mainly keratin). The stratum corneum lacks phospholipids, but is enriched in ceramides and neutral lipids that are arranged in a bilayers format and form so-called lipid channels. The barrier function of the skin is created by lamellar granules, which are synthesised in the granular layer and later become organised into the intercellular lipid bilayers domain of the stratum corneum. Barrier lipids are tightly controlled and any impairment to the skin, results in active synthetic processes to restore them. The skin's barrier function appears to depend on the specific ratio of various lipids; studies in which non-polar and relatively polar lipids were selectively extracted with petroleum-ether and acetone, respectively; indicating that the relatively polar lipids are more crucial to skin barrier integrity. Because of this highly organised structure, the stratum corneum is the major permeability barrier to external materials, and is regarded as the rate-limiting factor in the penetration of therapeutic agents through the skin. The ability of various agents to interact with the

intercellular lipid, therefore dictates the degree to which absorption is enhanced (Foldvari, 2000:417). The different layers of the epidermis are shown in Figure 2.6.

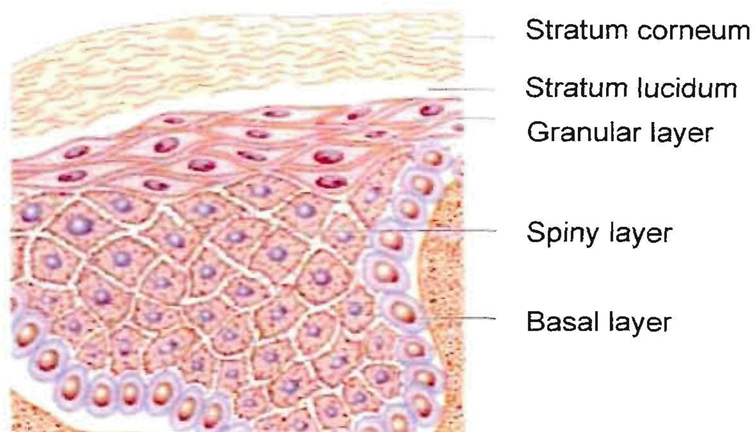


Figure 2.6: The different layers of the epidermis (Adapted from P & G, 2007)

2.4.1.2 THE DERMIS

The dermis (1 to 2 mm thick) is directly adjacent to the epidermis and provides the mechanical support for the skin (Bouwstra *et al.*, 2003:2). The dermis is largely acellular, but is rich in blood vessels, lymphatic vessels and nerve endings. An extensive network of dermal capillaries connects it to the systemic circulation, with considerable horizontal branching from the arterioles and venules in the papillary dermis, to form plexuses and to supply capillaries to hair follicles and glands. Dermal lymphatic vessels help to drain excess extracellular fluid and clear antigenic materials. The elasticity of the dermis is attributed to a network of protein fibres, including collagen and elastin, which are embedded in an amorphous glycosaminoglycan ground substance. The dermis also contains scattered fibroblasts, macrophages, mast cells and leukocytes. Hair follicles, sebaceous glands and sweat glands are found in the dermis and subcutis, and might serve as additional but limited pathways for drug absorption. In some cases hair follicles might act as target sites for drug delivery. Beneath the dermis is the hypodermis, a subcutaneous layer of loose areolar or fatty connective tissue (Foldvari, 2000:418).

2.4.2 FUNCTIONS OF THE SKIN

The numerous functions of the skin include the following:

1. Protective barrier with immunological and sensory functions (Foldvari, 2000:417);
2. Provide a multifunctional interface between us and our surroundings (Naik *et al.*, 2000:318);
3. Barrier to outward loss of water (Naik *et al.*, 2000:318);
4. Physical, chemical, immune, pathogen, UV radiation and free radical protection (Menon, 2002:4);
5. Thermo-regulation (Menon, 2002:4);
6. Performs endocrine functions (Vitamin D synthesis and peripheral conversion of prohormones) (Menon, 2002:4); and
7. Protect the body from unwanted influences from the environment (Bouwstra *et al.*, 2003:1).

2.5 Transdermal drug delivery

Percutaneous absorption of pharmaceuticals for either systemic or local delivery is a desirable process and can be attained by the combination of appropriate solute properties for skin transport with appropriate dosage form designs. Compounds have been applied to the skin for many centuries and, indeed, drugs in the form of plant or animal extracts, have been applied for the relief of a variety of local disorders. In recent years, systemic delivery through the transdermal route has led to the development and successful marketing of various pharmaceuticals in a patch form (Roberts *et al.*, 2002:89). When a treatment or product is applied to the skin's surface, some specific end result is desired. Depending on the goal and the mechanism of action, it will be necessary for active substances to reach target sites within the skin. In some cases, the benefit is due to the product as a whole, rather than any single ingredient (Zatz, 1993:13). According to Barry (2002:507), the eventual aim in dermatological biopharmaceutics is to design drugs with selective penetrability for incorporation into vehicles or devices that deliver the medicaments to the active site, at a controlled rate and concentration, for the necessary time. However, TDD has several advantages and limitations.

2.5.1 ADVANTAGES AND LIMITATIONS OF TRANSDERMAL DRUG DELIVERY

Controlled delivery of drugs into the body, is one of the major research topics in the pharmaceutical field. Most drugs are administered orally but, due to the high metabolic activity

in the gastro-intestinal tract and in the liver, for a variety of drugs this route of administration is not possible. Another problem with oral drug delivery is that for some drugs a continuous delivery is required, which is very difficult to achieve via the oral route. For these reasons there is a need for alternative routes of administration like the transdermal route (Bouwstra *et al.*, 2003:2). The primary reason for the TDD system is that they can provide sustained drug delivery and hence constant drug concentrations in plasma, over a prolonged period (Chong & Fung, 1989:135).

2.5.1.1 ADVANTAGES

Advantages of TDD include the following:

- The skin presents a relatively large and readily accessible surface area (1 to 2 m^2) for absorption (Naik *et al.*, 2000:319);
- For drugs, where oral administration is not possible due to the high metabolic activity in the gastro-intestinal tract and in the liver, transdermal administration is possible (Bouwstra *et al.*, 2003:2);
- The application of a patch-like device to the skin surface is a non-invasive (and thus a patient-compliant) procedure that allows continuous intervention (i.e. system repositioning, removal or replacement) (Naik *et al.*, 2000:319);
- Further benefits of TDD systems have emerged over the past years as technologies have evolved. These include the potential for sustained release which is useful for drugs with short biological half-lives, requiring frequent oral or parenteral administration and controlled input kinetics, which are particularly indispensable for drugs with narrow therapeutic indices (Naik *et al.*, 2000:319);
- The TDD system is easy to use (Shargel *et al.*, 2005:372); and
- TDD systems can be used for lipid-soluble drugs with low dose and low molecular weight (Shargel *et al.*, 2005:372).

2.5.1.2 LIMITATIONS

Limitations of TDD include the following:

- There remains a large pool of drugs for which TDD is desirable, but presently unfeasible. The nature of the stratum corneum is, in essence, the key to this problem. The excellent diffusional resistance offered by the membrane, means that the daily drug dose that can be systemically delivered through a reasonable 'patch-sized' area, remains in the range

of less than 10 mg. This limitation imposes the first criterion for a successful transdermal candidate (Naik *et al.*, 2000:319);

- Transdermal delivery is a challenging task for the pharmaceutical scientist and one that boasts significant progress (Naik *et al.*, 2000:318);
- Transdermal drugs must be pharmacologically potent, requiring therapeutic blood concentrations in the µg/ml range or less (Naik *et al.*, 2000:319);
- The stratum corneum is very selective with respect to the type of molecule that can be transported across this outer covering, and not all molecules that pass the 'potency' test, will have the necessary physicochemical properties (Naik *et al.*, 2000:319);
- Some irritation by applying a patch or drug may occur (Shargel *et al.*, 2005:372);
- Permeability of skin variable with condition, anatomic site, age and gender exists (Shargel *et al.*, 2005:372); and
- The type of cream or ointment base affects drug release and absorption (Shargel *et al.*, 2005:372).

2.5.2 PATHWAYS OF TRANSDERMAL PENETRATION

Based on the physiology of the skin, three possible pathways exist for passive transport of chemicals through the skin to the vascular network (Ho, 2003:50), which are the following:

1. intercellular diffusion through the lipid lamellae;
2. transcellular diffusion through both the keratinocytes and lipid lamellae; and
3. diffusion through appendages (hair follicles and sweat ducts).

The skin permeation routes are shown in Figure 2.7.

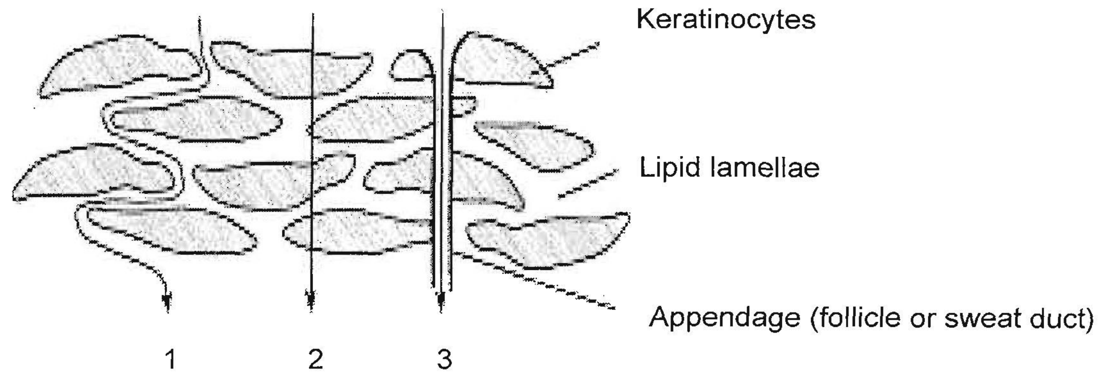


Figure 2.7: Skin permeation routes: (1) intercellular diffusion through the lipid lamellae; (2) transcellular diffusion through both the keratinocytes and lipid lamellae; and (3) diffusion through appendages (Ho, 2003:50)

2.5.2.1 INTERCELLULAR DIFFUSION THROUGH THE LIPID LAMELLAE

There has been much debate over the past decades regarding the route of penetration of drugs through the skin, but experimental evidence suggests that under normal circumstances, the predominant route is through the intercellular spaces. The diffusional path length is therefore much longer than the simple thickness of the stratum corneum (20 µm) and has been estimated to be as long as 500 µm. The intercellular spaces contain structured lipids and a diffusing molecule has to cross a variety of lipophilic and hydrophilic domains, before it reaches the junction between the stratum corneum and the viable epidermis. The nature of the barrier is thus very heterogeneous and it is perhaps surprising that diffusion through it can be described by a simple equation such as Fick's laws of diffusion (Hadgraft, 2004:292).

2.5.2.2 TRANSCELLULAR DIFFUSION THROUGH BOTH THE KERATINOCYTES AND LIPID LAMELLAE

It was originally believed that transcellular diffusion mechanisms dominated over the intercellular and transappendageal routes during the passage of solutes through the stratum corneum. However, transport by the transcellular route would involve the repeated partitioning of the molecule between lipophilic and hydrophilic compartments, including the almost impenetrable corneocyte intracellular matrix of keratin and keratohyaline (Roberts *et al.*, 2002:94).

2.5.2.3 DIFFUSION THROUGH APPENDAGES (HAIR FOLLICLES AND SWEAT DUCTS)

The amount of sebaceous glands on the total skin surface represents not more than 0.1% of the total surface. Therefore there are scientists who believe that this route is not a significant penetration pathway for most molecules. Others claim that the appendages can bypass the low diffusivity of the stratum corneum and may act as diffusional shunts. When the follicle is the site of action, such as in acne, scientists find ways to target a compound to this site, by developing delivery systems with specific physicochemical properties. When considering these openings as a possible route for penetration, it is important to understand the variations in follicle distribution among different body locations. Two types of liposomes were found to contribute significantly to penetration through shunt routes, namely classic multilamellar vesicles and flexible ultra-deformable liposomes (Dayan, 2007:32).

2.5.3 FACTORS INFLUENCING PERMEATION ACROSS THE SKIN

According to Dayan (2007:31), factors that may affect penetration include the size of the molecule, its affinity to the surface of the skin and its compatibility with the intercellular lipids. Also of significance for penetration are general skin condition, moisture content, temperature, thickness of the stratum corneum that can differ between races and body parts, and physical integrity. A few biological and physicochemical factors that may affect permeation across the skin are discussed in the following section:

2.5.3.1 BIOLOGICAL FACTORS

Some of the biological factors that may affect permeation across the skin include skin age, skin condition, regional skin sites, skin metabolism, circulatory effects and differences in species.

2.5.3.1.1 Skin age

According to Surber and Davis (2002:436), biological effect is decreased in the aged individual. Therefore pharmacodynamic parameters have to be used with care. Skin permeability is better in premature or newborn infants.

2.5.3.1.2 Skin condition

The intact, healthy skin is a tough barrier, but many agents can damage it. Vesicants such as acids and alkalis injure barrier cells and thereby promote penetration, as do cuts, abrasions and dermatitis. After injury or removal of the stratum corneum, the skin builds a temporary barrier within 3 days that persists until the regenerating epidermis can form normal keratinising cells.

Even the first complete layer of the new stratum corneum cells formed over a healing layer, can perceptibly reduce permeation (Barry, 2002:509).

2.5.3.1.3 Regional skin sites

Variation in cutaneous permeability around the body, depends on the thickness and nature of the stratum corneum and the density of skin appendages. However, the absorption rate varies widely for a specific substance passing through identical skin sites in different healthy humans (Barry, 2002:510). According to Surber and Davis (2002:435), variation between and within anatomical sites is likely to be the most important factor that influence the extent of absorption.

2.5.3.1.4 Skin metabolism

Far from being a passive membrane for the passage of drugs, the skin has a significant metabolic activity. Metabolic activity includes a wide range of oxidative, reductive, hydrolytic and conjugative reactions, making the skin a source of extrahepatic metabolism for many topically applied drugs (Surber & Davis, 2002:435).

2.5.3.1.5 Circulatory effects

Theoretically, changes in the peripheral circulation or blood flow through the dermis, can affect percutaneous absorption. Thus, an increased blood flow can reduce the time for which a penetrant remains in the dermis and also raise the concentration gradient across the skin (Barry, 1983:137).

2.5.3.1.6 Species differences

Human skin differs widely in characteristics such as the horny layer thickness, sweat glands and hair follicle densities. The capillary blood supply and the sweating ability also differ between humans and common laboratory animals. Such factors affect the routes of penetration and the resistance to permeation. Subtle biochemical differences between human and animal skins may alter reactions between penetrants and skin. Frequently, mice, rats and rabbits are used to assess percutaneous absorption, but their skins have more hair follicles than human skin and they lack sweat glands. In general, studies on skin penetration indicate that monkey- and pig skins are most similar to that of humans. Hairless mouse skin has some similar characteristics and it has been widely used during different studies, but its stratum corneum is very fragile. Hairless rats and fuzzy guinea pig may be better models for humans. Although animal skin has been used to obtain skin penetration data, it is better to use human skin whenever possible (Barry, 2002:510).

2.5.3.2 PHYSICOCHEMICAL FACTORS

Physicochemical factors that may affect permeation across the skin, include skin hydration, temperature, pH, penetration enhancers, molecular size, partition coefficient and diffusion coefficient.

2.5.3.2.1 Skin hydration

When water saturates the skin, the tissue softens, swells and wrinkles, and its permeability dramatically increases (Barry, 1983:145). According to Barry (2002:511), hydration of the stratum corneum is one of the most important factors to increase the penetration rate of most substances through the skin. Hydration may result from water diffusing from underlying epidermal layers, or from perspiration that accumulates after the application of an occlusive vehicle or dressing.

2.5.3.2.2 Temperature

According to Watkinson and Brain (2002:85), temperature does not tend to affect partitioning phenomena, phase volumes or membrane thicknesses. However, in certain membranes containing structures that are subject to phase changes on heating or cooling, there may be a concurrent effect on the diffusion. For example, the flux of water through human skin increases with temperature and this increase has been linked to an increasingly fluid environment within the stratum corneum lipid bilayers.

2.5.3.2.3 pH

Surber and Davis (2002:433) demonstrate that the pH of topical vehicles affects the extent of dissociation of ionisable drug molecules and, thus, their thermodynamic activity, partitioning, and skin penetration. According to the simple form of the pH-partition hypothesis, only unionised molecules pass readily across lipid membranes. Weak acids and bases dissociate to different degrees, depending on the pH and their pKa or pKb values. Thus, the proportion of unionised drug in the applied phase mainly determines the effective membrane gradient, and this fraction depends on pH. However, ionised molecules do penetrate the stratum corneum to a limited extent, because they usually have a much greater aqueous solubility than the neutral species. The stratum corneum is remarkably resistant to alteration in pH, tolerating a range of 3 to 9 (Barry, 2002:511).

2.5.3.2.4 Penetration enhancers

According to Hsieh (1994:10), methods of enhancing drug transport across the biological barriers, can be divided into two major categories, namely physical- and chemical enhancement. Penetration enhancement is discussed in 2.5.4.

2.5.3.2.5 Molecular size

According to Barry (2002:513), absorption is apparently related to molecular weight where small molecules penetrate faster than large ones. However, the specific effect of the size of the penetrating molecule on the flux, can only be determined if the effect of size can be separated from the resultant change in solubility characteristics. Ideal properties that molecules would require to penetrate the stratum corneum are:

- a low molecular mass, preferably less than 600 Da;
- an adequate solubility in oil and water;
- a balanced partition coefficient; and
- a low melting point.

Nicotinamide, with a molecular mass of 122.1 Da and good solubility in water and oil therefore shows ideal properties to penetrate the stratum corneum (Reynolds, 1993:1045).

2.5.3.2.6 Partition coefficient

The partition coefficient (K) is important in establishing the flux of a drug through the stratum corneum. When the membrane provides the sole or major source of diffusional resistance, then the magnitude of the partition coefficient is very important, because it can make a difference by a factor of 10^8 (Barry, 2002:512). According to Wells (2002:119), partition coefficient has a number of applications which are relevant to preformulation and include the following:

- solubility in both aqueous and in mixed solvents;
- drug absorption *in vivo* when applied to a homologous series for structure activity relationships; and
- partition chromatography in the choice of column (HPLC) or plate and choice of mobile phase.

A partition coefficient of between 1 and 3 indicates the ability of a drug to dissolve both in water and oil, ensuring that the compound will permeate the skin comparatively fast (Roberts &

Walters, 1998). In this study the partition coefficient of nicotinamide was determined to be -0.32, which predicted that permeation across the skin might not be optimal.

2.5.3.2.7 Diffusion coefficient

The diffusional speed of a molecule depends mainly on the state of matter of the medium. In gases and air, diffusion coefficients are large because the void space available to the molecules is large compared to their size, and the mean free path between molecular collisions is large. In liquids, the free volume is much smaller, mean free paths are decreased and diffusion coefficients much reduced to a great extend. In skin, the diffusion drops progressively and reaches its lowest values within the compacted stratum corneum matrix. The diffusion coefficient of a drug in a topical vehicle or in skin at a constant temperature, depends on the properties of the drug and the diffusion medium, as well as the interaction between them. The value *D* measures the penetration rate of a molecule under specified conditions. The measured value *D* may reflect influences other than intrinsic mobility. For example, some drug may bind and become immobilised within the stratum corneum, and this process affects the magnitude of *D* as determined from the lag time (Barry, 2002:512).

2.5.3.3 MATHEMATICAL APPROACH TO DRUG PERMEATION

2.5.3.3.1 Fick's law of diffusion

According to Rieger (1993:38), Fick's laws are generally viewed as the mathematical description of diffusion processes through membranes and are applicable whenever the chemical or physical nature of the membrane controls the rate of diffusion. In passive diffusion, matter moves from one region of a system to another, following random molecular motions. The basic hypothesis underlying the mathematical theory for isotropic materials is that the rate of transfer of diffusing substance per unit area of a section is proportional to the concentration gradient measured normal to the section. This is expressed as Fick's first law of diffusion (Barry, 2002:506). Fick's second law relates the rate of change in concentration with time at a given point in a system, to the rate of change in concentration gradient at that point (Watkinson & Brain, 2002:64). Fick's law of diffusion can be written as follows (Rieger, 1993:39):

$$J = \frac{K.D}{l} \Delta C$$

Equation 2.1

Where:

J	=	flux (µg/cm^2.h)
K	=	partition coefficient
D	=	diffusion coefficient (cm^2/h)
ΔC	=	concentration difference (µg/cm^3)
l	=	*membrane thickness* (cm)

2.5.4 PENETRATION ENHANCEMENT

According to Barry (1983:160) clinical investigators and chemical warfare experts suggested that substances which could temporarily diminish the impermeability of the skin, must exist. Such materials, if they are safe and non-toxic, could be used in dermatology to enhance the penetration rate of drugs and even to treat patients systemically by means of the dermal route. The following are properties which such a material ideally should possess:

- The material should be pharmacologically inert and it should have no action of itself at the receptor sites in the skin or in the body in general;
- The material should not be toxic, irritating or allergenic;
- On application, the onset of penetration enhancing action should be immediate. The duration of the effect should be predictable and should be suitable;
- When the material is removed from the skin, the tissue should immediately and fully recover its normal barrier property;
- The barrier function of the skin should reduce in one direction only, as to promote penetration into the skin. Body fluids, electrolytes or other endogenous materials should not be lost to the atmosphere;
- The enhancer should be chemically and physically compatible with a wide range of drugs and pharmaceutical adjuvants;
- The substance should be an excellent solvent for drugs;
- The material should spread well on the skin and it should possess a suitable skin "feel";
- It must be possible to formulate the chemical into lotions, suspensions, ointments, creams, gels, aerosols and skin adhesives; and
- It should be inexpensive, odourless, tasteless, colourless and thus cosmetically acceptable (Barry, 1983:160).

2.5.4.1 CHEMICAL PENETRATION ENHANCEMENT

According to Naik *et al.* (2000:321) the most extensively investigated enhancement strategy, involves the use of chemicals that can reversibly compromise the skin's barrier function and consequently allow the entry of otherwise poorly penetrating molecules into the membrane and through to the systemic circulation. According to Foldvari (2000:419) the specific mechanism can fall into one of the following three categories:

1. Disruption of the highly ordered structure of intercellular lipid channels;
2. Interaction with corneocyte intracellular protein components; or
3. Enhanced partitioning of the drug in the presence or absence of the enhancer compound.

Most chemical enhancers affect the intercellular lipid bilayers in the stratum corneum. This creates various types of "openings" in the bilayers. The nature of these "openings" can vary. It can be triggering of a thermodynamic imbalance within the lipid domains leading to increased lipid fluidity or creation of actual microscopically visual pores (Dayan, 2007:37). The following are examples of chemical penetration enhancers:

2.5.4.1.1 Water

From the viewpoint of safety and effectiveness, water is probably the best penetration enhancer (Barry, 1983:172). Under normal conditions the stratum corneum is a relatively dry tissue with a water content amount of approximately 20% w/w. Increased hydration of the stratum corneum, which can be achieved by occlusion of the skin, generally increases transdermal delivery of topically applied drugs. Because the stratum corneum lipids play an important role in the skin barrier function, the information on the effect of hydration on stratum corneum lipid organisation is very important. Water has been found to exert only a minor effect on the temperatures of lipid transitions in the stratum corneum, in contrast to that observed for phospholipid membranes (Bouwstra *et al.*, 2003:22).

2.5.4.1.2 Isopropyl myristate and Isopropyl alcohol

Both isopropyl myristate and isopropyl alcohol were shown to increase permeation through the skin. When applied together, they demonstrate a synergetic enhancement of permeation. Isopropyl myristate generate disordered bilayers in the corneocyte-bound lipids and isopropyl alcohol disorders the free bilayer structures of the intercellular lipids. When combining two or more skin penetration enhancers to improve skin permeation, it is recommended to choose

enhancers with different mechanisms of action. This will prevent competition at the site of action and has a better probability of not provoking irritation (Dayan, 2007:37).

2.5.4.1.3 Oleic acid

According to Dayan (2007:38) oleic acid was found to increase epidermal permeability through a mechanism involving the perturbation of the stratum corneum lipid bilayers. The magnitude of enhancement is affected by several factors, including fatty-acid chain length, the presence of double bonds and the solvent or vehicle in which the fatty acid is dissolved (Foldvari, 2000:420).

2.5.4.1.4 Azone

Azone was the first molecule specifically designed as a skin penetration enhancer. Chemically it may be considered to be a hybrid of a cyclic amide, as with pyrrolidone structures with an alkylsulphoxide, but is missing the aprotic sulphoxide group. Azone is a colourless, odourless liquid and it possesses a smooth, oily yet non-greasy feel (Williams & Barry, 2004:608).

2.5.4.1.5 Vesicular structures

Vesicles are microscopic spheres, usually composed of amphiphilic molecules. Classic liposomes are composed of phospholipids. Topically applied vesicles can either mix with the stratum corneum lipid matrix or penetrate the stratum corneum by using the lipid-water interface of the intercellular matrix. A major force driving vesicle penetration through the skin may be the water gradient across the epidermis (Dayan, 2007:38). According to Williams and Barry (2004:614) many studies have employed phospholipids as vesicles to carry drugs into and through human skin. However, a few studies have used phospholipids in the non-vesicular form as a penetration enhancer.

2.5.4.2 PHYSICAL PENETRATION ENHANCEMENT

According to Thomas and Finnin (2004:699), physical enhancement methods have been studied that involve the use of an energy source to overcome the barrier properties of the skin. These methods rely on providing a reservoir of drug on the skin surface, from which the required levels of delivery can be achieved. The following are examples of physical penetration enhancers:

2.5.4.2.1 Ultrasound

According to Barry (2001:108) this technique was originally used in physiotherapy and sports medicine and was extended to TDD studies. Naik *et al.* (2000:324) define the use of ultrasound

as sound of frequency higher than 20 kHz, to compromise the skin's barrier function. The use of ultrasound has received considerable attention and is also known as sonophoresis. The ultrasonic energy (at low frequency) disturbs the lipid packing in the stratum corneum by cavitation. Shock waves of collapsing vacuum cavities increase free volume space in bimolecular leaflets and thus enhance drug penetration into the tissue (Barry, 2001:108). Cavitation is thought to be the predominant mechanism by which low-frequency ultrasound promotes skin penetration enhancement and probably accounts for the enhanced transport of polar macromolecules such as insulin, interferon-γ and erythropoietin across human skin. Mechanistically, sonophoresis is considered to enhance drug delivery through a combination of thermal, chemical and mechanical alterations within the skin tissue (Naik *et al.*, 2000:324).

2.5.4.2.2 Iontophoresis

According to Naik *et al.* (2000:324), iontophoresis is the most evolved technology of physical penetration enhancer. It uses a small electrical current to facilitate the transfer of drugs across the skin. Charged species are repelled into and through the skin as a result of an electrical potential across the membrane. The efficiency of this process is dependent on the polarity, valency and ionic mobility of the permeant, as well as on the composition of the delivery formulation and the current profile. Two electrolyte chambers containing electrodes are placed on the skin surface and driven by a constant current source. The magnitude of current determines the amount of charge generated in the circuit and, in turn, the number of ions transported across the skin. This ensures a controlled and efficient method of drug delivery because the amount of compound delivered, is directly proportional to the quantity of charge passed. Barry (2001:108) gives three main mechanisms that enhance molecular transport namely:

1. Charged species are driven primarily by electrical repulsion from the driving electrode;
2. The flow of electric current may increase the permeability of skin; and
3. Electro-osmosis may affect uncharged molecules and large polar peptides.

2.5.4.2.3 Electroporation

Skin electroporation creates transient aqueous pores in the lipid bilayers by application of short electrical pulses of approximately 100 to 1000 V/cm. These pores provide pathways for drug penetration that travel straight through the horny layer (Barry, 2001:109). Naik *et al.* (2000:324) describe that although this mode of electrical transdermal enhancement has been shown to be more effective relative to iontophoresis for several molecules *in vitro*, and to produce

significantly elevated levels of transport compared with passive delivery, the limited data from *in vivo* and skin toxicological studies means that its clinical value remains to be established.

2.5.4.2.4 Magnetophoresis

According to Barry (2001:109) limited work has been done on the ability of magnetic fields to move diamagnetic materials through the skin, but he discussed the employing of an intelligent system based on magnetism or microchip technology to deliver drugs in controlled, pulsatile mode.

2.5.4.2.5 Photomechanical wave

With this technique a drug solution is placed on the skin and covered by a black polystyrene target, after which it is irradiated with a laser pulse. The resultant photomechanical wave stresses the horny layer and enhances drug delivery (Barry, 2001:109).

2.5.4.3 PENETRATION ENHANCEMENT BY BYPASSING OR REMOVING THE STRATUM CORNEUM

Different techniques exist by which drug penetration could be enhanced by means of bypassing or removal of the stratum corneum.

2.5.4.3.1 Micro-needle array

The stratum corneum can be bypassed by means of an injection. One development in this approach is a device of 400 micro-needles which insert the drug just below the barrier. The solid silicon needles which are coated with the drug, or hollow metal needles which are filled with drug solution, penetrate the horny layer without breaking it or stimulating the nerves in deeper tissues (Barry, 2001:107).

2.5.4.3.2 Stratum corneum ablated

As the horny layer usually provides the permeation barrier, we could consider simply removing it. Chemical peels may provide superficial or light, medium or deep treatments. Microdermabrasion uses a stream of aluminium oxide crystals and dermabrasion employs a moto-driven abrasive fraise of cylinder. Laser ablation applies high powered pulses to vaporise a section of the horny layer in order to produce permeable skin regions. The apparatus is costly and requires expert operation to prevent damage such as burns (Barry, 2001:107).

2.6 Pheroid™ Technology

Pheroid™ technology consists mainly of modified essential fatty acids. It generally contains a lipid bilayer, but it contains no phospholipids or cholesterol. The basic Pheroid™ has a vesicular structure with sizes ranging from 200 to 440 nm and the membranes of the Pheroid™ contain pores (Grobler *et al.*, 2008:283).

2.6.1 PHEROID™ TECHNOLOGY FOR TRANSDERMAL DELIVERY

It is widely recognised that inadequate delivery is the single most important factor delaying optimised application of new classes of molecular therapeutics or cosmeceuticals. Pheroid™ technology (previously called Emzaloid™ technology), is able to enhance the absorption and/or efficacy of various categories of active ingredients and other compounds leading to major improvements in the control of size, charge and the hydrophilic-lipophilic characteristics of therapies, when compared to other systems (Grobler *et al.*, 2008:284).

2.6.1.1 STRUCTURAL CHARACTERISTICS

According to Grobler *et al.* (2008:284), the Pheroid™ delivery system is a colloidal system that contains unique and stable lipid-based submicron- and micron-sized structures, called Pheroid™. It is distributed uniformly in a dispersion medium that may be adapted according to the application. The dispersed structures can be manipulated in terms of morphology, structure, size and function. The ultrafine particles that remain suspended in the dispersion medium are usually between 1 to 100 nm in diameter. Various types of Pheroid™ are typically formulated to have a diameter of between 200 nm and 2 µm. The intention in using colloidal systems as carriers of drugs or cosmetic compounds, is to enhance the efficacy of the administered compounds while reducing the unwanted side effects. Colloidal systems are typically classified as:

- Simple colloids where a clear distinction between the dispersed phase and dispersion medium is found;
- Multiple colloids in which three phases co-exist; and as
- Network colloids that have two phases, forming an inter-penetrating network, as is found in polymer matrices.

2.6.1.2 FUNCTIONAL CHARACTERISTICS

The efficacy of the delivery of an active compound to skin involves different processes, and depends on the target of delivery. Delivery of an active compound to the epidermis will involve at least the following processes:

1. Entrapment of an active compound in the carrier;
2. Penetration of an entrapped compound across the stratum corneum to the epidermis;
3. Uptake of the entrapped compound by the corneocytes; and
4. Release of the active compound from the carrier (Grobler *et al.,* 2008:293).

2.6.1.2.1 Pliable system design and versatility

Due to the use of a gas as well as the pliable pegylated tails added to the fatty acids, extremely elastic structures are formed. Pheroid™ are not shattered under moderate pressure or extravascation. The pegylation serves to sterically stabilise the Pheroid™ and maintain their interior spaces. Furthermore, polyethylene glycol has been shown to contribute to increased bio-availability, increased drug stability and extended circulating life, lower toxicity and enhanced drug solubility (Grobler *et al.*, 2008:294).

2.6.1.2.2 Entrapment efficiency

The efficiency of entrapment of a compound can be expressed as the percentage of the initial amount of the compound added to the formulation that is entrapped. An entrapment efficiency of more than 90% has been aimed at for all products in development. The number of molecules of the active compound that are entrapped within one Pheroid™, depends mainly on the size, charge and solubility of the active compounds (Grobler *et al.,* 2008:294).

2.6.1.2.3 Penetration efficiency

The percentage of active compound delivered to the skin is enhanced by entrapment in Pheroid™. The easiest way to measure the efficiency of penetration is a comparative investigation, where the enhancement caused by the carrier, is determined by comparing it to an existing commercial product (Grobler *et al.*, 2008:296).

2.6.1.2.4 Uptake of Pheroid™ and entrapped compounds by cells

According to Grobler *et al.* (2008:297), the Pheroid™ is sterically stabilised by electro-chemical interaction and not by cholesterol, as in the case in most lipid-based delivery systems. The

uptake of Pheroid™ by cells, may be influenced by the Pheroid™ formulation and by the mechanism of uptake by the cells. The permeation of the Pheroid™ formulation is determined by one or more of the following factors, namely:

- The size of the Pheroid™;
- The morphology of the Pheroid™;
- The molecular geometry of the fatty acids themselves;
- The concentration and ratios of the various fatty acids;
- The hydration medium;
- The pH of the preparation;
- The presence of charge-changing molecules;
- The presence of molecules that influence the electrostatic milieu;
- The character and concentration of the active or drug; and
- The state of the Pheroid™.

2.6.1.2.5 Metabolism, targeting and distribution

Depending on the type and extent of the fatty acid modifications, the distribution of Pheroid™ can be influenced. The cellular uptake of the Pheroid™ is based on various interactions between fatty acids and cells. Depending on the composition of the Pheroid™, they are metabolised in either the mitochondria or the peroxisomes of the cell (Grobler *et al.*, 2008:300).

2.6.1.3 THERAPEUTIC EFFICACY

Although the efficiency of entrapment and the rate of transport and release of active compounds into and across the skin, can be an indication of the performance of a delivery system, the effect of a delivery system or carrier should ideally be measured by its contribution to therapeutic efficacy. The formulation of active compounds in Pheroid™ has shown to increase the efficacy of a number of such active compounds (Grobler *et al.*, 2008:300).

2.6.1.4 POSSIBLE APPLICATIONS OF PHEROID™ TECHNOLOGY IN COSMETICS

Retinoids have been formulated with Pheroid™ and are specifically used for the treatment of skin *eruption and the enhancement of healthy and glowing skin.* Other molecules that have an influence on the proliferation and response pathways, and that can be used with success in Pheroid™, are vitamin D, hydrocortisone, epinephrine, extracellular adhesion and signalling

molecules and calcium. The efficacy of a number of anti-infective agents used in topical applications was shown to be enhanced by entrapment in Pheroid™ in either *in vitro* or *in vivo* studies. These include gentamycin, sulphamethoxazole, trimethoprim, cloxacillin, acyclovir, ciprofloxacin, miconazole nitrate and neomycin. Frequency of dosing can be reduced without diminishing potency, or higher doses can be given to enhance therapeutic impact. The Pheroid™ system has the potential to minimise cytotoxicity, such as that symptomised by the membrane damage caused by active compounds (Grobler *et al.*, 2008:307).

2.6.1.5 ADVANTAGES OF PHEROID™ TECHNOLOGY AS A TRANSDERMAL DRUG DELIVERY SYSTEM

According to Grobler (2004:6), the key advantages of the Pheroid™ technology are the following:

- Consists mainly of essential fatty acids that are a natural and essential ingredient of the body;
- Studies demonstrated no immune responses in man;
- A variety of types can be formulated, depending on the composition and manufacturing method;
- Can be manipulated in terms of size, charge, lipid composition and membrane packing;
- Causes no cytotoxicity and assists with maintenance of cell membrane;
- Drug resistance can be reduced;
- Insoluble drugs can be entrapped into Pheroid™;
- Protects drugs from metabolism and inactivation in the plasma or other body fluids; and
- Enhances bio-availability of oral, topical and buccal administration of active compounds.

2.6.1.6 CONCLUSION

The vast majority of delivery systems can structurally be characterised as colloids. To a large extent the surface properties determine the therapeutic profiles of the delivered compounds. The Pheroid™ system mainly consists of essential fatty acids (EFA). EFA are the primary component of the delivery system and are inherent components of the skin, and should be recognised as such by the immune system with no immunological implications. The presence of the EFA also contribute to the normalisation of the physiological micro-environment, resulting in added anti-inflammatory action, suppression of epidermal hyper-proliferation, normalising the water barrier of the skin, as well as fast and efficient delivery of the drug. The Pheroid™ is

probably one of the most effective, versatile and inexpensive delivery systems in commercial use. All components used in the manufacturing of Pheroid™ are pharmaceutically safe and the system is based on naturally occurring molecules of the body (Grobler *et al.*, 2008:308), thus promising to be an excellent penetration enhancer.

2.7 Summary

Acne is a very common skin disease and affects most adolescents and young adults. It is a chronic inflammatory disease characterised by comedones, papules, pustules and sometimes cysts. It commonly affects the face, chest and upper back. Factors that influence the development of acne include an increase in sebum production, abnormal follicular desquamation, bacterial proliferation and inflammation. Acne can be divided into three main groups according to the severity of the disease, namely mild acne, moderate acne and severe acne. The treatment of acne depends on the severity of the condition, and topical as well as oral treatments are available. Treatment of acne begins with patient education and it is very important that patients know how acne is caused and how it can be treated. Reasonable expectations must be established and questions must be answered. Oral treatments include antibiotics, isotretinoin and hormonal therapy. Topical treatments include antibiotics, retinoids, azelaic acid, benzoyl peroxide and nicotinamide. Nicotinamide is a form of vitamin B_3 and new studies have shown great benefits when nicotinamide is applied to the skin, especially for its moisturising, depigmenting and anti-inflammatory properties. Because of nicotinamide's anti-inflammatory properties it is a good alternative in the treatment of acne. The widespread use of antimicrobial drugs during the treatment of acne has led to resistance of micro-organisms and therefore nicotinamide may be a wonderful new approach to the management of acne.

Although most drugs are administered orally, this route of administration is not possible for a variety of drugs. Therefore the skin can be used as an alternative route for administration of drugs, and it is called TDD. TDD has exceptional advantages, but also a few limitations. One of these limitations is the barrier function of the skin. Although the stratum corneum gives the skin outstanding protection against unwanted substances from its surroundings, it is possible for drugs to be administered transdermally. There are basically three possible pathways of transdermal penetration across the skin, namely intercellular diffusion, transcellular diffusion and appendageal diffusion. Penetration across the skin can be enhanced by using different approaches. Chemical- as well as physical penetration enhancers can be used. A new technology, named Pheroid™ technology, is also a way to enhance penetration across the skin. This technology uses vesicular structures with no phospholipids or cholesterol to enhance penetration. The potential of using the skin as an alternative route for the administration of

drugs, has attracted considerable interest in recent years, and I believe that TDD can become one of the best methods of administering drugs in the future.

In this study the aims and objectives were to formulate a nicotinamide containing cream and emulgel, with and without Pheroid™ vesicles, for the treatment of acne; to develop and validate a HPLC method to quantitatively determine concentrations of the different ingredients in the formulations; to determine the aqueous solubility and partition coefficient of nicotinamide; to determine whether nicotinamide diffuses through the skin or into the dermis and epidermis and to do stability tests on the different products formulated.

The log D value of nicotinamide (-0.32) predicted that diffusion through the skin may not be optimal. Therefore the use of penetration enhancers were essential. In this study Pheroid™ technology were used to enhance penetration.

2.8 References

AMERICAN ACADEMY OF DERMATOLOGY. 2009. Acne. http://www.aad.org/public/publications/pamphlets/common_acne.html Date of access: 28 Oct. 2009.

BARRY, B. 2002. Transdermal drug delivery. (*In* Aulton, M.E., *ed.* Pharmaceutics: The science of dosage form design. 2nd ed. London: Churchill Livingston. p. 499-533.)

BARRY, B.W. 1983. Dermatological formulations. New York: Marcel Dekker. 480p.

BARRY, B.W. 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. *European journal of pharmaceutical sciences,* 14(2001):101-114.

BOUWSTRA, J.A. & HONEYWELL-NGUYEN, P.L. 2002. Skin structure and mode of action of vesicles. *Advanced drug delivery reviews,* 54(1):41-55.

BOUWSTRA, J.A., HONEYWELL-NGUYEN, P.L., GOORIS, G.S. & PONEC, M. 2003. Structure of the skin barrier and its modulation by vesicular formulations. *Progress in lipid research,* 42(1):1-36.

CHOI, C.M. & BERSON, D.S. 2006. Cosmeceuticals. *Seminars in cutaneous medicine and surgery,* 2006(25):163-168.

CHONG, S. & FUNG, H. 1989. Transdermal drug delivery systems: Pharmacokinetics, clinical efficacy and tolerance development. (*In* Hadgraft, J. & Guy, R.H. *eds.* Transdermal Drug Delivery – Developmental issues and research initiatives. Vol. 35. New York: Marcel Dekker. p. 135-153.)

CORDAIN, L. 2005. Implication for the role of diet in acne. *Seminars in cutaneous medicine and surgery,* 24(2):84-91.

DAYAN, N. 2007. Pathways for Skin Penetration. (*In* Kozlowski, A. *ed.* Biotechnology in cosmetics: Concepts, tools and techniques. USA: Allured Publishing Corporation. p. 29-42.)

DEBENEDICTIS, C., JOUBEH, S., ZHANG, G., BARRIA, M. & REZA, F.G. 2001. Immune functions of the skin. *Clinics in dermatology,* 19(5):573-585.

FOLDVARI, M. 2000. Non-invasive administration of drugs through the skin: challenges in delivery system design. *Pharmaceutical science & technology today,* 3(12):417-425.

GIBBON, C.J. *ed.* 2005. South African medicines formulary. 7th ed. Cape Town: Health and Medical publishing group. 581p.

GOLLNICK, H. & CUNLIFFE, W. 2003. Management of acne – A report from a global alliance to improve outcomes in acne. *Journal of the American academy of dermatology,* 2003(49):1-38.

GROBLER, A. 2004. Emzaloid™ technology. (Confidential concept document presented to Ferring Pharmaceuticals) 20p.

GROBLER, A., KOTZE, A. & DU PLESSIS, J. 2008. The design of a skin-friendly carrier for cosmetic compounds using Pheroid™ technology. (*In* Wiechers, J. *ed.* Science and applications of skin delivery systems. Wheaton, IL: Allured Publishing. p. 283-311.)

HADGRAFT, J. 2004. Skin deep. *European journal of pharmaceutics and biopharmaceutics,* 58(2):291-299.

HEALTH TIPS REVIEW. 2009. Causes and symptoms of acne. http://healthproductsreviewed.org/category/acne Date of access: 28 Oct. 2009.

HO, C.K. 2003. Probabilistic modelling of percutaneous absorption for risk-based exposure assessments and transdermal drug delivery. *Statistical methodology,* 1(2004): 47-69.

HSIEH, D.S. 1994. Understanding permeation enhancement technologies. (*In* Hsieh, D.S., *ed.* Drug permeation enhancement – Theory and application. Vol. 63. New York: Marcel Dekker. p. 3-17.)

HUBER, J. & WALCH, K. 2006. Treating acne with oral contraceptives: use of lower doses. *Contraception,* 2006(73):23-29.

KNIP, M., DOUEK, I.F., MOORE, W.P.T., GILLMOR, H.A., MCLEAN, A.E.M., BINGLEY, P.J. & GALE, E.A.M. 2000. Safety of high-dose nicotinamide: a review. *Diabetologia,* 2000(43):1337-1345.

KNUTSON, D.D. 1974. Ultrastructural observations in acne vulagaris: the normal sebaceous follicle and acne lesions. *The journal of investigative dermatology,* 1974(62):288-307.

KRAUTHEIM, A. & GOLLNICK, H.P.M. 2004. Topical treatment of acne. *Clinics in dermatology,* 2004(22):398-407.

KROWCHUK, D.P. 2000a. Managing acne in adolescents. *Pediatric clinics of North America,* 47(4):841-857.

KROWCHUK, D.P. 2000b. Topical therapies for acne. *Current problems in dermatology,* 12(5):213-215.

LANDOW, K. 1997. Dispelling myths about acne. *Postgraduate medicine online,* 102(2):94-112.

LONG, C.C. 2002. Common skin disorders and their topical treatment. (*In* Walters, K.A. *ed.* Dermatological and transdermal formulation. Vol.119. New York: Marcel Dekker. p.41-60.)

LUPO, M.P. 2001. Antioxidants and Vitamins in cosmetics. *Clinics in dermatology,* 2001(19):467-473).

MENON, G.K. 2002. New insights into skin structure: scratching the surface. *Advanced drug delivery reviews,* 54(1):3-17.

NAIK, A., KALIA, Y.N. & GUY, R.H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical science & technology today,* 3(9):318-326.

NICOLI, S., ZANI, F., BILZI, S., BETTINI, R. & SANTI, P. 2008. Association of nicotinamide with parabens: Effect on solubility, partition and transdermal permeation. *European journal of pharmaceutics and biopharmaceutics,* 2008(69):613-621.

OTTE, N., BORELLI, C. & KORTING, H.C. 2005. Nicotinamide – biologic action of an emerging cosmetic ingredient. *International journal of cosmetic science,* 2005(27):255-261.

P & G. 2007. The layers of the epidermis. http://www.pgbeautygroomingscience.com/the-epidermis.html Date of access: 21 Sept. 2009.

REDMOND, G.P. 1998. Effectiveness of oral contraceptives in the treatment of acne. *Contraception,* 1998(58):29-33.

REYNOLDS, J.E.F. *ed.* 1993. Martindale: The extra Pharmacopoeia. 13th edition. London: The pharmaceutical press. 2363p.

RIEGER, M.M. 1993. Factors affecting sorption of topically applied substances. (*In* Zatz, J.L., *ed.* Skin permeation – fundamentals and application. Wheaton: Allured Publishing Corp. p.33-72.)

ROBERTS, M.S. & WALTERS, K.A. 1998. The relationship between structure and barrier function of skin. (*In* Roberts, M.S. & Walters, K.A. *eds.* Dermal absorption and toxicity assessment. New York: Marcel Dekker. p. 1-42.)

ROBERTS, M.S., CROSS, S.E. & PELLETT, M.A. 2002. Skin Transport. (*In* Walters, K.A. *ed.* Dermatological and transdermal formulation. Vol 119. New York: Marcel Dekker. p. 89-195.)

SANDERS, J.E., GOLDSTEIN, B.S., LEOTTA, D.F. & RICHARDS, K.A. 1999. Image processing techniques for quantitative analysis of skin structures. *Computer methods and programs in biomedicine,* 59(2):167-180.

SHARGEL, L., WU-PONG, S. & YU, A.B.C. 2005. Applied biopharmaceutics and pharmacokinetics. Boston: Mc Graw Hill. 372p.

SHAW, L. & KENNEDY, C. 2007. The treatment of acne. *Pediatrics and child health,* 17(10):385-389.

SKIN10. 2005. Dermis. http://www.skin10.com Date of access: 21 Sept. 2009.

SKINSPOTS. 2009. How acne develops. http://www.skinspots.co.uk. Date of access: 28 Oct. 2009.

SURBER, C. & DAVIS, A.F. 2002. Bioavailability and Bioequivalence of Dermatological Formulations. (*In* Walters, K.A. *ed.* Dermatological and Transdermal Formulation. Vol. 119. New York: Marcel Dekker. p. 401-498.)

SWEETMAN, S. 2008. Martindale: the complete drug reference. http://www.medicinescomplete.com/mc/martindale/2008 Date of access: 20 Sept. 2009.

TALK ACNE. 2009. Skin structure diagram. http://www.talkacne.com Date of access: 21 Sept. 2009.

THOMAS, B.J. & FINNIN, B.C. 2004. The transdermal revolution. *Drug discovery today* 9(16):697-703.

TURNER, N.G. & NONATO, L.B. 1997. Drugs and the pharmaceutical sciences. (*In* Potts, R.O. & Guy, R.H. *eds.* Mechanisms of Transdermal Drug Delivery. Vol. 83. New York: Marcel Dekker. p. 1-40.)

WATKINSON, A.C. & BRAIN, K.R. 2002. Basic mathematical principles in skin permeation. (*In* Walters, K.A. *ed.* Dermatological and transdermal formulation. Vol 119. New York: Marcel Dekker. p. 61-88.)

WEBSTER, G.F. & GRABER, E.M. 2008. Antibiotic treatment for acne vulgaris. *Seminars in cutaneous medicine and surgery,* 2008(27):183-187.

WELLS, J. 2002. Pharmaceutical preformulation: the physicochemical properties of drug substances. (*In* Aulton, M.E., *ed.* Pharmaceutics: The science of dosage form design. 2nd ed. London: Churchill Livingston. p. 113-138.)

WERTZ, P.W. & MICHNIAK, B.B. 2000. Sebum. (*In* ELSNER, P. & MAIBACH, H.I., *eds.* Cosmeceuticals. Vol.23. New York: Marcel Dekker. p. 45-56.)

WHITE, G.M. 1999. Acne therapy. *Disease-a-month,* 45(8):304-330.

WICKETT, R.R. & VISSCHER, M.O. 2006. Structure and function of the epidermal barrier. *American journal of infection control,* 34(10):98-110.

WILLIAMS, A.C. & BARRY, B.W. 2004. Penetration enhancers. *Advanced drug delivery reviews,* 56(5):603-618.

ZATZ, J.L. 1993. Rationale and approaches to skin permeation. (*In* Zatz, J.L., *ed.* Skin permeation – fundamentals and application. Wheaton: Allured Publishing Corp. p.11-31.)

CHAPTER 3

ARTICLE FOR PUBLICATION IN THE INTERNATIONAL JOURNAL OF PHARMACEUTICS

Formulation, in vitro release and transdermal diffusion of Vitamin B_3 for treatment of acne

Telanie Venter, Minja Gerber, Jan L. du Preez, M.M. Malan, Jeanetta du Plessis*

Unit for Drug Research and Development, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

* Corresponding author. Tel.: +2718 299-2274; Fax: +2718 299-2225. E-mail address: Jeanetta.duPlessis@nwu.ac.za (J du Plessis)

Abstract

Acne is a common condition, affecting most adolescents and young adults. It is an inflammatory disease, characterised by comedones, papules and pustules. Because of the widespread use of antimicrobial agents in the treatment of acne, resistance of disease-related micro-organisms developed. Therefore new strategies for the treatment are necessary. Nicotinamide is a new approach to the topical treatment of acne, without the risk of emerging resistant micro-organisms. The aim of this study was to formulate four different semi-solid formulations with nicotinamide as the active ingredient, and to determine which formulation delivers nicotinamide best to the target site. Franz cell diffusion studies were performed over a 12 h period, followed by tape stripping experiments. A new technology named Pheroid™ technology was used to enhance transdermal penetration. This technology uses vesicular structures without phospholipids or cholesterol to enhance penetration. Another aim of this study was to perform stability tests over a six months period on the formulations. Products were stored at 25°C/60% RH, 30°C/60% RH and 40°C/70% RH. HPLC analysis was used to determine the concentrations of the ingredients in the products. Other stability tests included appearance, pH, viscosity, mass loss and confocal laser scanning microscopy.

Keywords: Nicotinamide, Acne, Transdermal delivery, Stability testing, Formulation, Pheroid™

1 Introduction

Acne vulgaris, more commonly known as acne, is the most common skin disease treated by physicians (Krowchuk, 2000). According to Gollnick and Cunliffe (2003) acne affects almost 80% of adolescents and young adults aged 11 to 30. Acne is a chronic condition that may last for years and cause emotional distress and permanent scarring (Krowchuk, 2000). It is characterised by comedones, papules, pustules and sometimes cysts, and commonly affects the face, chest and upper back (Long, 2002). According to Brajac et al. (2004), the four etiological factors of acne are enhanced sebum excretion, hypercornification of the sebaceous duct, ductal colonization with *Propionibacterium acnes* and production of inflammation. The assessment of acne severity divides acne into four main groups, namely mild, moderate, severe and very severe cases. The type of therapy and treatment needed depends on the severity of the case (Shaw and Kennedy, 2007). Mild acne requires topical therapy, while moderate acne requires topical and oral antibiotic therapy. Severe acne requires topical and oral therapy with very regular review and very severe acne requires urgent referral to a specialist for large doses of antibiotics, steroids or oral isotretinoin.

The widespread use of antimicrobial drugs in the treatment of acne led to resistance of micro-organisms and therefore new strategies are needed in the treatment of acne. A wonderful new approach to the management of acne is treatment with nicotinamide.

Nicotinamide is used in the treatment of moderate inflammatory acne without posing the risk of the emergence of resistant micro-organisms (Otte et al., 2005). Nicotinamide is a form of vitamin B_3, and has shown great and exciting benefits when applied to the skin (Lupo, 2001). It essentially acts as an antioxidant, thus increasingly gains interest in the prevention and treatment of skin diseases like acne (Otte et al., 2005).

Using the skin as an alternative route for administering drugs has become very popular over the last few decades. Although most drugs are administered orally, for a variety of drugs this route of administration is not possible. Therefore administration through the skin (also known as transdermal drug delivery) is a possible answer and has great advantages (Bouwstra et al., 2003). Unfortunately, using the skin as an alternative route for administering drugs is a

challenge and has numerous limitations. One of these limitations is the barrier function of the skin (Naik et al., 2000). Although the stratum corneum gives the body outstanding protection against unwanted substances from its surroundings, it is possible for drugs to be administered transdermally. There are three possible pathways for transport of drugs through the skin (Ho, 2003) namely, intercellular diffusion through the lipid lamellae, transcellular diffusion through the keratinocytes and lipid lamellae and lastly, diffusion through hair follicles and sweat ducts. Although there are possible pathways for drugs to permeate the skin, there are several factors that may affect the permeation. A few of these factors are: skin age, skin condition, skin site, skin metabolism, skin hydration, temperature, pH and penetration enhancers (Dayan, 2007. Penetration enhancement can be divided into chemical and physical penetration enhancers. Chemical penetration enhancement involves the use of chemicals that can reversibly compromise the skin's barrier function and allow the entry of otherwise poorly penetrating molecules into the membrane and through to the systemic circulation (Naik et al., 2000). Physical penetration enhancement relies on providing a reservoir of drug on the skin surface from which the required levels of drug delivery can be achieved (Thomas and Finnin, 2004). A new technology named Pheroid™ technology is also a way to enhance penetration across the skin. This technology uses vesicular structures with no phospholipids or cholesterol to enhance penetration (Grobler et al., 2008).

2 Materials and methods

2.1 Materials

The active ingredient nicotinamide was obtained from Sigma-Aldrich Corporation (Johannesburg, South Africa). The other ingredients used in the formulation of the semi-solid products were obtained as follows: liquid paraffin, tween, methylparaben, propylparaben and cetyl alcohol were obtained from Merck Laboratory Supplies (Midrand, South Africa). Span 60 was obtained from Fluka Analytical (Germany). Xanthan gum was supplied by Warren Chem Specialities (Johannesburg, South Africa) and tocopherol was ordered from Chempure (Pretoria, South Africa). Potassium dihydrogen orthophosphate and sodium hydroxide used for the preparation of phosphate buffered solution (PBS) were supplied by Merck Laboratory Supplies (Midrand, South Africa). Nile red and THF (tetrahydrofuran) from Sigma-Aldrich Corporation (Johannesburg, South Africa) was used. Octanesulphonic acid and HPLC (High Performance Liquid Chromatograph) analytical grade methanol was obtained from Merck Laboratory Supplies (Midrand, South Africa). Deionised HPLC grade water prepared with a Milli-Q® water purification system (Millipore, Milford, USA) was used throughout the entire study.

2.2 Methods

2.2.1 Formulation of semi-solid products

Four semi-solid products were formulated with nicotinamide as the active ingredient for the treatment of acne. A cream, Pheroid™ cream, emulgel and Pheroid™ emulgel were formulated.

2.2.2 Standard preparation

The following ingredients were weighed off and dissolved in a 100 ml volumetric flask: nicotinamide (60.0 mg); methyl paraben (8.0 mg); propyl paraben (1.6 mg); BHT (butylated hydroxytoluene) (4 mg) and tocopherol (4 mg). It was then made up to volume with methanol/HPLC water and injected into the HPLC in triplicate.

2.2.3 Sample preparation

2 g of each formulation at each condition was weighed off in 100 ml volumetric flasks in duplicate. The cream and Pheroid™ cream samples were made up to volume with THF, and

the emulgel and Pheroid™ emulgel samples were made up to volume with HPLC water. The solutions were filtered and injected into the HPLC in duplicate.

2.2.4 Stability testing

In this study the four different semi-solid formulations that were formulated, were stored at 25°C/60% RH (relative humidity), 30°C/60% RH and 40°C/70% RH. The following stability tests were done on months 0, 1, 2, 3 and 6: concentration assay, pH, viscosity, confocal laser scanning microscopy (CLSM), visual appearance and mass loss. According to the ICH (2003) a significant change for a drug product is defined as a 5% change in assay from its initial value; any degradation product's exceeding its acceptance criterion; failure to meet the acceptance criteria for appearance (e.g., colour, phase separation, caking, hardness); and failure to meet the acceptance criterion for pH.

2.2.4.1 Concentration assay

An Agilent® 1100 Series HPLC system was used for the analysis (Agilent Technologies, Palo Alto, CA). The instrument is designed with an Agilent® 1100 pump, diode array detector, auto sampler injection mechanism and Chemstation Rev. A.06.02 software for data acquisition and analysis. Analysis was performed in a controlled laboratory environment at 25°C. High performance silica based, reversed phase Phenomenex® Luna C18 (2) column, (250 × 4.6 mm) with a 5 µm particle size was used (Phenomenex®, Torrance, CA). The mobile phases consist of a filtered and degassed mixture of 1 g octanesulphonic acid in 1000 ml of HPLC water - the pH was set at 3.5 with 10% phosphoric acid, and methanol. The solvents of the standard solution were methanol and HPLC water. For the preparation of samples, THF was used for the preparation of cream samples and HPLC water was used for the preparation of gel samples. The flow rate was set to 1.0 ml/min and the injection volume was 5 µl. The detection wavelength was set to 220 nm. The retention times of the active ingredients that were analysed were as follows: nicotinamide 2.5 min, methyl paraben 6.8 min, propyl paraben 9.0 min, BHT 12.9 min and tocopherol 19.5 min. The run time was 25 min with a post runtime of 5 min.

2.2.4.2 pH

The apparatus that was used to measure the pH of the formulations was a Mettler Toledo pH meter (made in Switzerland). A Mettler Toledo Inlab® 410 electrode was used and the apparatus was calibrated each time before use. The pH of each formulation at each condition was measured in triplicate.

2.2.4.3 Viscosity

A Brookfield Viscometer (model DV II, Stoughton, Massachusetts, USA) was used. The formulation was placed in the water bath to reach a temperature of 25°C. The spindle (Stoughton, MA) was placed in the formulation and the rate was specified. The viscosity reading was measured every 10 sec for 5 min. Approximately 32 readings were obtained and the average viscosity was determined.

2.2.4.4 Confocal laser scanning microscopy

Samples were prepared by weighing off 0.1 g of each formulation under each condition. 2 µl nile red and 1000 µl HPLC water were added. The samples were vortexed and placed in a dark cupboard. 25 µl of the solution were placed on a microscope plate and micrographs were taken with a Nikon PCM 2000 CLSM with a He/Ne laser-543 nm and an Argon ion laser with 457 – 517 nm.

2.2.4.5 Visual appearance

The visual appearance of each formulation was assessed by comparing the colour of the cream or emulgel to paint colour cards. Photos were taken with a camera (Canon E240) and compared to each other.

2.2.4.6 Mass loss

The apparatus that was used to determine the mass loss of each formulation was a Shimadzu (Japan) scale. The mass of each formulation at each condition was determined in triplicate. After the indicated time intervals the mass of each formulation was determined and was subtracted from the original mass to determine the mass loss.

2.2.5 Physicochemical properties

2.2.5.1 Aqueous solubility

The aqueous solubility of nicotinamide was determined by preparing saturated solutions in PBS with a pH of 7.4. The solutions were stirred with magnetic bars in a 32 °C water bath for 24 h. An excess amount of nicotinamide was inserted in order to keep the solution saturated at all times. The solutions were then filtered, diluted and analysed by HPLC. This experiment was performed in triplicate.

2.2.5.2 Partition coefficient (Log D)

The experimental *n*-octanol-PBS partition coefficient (log D) was performed as follows: pre-saturated *n*-octanol and PBS (pH 7.4) were prepared by vigorously stirring equal amounts of each for 24 h, after which the two layers were separated. Nicotinamide (9.9 mg) was dissolved in 20 ml pre-saturated PBS. The nicotinamide pre-saturated PBS (3 ml) was placed in a test-tube and pre-saturated *n*-octanol (3 ml) was added. It was shaken for 3 h at 32°C and then centrifuged at 4500 rpm for 10 min. This experiment was performed in quaternary. The aqueous phase was analysed by HPLC. The logarithmic ratio of the concentration in the *n*-octanol phase to the concentration in the PBS was used to calculate the log D.

2.2.6 Diffusion experiments

2.2.6.1 Skin preparation

Abdominal skin of Caucasian female patients was obtained after cosmetic abdominoplastic surgery. Ethical approval for obtaining and preparing the skin was provided by the Research Ethics Committee of the North-West University under the reference number 04D08. The surgeon's permission was asked before asking consent from the patient. To ensure anonymity, the identity of the patient will not be published. The skin was frozen at -20°C not longer than 24 h after it had been surgically removed. Before preparation, the skin was thawed to room temperature. The adipose layer was carefully removed with a scalpel. The skin was placed on Whatman® filter paper with the stratum corneum facing upwards. Circles with a diameter of ±15 mm were punched into the skin. The skin circles were placed on aluminium foil before the foil was carefully closed and put into a Ziploc® bag. It was kept frozen at -20°C until used. Prior to

the diffusion study, the skin circles were thawed to room temperature before mounting them in the diffusion apparatus.

2.2.6.2 Preparation of PBS (pH 7.4) buffer

Potassium dihydrogen orthophosphate (13.62 g) was weighed off and dissolved in 500.0 ml HPLC water. Sodium hydroxide (3.15 g) was weighed off and dissolved in 786.8 ml HPLC water. The two solutions were mixed and the pH was set to 7.4 with 10% orthophosphoric acid. The solution was filtered and degassed.

2.2.6.3 Diffusion studies

Vertical Franz diffusion cells with a receptor capacity of approximately 2 ml and a diffusion area of 1.075 cm^2 were used in the study. In each study 10 Franz cells were used. These cells consist of a donor (top; contained semi-solid formulation with active) and receptor (bottom; filled with PBS at pH 7.4) compartment. A small magnetic stirring bar was placed in the receptor compartment of each Franz cell to maintain stirring throughout the experiment. The skin circles or membranes were mounted between the receptor and donor compartment with the stratum corneum facing upwards (towards the donor compartment). Dow Corning® high vacuum grease was used to seal the cells to prevent any leakage. The donor and receptor compartments were secured by a horseshoe clamp. The receptor compartments were filled with PBS (pH 7.4) while making sure that no air bubbles had formed. The donor compartments were filled with enough of the semi-solid formulation to keep the skin saturated. It was then covered with Parafilm® to prevent evaporation. The Franz cells were placed in a 37°C water bath in order to attain a skin temperature of 32°C. The entire content of the receptor phases was withdrawn at specific time intervals and replaced with fresh PBS (pH 7.4) that was pre-heated to 32°C. HPLC vials were then filled with the withdrawn PBS (receptor phase). The concentration of nicotinamide was immediately analysed by HPLC.

2.2.6.4 Skin diffusion

The method as discussed in 2.2.6.3 was used for the skin diffusion studies. The entire content of the receptor phases was withdrawn and replaced with fresh PBS (pH 7.4) after 20, 40, 60,

80, and 100 min, as well as 2, 4, 6, 8, 10 and 12 h. Tape stripping was performed after the 12 h withdrawal (see section 2.2.6.6).

2.2.6.5 Membrane permeation

The method as discussed in 2.2.6.3 was used for the membrane diffusion studies. The entire content of the receptor phases was withdrawn and replaced with fresh PBS (pH 7.4) after 30, 60, and 90 min, as well as 2, 4 and 6 h. The aim of the membrane studies was to determine whether nicotinamide was released from the formulations. Therefore there is a difference in withdrawal times from the diffusion studies.

2.2.6.6 Tape stripping

According to Lademann et al. (2009) tape stripping is a simple and efficient method for the assessment of quality and efficacy of cosmetical and dermatological formulation. After topical application and penetration of formulations, the cell layers of the stratum corneum are successively removed from the same skin area using adhesive films. The tape strips contained the amount of penetrated formulation, which can be analysed by chemical methods (Lademann et al., 2009). At the end of the diffusion study the diffusion cells were carefully taken apart and the pieces of skin were pinned onto a piece of Parafilm®, stapled to a solid surface. The diffused area could clearly be seen. The pieces of skin were dabbed dry with tissue. Pieces of 3M Scotch® Magic™ Tape were cut into sizes big enough to cover the diffused area, and small enough not to overlap the areas outside the diffused area. The first tape strip was discarded, as it is seen as part of the cleaning procedure. The next 15 strips (epidermis) were placed in a vial filled with enough PBS (pH 7.4) to cover the strips. An indication of the complete removal of the stratum corneum is when the viable epidermal layer glistens. The vials were kept overnight at 4°C. The remaining skin was cut into pieces to enlarge the surface area. It was placed in vials filled with enough PBS (pH 7.4) to cover the skin pieces and were kept overnight at 4°C. The tape samples were filtered and analysed by HPLC. The skin samples were homogenised and filtered in turn to be analysed by HPLC.

2.2.6.7 HPLC analysis of nicotinamide

A HPLC method had already been developed and validated in conjunction with Prof Jan du Preez from the Analytical Technology Laboratory at the North-West University, Potchefstroom Campus, South Africa. An Agilent® 1200 Series HPLC equipped with an Agilent® 1200 pump, auto sampler injection mechanism and UV detector was used (Agilent Technologies, Palo Alto, CA). The same analysis software and column was used as described in section 2.2.4.1. The mobile phase consisted of octanesulphonic acid. The flow rate, injection volume was the same as described in section 2.2.4.1. The UV-detector was set at 220 nm for the detection of nicotinamide. The retention time of nicotinamide was between 2 and 3 min, and the runtime was 8 min. The solvent used was PBS. The PBS was prepared by accurately weighing and dissolving potassium dihydrogen orthophosphate (2.7 g) and potassium hydroxide (0.95 g) in 1000 ml HPLC water. The pH of the PBS was established by 10% orthophosphoric acid to 7.4. Analyses were performed in a controlled laboratory environment at 25°C.

2.2.6.8 Data analysis

For the diffusion studies the cumulative concentration of the drug that permeated the skin was plotted against time. The linear portion of the graph represented the flux of nicotinamide. Average flux values were obtained by the slope of the straight line. The profiles exhibited character with a clear steady-state flux between 0 and 2 h. The yield of each cell was expressed as a percentage of the applied concentration. The percentage yield after 6 and 12 h was also determined for skin and membrane studies diffusion, respectively.

2.2.6.9 Statistical analysis

For statistical analysis the median (statistically calculated centre of a set of data) of the flux was examined using Statistica (Statsoft, 2008) and SAS (SAS Institute Inc., 2005). If there is a huge variation in the experimental flux values, the median flux is a more exact method to determine flux (Gerber et al., 2008). A Kruskal-Wallis test was performed to determine a p-value. A p-value less than 0.05 would indicate a statistical significant difference between the data in the different groups (Steyn et al., 1994).

3 Results and discussion

3.1 Formulation of semi-solid products

A 3% nicotinamide cream and 3% nicotinamide Pheroid^{TM} cream were prepared in sufficient quantities and stored at different temperatures to perform stability tests. The cream and Pheroid^{TM} cream applied easily and was not too oily. Both had a homogeneous white texture. The Pheroid^{TM} cream was a little bit oilier than the cream.

A 3% nicotinamide emulgel and 3% nicotinamide Pheroid^{TM} emulgel were prepared in sufficient quantities and stored at different temperatures to perform stability tests. The emulgel and Pheroid^{TM} emulgel had a clear white colour and applied easily.

3.2 Stability tests

3.2.1 Concentration assay

Table 1: Indication of which formulations were stable during the concentration assay over the whole stability testing period

In the cream, the decrease in concentration after 6 months when stored at 25°C/60% RH was: nicotinamide (9%), methyl paraben (7%), propyl paraben (6%) and BHT (17%); at 30°C/60% RH it was: nicotinamide (5%), methyl paraben (10%), propyl paraben (3%) and BHT (9%); and at 40°C/70% RH it was: nicotinamide (7%), methyl paraben (6%), propyl paraben (8%) and BHT (10%).

In the Pheroid^{TM} cream, the decrease in concentration after 6 months when stored at 25°C/60% RH was: nicotinamide (9%), methyl paraben (4%), propyl paraben (9%), BHT (1%) and tocopherol (2%); at 30°C/60% RH it was: nicotinamide (6%), methyl paraben (2%), propyl paraben (7%), BHT (12%) and tocopherol (7%); and at 40°C/70% RH it was: nicotinamide (5%), methyl paraben (2%), propyl paraben (2%), BHT (1%) and tocopherol (21%).

In the emulgel, the decrease in concentration after 6 months when stored at 25°C/60% RH was: nicotinamide (6%), methyl paraben (5%), propyl paraben (35%) and BHT (60%); at 30°C/60% RH it was: nicotinamide (6%), methyl paraben (2%), propyl paraben (14%) and BHT (56%); and

at 40°C/70% RH it was: nicotinamide (7%), methyl paraben (1%), propyl paraben (39%) and BHT (36%).

In the Pheroid^{TM} emulgel, the decrease in concentration after 6 months when stored at 25°C/60% RH was: nicotinamide (7%), methyl paraben (8%), propyl paraben (29%), BHT (25%) and tocopherol (61%); at 30°C/60% RH it was: nicotinamide (5%), methyl paraben (10%), propyl paraben (6%), BHT (10%) and tocopherol (50%); and at 40°C/70% RH it was: nicotinamide (8%), methyl paraben (8%), propyl paraben (12%), BHT (15%) and tocopherol (17%).

The following ingredients in the different formulations stayed within the acceptable limits during the six months period: methyl paraben in the Pheroid^{TM} cream (25°C/60% RH and 30°C/60% RH), methyl paraben in the emulgel (30°C/60% RH and 40°C/70% RH), propyl paraben in the cream (30°C/60% RH), BHT in the Pheroid^{TM} cream (25°C/60% RH and 40°C/70% RH) and tocopherol in the Pheroid^{TM} cream (25°C/60% RH).

Nicotinamide broke down (5% or more) in all the formulations at 25°C/60% RH, 30°C/60% RH and 40°C/70% RH) and therefore did not meet the requirements of the ICH. The degradation of all the active ingredients could be due to insufficient protection by the anti-oxidants and preservatives.

3.2.2 pH

The pH of the cream, Pheroid^{TM} cream, emulgel and Pheroid^{TM} emulgel remained relatively stable over six months. The biggest decrease in pH was that of the formulations stored at 40°C/70% RH with a decrease of 11.8% (cream), Pheroid^{TM} cream (18.2%), emulgel (19%) and Pheroid^{TM} emulgel (23.5%).

3.2.3 Viscosity

The viscosity of the cream, Pheroid^{TM} cream, emulgel and Pheroid^{TM} emulgel changed radically over the six months period. The cream showed a decrease in viscosity of 74.3%, the Pheroid^{TM} cream a decrease of 93.5% and in the emulgel a decrease of 79.0%. The decrease in viscosity can be explained as a reaction between some ingredients. The large decrease in viscosity is a warning of instability. In the Pheroid^{TM} emulgel the viscosity relatively stayed the same.

3.2.4 Confocal laser scanning microscopy

There was no significant change in the formulations' particle sizes over the six months period. The particle sizes ranged between 1 and 10 µm. The homogeneity of the products also stayed the same.

3.2.5 Visual appearance

The cream's and emulgel's colour didn't show any significant change over the six month period. The colour of the Pheroid™ cream and Pheroid™ emulgel showed a radical change from white to yellow over the six months period. This showed instability in the Pheroid™ products.

3.2.6 Mass loss

The mass of the cream, Pheroid™ cream, emulgel and Pheroid™ emulgel remained stable over the six months period. The biggest decrease in mass after 6 months was in the formulations stored at 40°C/70% RH. A decrease in mass was observed for cream (0.7%), Pheroid™ cream (1.1%), emulgel (2.8%) and Pheroid™ emulgel (1.5%) after 6 months. This shows that the containers used for storage of the products sealed well enough for limiting evaporation and preventing moisture absorption.

3.3 Physicochemical properties

3.3.1 Aqueous solubility and Log D

The aqueous solubility for a drug to ideally permeate the skin, is more than 1 mg/ml (Naik *et al.*, 2000). The solubility of nicotinamide was determined to be 212.92 mg/ml in PBS (pH 7.4) at 32 °C.

A log P of between 1 and 3 indicates the ability of a drug to dissolve in both water and oil, ensuring that the compound would permeate the skin comparatively fast (Roberts & Walters, 1998). The log D for nicotinamide was determined to be -0.32.

When looking at the solubility of nicotinamide of 212.92 mg/ml, it was expected that nicotinamide would permeate excellently through the skin. In contrast, the log D value (-0.32) indicated that permeation might not be optimal.

3.4 Diffusion experiments

3.4.1 Membrane release studies

The Pheroid^{TM} emulgel released the highest % nicotinamide (2.938%) after 6 h, followed by the emulgel (1.208%), Pheroid^{TM} cream (0.636%) and cream (0.578%). The Pheroid^{TM} emulgel released more than twice the amount of the emulgel and five times more than the Pheroid^{TM} cream and cream. This can be because of the emulgel's bigger water content that makes it more water soluble and increases the permeability (Bouwstra et al., 2003:22). There was a slight difference between the % nicotinamide released from the Pheroid^{TM} cream and cream. The Pheroid^{TM} cream released 10% more nicotinamide than the cream. This could be because of the penetration enhancing effect of the Pheroid^{TM} vesicles (Grobler et al., 2008).

3.4.2 Diffusion studies

The emulgel showed the highest average % nicotinamide diffused (0.069%) after 12 h followed by the Pheroid^{TM} emulgel (0.048%), Pheroid^{TM} cream (0.038) and cream (0.025). There was not a significant difference in the average % nicotinamide diffused after 12 h of each formulation.

The emulgel showed the highest average cumulative concentration (29.068 µg/ml) of nicotinamide after 12 h, followed by the Pheroid^{TM} emulgel (23.604 µg/ml), Pheroid^{TM} cream (21.409 µg/ml) and cream (13.913 µg/ml). There was a significant difference between the cream and the rest of the formulations. The Pheroid^{TM} cream showed very poor diffusion over the 12 h period. This can be because of the high oil content in the cream formulations. Absorption is related to molecular weight, where small molecules penetrate faster than larger ones. Therefore oil molecules, which are larger than water molecules, will penetrate slower. The emulgel formulation with the lowest oil content showed the best diffusion. With an increase in the oil content a decrease in diffusion was seen (Barry, 2002).

In all the formulations a definite flux could be seen between 0 and 2 h. The flux of each formulation is illustrated in Figure 1.

Figure 1: Average cumulative amount that penetrated through the skin as a function of time to illustrate the average flux (0-2 h) value in Pheroid™ cream, cream, Pheroid™ emulgel and emulgel

The emulgel (3.166 ± 0.079 μg/cm^2.h) gave the best average flux, followed by the Pheroid™ emulgel (2.918 ± 0.282 μg/cm^2.h). This could be because of rapid penetration into the skin via the hydrophilic transappendageal route (Dayan, 2007). The cream (1.849 μg/cm^2.h ± 0.080) and Pheroid™ cream (1.324 μg/cm^2.h ± 0.040) gave the smallest flux values. This could be because of the big oil molecules that made it difficult for permeation through the skin (Barry, 2002:513).

Figure 2: Box-plots and red lines of the flux values in Pheroid™ emulgel, Pheroid™ cream, emulgel and cream after application to the skin to illustrate median and average flux, respectively

Average values are determined by the sum of the data divided by the number of data points. The median values determine the centre point of the data points. The median flux values give a more accurate representation of the true flux since it takes all the data into consideration and is not affected by a distortion in the spread of the data, as in the case with average flux values (Gerber et al., 2008). The average flux values didn't differ much from the median flux values. This is because of the small variation in data points and therefore a small amount of outliers in the data. Hence either average or median flux values can be used to represent flux in this study.

3.4.3 Tape stripping

Figure 3: Comparison of the concentration (μg/ml) nicotinamide in the dermis and epidermis between the different formulations

Nicotinamide in the Pheroid™ emulgel (6.767 μg/ml) had the greatest concentration into the epidermis followed by the cream (5.044 μg/ml), the emulgel (4.816 μg/ml) and lastly, the Pheroid™ cream (3.655 μg/ml). The Pheroid™ emulgel had 25% more nicotinamide in the

epidermis than the cream, 29% more than the emulgel and 46% more than in the Pheroid™ cream. This shows that the Pheroid™ vesicles in the Pheroid™ emulgel increased the penetration to the epidermis. The Pheroid™ vesicles in the Pheroid™ cream on the other hand, didn't show any increase in penetration into the epidermis when comparing it to the other formulations.

Nicotinamide in the Pheroid™ cream (26.029 μg/ml) had the greatest concentration in the dermis, followed by the Pheroid™ emulgel (15.147 μg/ml), the cream (12.616 μg/ml) and finally, the emulgel (5.393 μg/ml). The Pheroid™ cream showed almost twice the concentration nicotinamide in the dermis than the Pheroid™ emulgel and cream and five times more than in the emulgel. This shows that the Pheroid™ vesicles in the Pheroid™ cream increased the penetration to the dermis (Grobler et al., 2008).

The concentration nicotinamide in the dermis is considerably higher than the concentration nicotinamide in the epidermis in all the formulations. This shows that nicotinamide penetrated deeper than just the top layer of the skin. It penetrated into the dermis. For the treatment of acne this is exactly where the action of treatment is needed, because acne develops from the sebaceous glands and follicles which are situated in the dermis (Barry, 1983).

When comparing the concentration in the dermis with the average cumulative concentration that diffused through the skin after 12 h, it is seen that the cream and Pheroid™ cream didn't show a great difference in the concentration nicotinamide. For the Pheroid™ emulgel there was a small difference between the concentration in the dermis and the average cumulative concentration that diffused through the skin after 12 h, but for the emulgel there was a great difference. The concentration nicotinamide in the epidermis is considerably lower than the average cumulative concentration that diffused through the skin after 12 h. This also shows a deeper penetration of nicotinamide, although it is not necessary for nicotinamide in the treatment of acne to be systemically absorbed (Barry, 1983).

3.4.4 Statistical analysis

When the data of the membrane release studies were statistically analysed and compared to each other with the Kruskal-Wallis test, certain p-values were obtained to indicate whether there

was a significant difference or not between the different groups. When the average cumulative concentration of nicotinamide after 6 h of each formulation was compared to each other, the p-value was 0.0001. This means that there is a significant difference between the different formulations. There was a significant difference between the cream and Pheroid™ gel with a p-value of 0.000035. There was also a significant difference between the gel and Pheroid™ gel with a p-value of 0.022 and between the Pheroid™ cream and Pheroid™ gel with a p-value of 0.018.

When the data of the skin diffusion studies were statistically analysed and compared to each other, certain p-values were obtained to indicate whether there was a significant difference or not, between the different groups. When the median cumulative concentration of nicotinamide after 12 h of each formulation was compared to each other the p-value was 0.0164. This means that there was a significant difference between the different formulations. There was a significant difference between the cream and gel with a p-value of 0.010795.

When the tape stripping data was statistically analysed and compared to each other, certain p-values were obtained to indicate whether there was a significant difference or not between the different groups. When the median concentration of nicotinamide in the epidermis of each formulation was compared to each other, the p-value was 0.3368. This means that there was no significant difference between the different formulations.

When the median concentration of nicotinamide in the dermis of each formulation was compared to each other, the p-value was 0.0130. This means that there was a significant difference between the different formulations. There was a significant difference between the gel and Pheroid™ cream (p = 0.0292) and between the gel and Pheroid™ gel (p = 0.0265).

When the median concentration of nicotinamide in the epidermis of all the formulations was compared to the average flux value, the p-value was 0.4377. This means that there was no significant difference between the average concentration in the epidermis and the average flux value.

When the median concentration of nicotinamide in the dermis of all the formulations was compared to the average flux value, the p-value was 0.0796. This means that there was no

significant difference between the average concentration in the dermis and the average flux value.

4 Conclusion

Nicotinamide's aqueous solubility value of 212.92 mg/ml predicted good permeation through the skin in contrast to the log D value of -0.32 that predicted that permeation might not be optimal. In the membrane release studies the Pheroid™ emulgel released the highest % nicotinamide after 6 h. This could be because of the emulgels' greater water content that makes it more water soluble and increases permeation. The nicotinamide released from the Pheroid™ cream and Pheroid™ emulgel (when compared to the formulations without Pheroid™) showed the enhancing effect of the Pheroid™ vesicles.

In the skin diffusion studies the emulgel showed the highest average cumulative concentration of nicotinamide as well as the highest average % nicotinamide diffused after 12 h. The cream formulations showed very poor diffusion over the 12 h period. The high oil content in the cream formulations could be the reason for this problem. With an increase in the oil content a decrease in diffusion was seen.

The emulgel had the highest average flux value which might be attributed to rapid penetration into the skin via the hydrophilic transappendageal route.

According to Barry (2002), the eventual aim in dermatological biopharmaceutics is to design drugs with selective penetrability for incorporation into vehicles or devices that deliver the medicaments to the active site, at a controlled rate and concentration, for the necessary time. Therefore the aim was to deliver nicotinamide to the dermis and epidermis where the drug action is most needed for the treatment of acne.

The Pheroid™ vesicles in the Pheroid™ emulgel showed an increase in the penetration into the epidermis while the Pheroid™ vesicles in the Pheroid™ cream didn't show any increase. Although nicotinamide in the Pheroid™ cream showed the lowest concentration in the epidermis, it showed the highest concentration in the dermis. This shows that although Pheroid™ technology has advantages in enhancing skin penetration, it differs from formulation to formulation. Pheroid™ technology has great advantages but unfortunately can't be set as a rule for all formulations.

The concentration nicotinamide in the dermis was considerably higher than the concentration nicotinamide in the epidermis in all the formulations. This shows that nicotinamide penetrated deeper than just the top layer of the skin. It penetrated into the dermis where the action of treatment for acne is needed.

The concentration nicotinamide in the epidermis was considerably lower than the average cumulative concentration that diffused the skin after 12 h. This also shows a deeper penetration of nicotinamide.

Generally, the concentration nicotinamide in the dermis didn't differ much from the average cumulative concentration that diffused the skin after 12 h. As it is not necessary for nicotinamide to be systemically absorbed in the treatment of acne, it would be best to find a way to prevent systemically absorption and rather try to get a bigger concentration nicotinamide to the dermis and epidermis, where treatment is needed most.

Thus it can be said that the emulgel is the best formulation to use when transdermal delivery of nicotinamide is wanted and when a quick delivery of nicotinamide is needed. The Pheroid^{TM} emulgel is the best formulation to use when nicotinamide must be delivered in the epidermis and the Pheroid^{TM} cream is the best formulation to use when nicotinamide must be delivered in the dermis like in the treatment of acne.

Unfortunately the change in colour, viscosity and concentration of the active ingredients in the formulations showed instability of the products over the six month period. In future different anti-oxidants and preservatives should be considered and a higher concentration thickening agent should be used.

5 Acknowledgements

The authors wish to express their gratitude towards the National Research Foundation (NRF) of South Africa for the financial support as well as the Unit for Drug Research and Development of the North-West University, Potchefstroom Campus, South Africa. We would like to thank Prof. F. Steyn for the statistical analysis of the data. Special thanks to Prof. L.A Chait from Park Lane Clinic (Johannesburg – South Africa) for donation of the abdominal skin after cosmetic abdominoplastic surgery that was used during the transdermal diffusion studies.

6 References

Barry, B.W., 1983. Dermatological formulations, New York, Marcel Dekker, 480p.

Barry, B., 2002. Transdermal drug delivery. In: Aulton, M.E., (Ed.), Pharmaceutics: The science of dosage form design, 2nd ed, London, Churchill Livingston, pp. 499-533.

Bouwstra, J.A., Honeywell-Nguyen, P.L., Gooris, G.S., Ponec, M., 2003. Structure of the skin barrier and its modulation by vesicular formulations. Prog. Lipid Res. 42, 1-36.

Brajac, I., Zulle-Bilic, L., Tkalcic, K.L., Gruber, F., 2004. Acne vulgaris: myths and misconceptions among patients and family physicians. Patien. Educ. Couns. 54, 21-25.

Dayan, N., 2007. Pathways for Skin Penetration. In: Kozlowski, A., (Ed.), Biotechnology in Cosmetics: Concepts, Tools and Techniques, USA, Allured Publishing Corporation, pp. 29-42.

Gerber, M., Breytenbach, J.C., Du Plessis, J., 2008. Transdermal penetration of zalcitabine, lamivudine and synthesized N-acyl lamivudine esters. Int. J. Pharm., 351, 186-193.

Gollnick, H. and Cunliffe, W., 2003. Management of acne – A report from a global alliance to improve outcomes in acne. J. am. Acad. Dermatol., 49, 1-38.

Grobler, A., Kotze, A., Du Plessis, J., 2008. The design of a skin-friendly carrier for cosmetic compounds using Pheroid™ technology. In: Wiechers, J. (Ed.), Science and applications of skin delivery systems, Allured, Wheaton, IL., pp. 283-311.

Ho, C.K., 2003. Probabilistic modelling of percutaneous absorption for risk-based exposure assessments and transdermal drug delivery. Transport. Res. Rec., 1, 47-69.

International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH) Q1A(R2)., 2003. Stability testing of new drug substances and products. pp.1-22.

Krowchuk, D.P., 2000. Managing acne in adolescents. Pediatr. Clin. N. Am., 47, 841-857.

Lademann, J., Jacobi, U., Surber, C. Weighmann, H.J. & Fluhr, J.W., 2009. The tape stripping procedure – evaluation of some critical parameter. Eur J Pharm, 72, 317-323.

Long, C.C., 2002. Common skin disorders and their topical treatment. In: Walter, K.A., (Ed.), Dermatological and Transdermal formulation, Vol. 119, Marcel Dekker, New York, pp. 41-60.

Lupo, M.P., 2001. Antioxidants and Vitamins in cosmetics. Clin. Dermatol., 19, 467-473.

Naik, A., Kalia, Y.N., Guy, R.H., 2000. Transdermal drug delivery: overcoming the skin's barrier function. Pharm. Sci. Technol. To., 3, 318-326.

Otte, N., Borelli, C., Korting, H.C., 2005. Nicotinamide – biologic action of an emerging cosmetic ingredient. Int. J. Cosmetic. Sci., 27, 255-261.

Roberts, M.S. & Walters, K.A., 1998. The relationship between structure and barrier function of skin. In Roberts, M.S. & Walters, K.A. (Eds.), Dermal absorption and toxicity assessment. Marcel Dekker, New York, pp. 1-42.

SAS Institute., 2005. SAS OnlineDoc, version 9.1. http://www.sas.com Date of access: 10 Nov. 2009.

Shaw, L. and Kennedy, C., 2007. The treatment of acne. Paediatr. Child Healt., 17, 385-389.

Statsoft., 2008. Statistica, version 8.0. http://www.statsoft.co.za Date of access: 10 Nov. 2009.

Steyn, A.G.W., Smit, C.F., Du Toit, S.H.C. & Strasheim, C., 1994. Modern Statistics in Practice. Pretoria: J.L. van Schaik. 640p.

Thomas, B.J. and Finnin, B.C., 2004. The transdermal revolution. Drug Discov. Today, 9, 697-703.

FIGURE LEGENDS:

Figure 1: Average cumulative amount that penetrated through the skin as a function of time to illustrate the average flux (0-2 h) value in Pheroid^{TM} cream, cream, Pheroid^{TM} emulgel and emulgel

Figure 2: Box-plots and red lines of the flux values in Pheroid^{TM} emulgel, Pheroid^{TM} cream, emulgel and cream after application to the skin to illustrate median and average flux, respectively

Figure 3: Comparison of the concentration (μg/ml) nicotinamide in the dermis and epidermis between the different formulations

TABLES:

Table 1: Indication of which formulations were stable during the concentration assay over the whole stability testing period

	Cream			**Pheroid™ cream**		
	25°C/60% RH	**30°C/60% RH**	**40°C/70% RH**	**25°C/60% RH**	**30°C/60% RH**	**40°C/70% RH**
Nicotinamide				X	X	
Methyl paraben						
Propyl paraben		X				
BHT				X		X
Tocopherol				X		
	Emulgel			**Pheroid™ emulgel**		
	25°C/60% RH	**30°C/60% RH**	**40°C/70% RH**	**25°C/60% RH**	**30°C/60% RH**	**40°C/70% RH**
Nicotinamide						
Methyl paraben		X	X			
Propyl paraben						
BHT						
Tocopherol						

FIGURES:

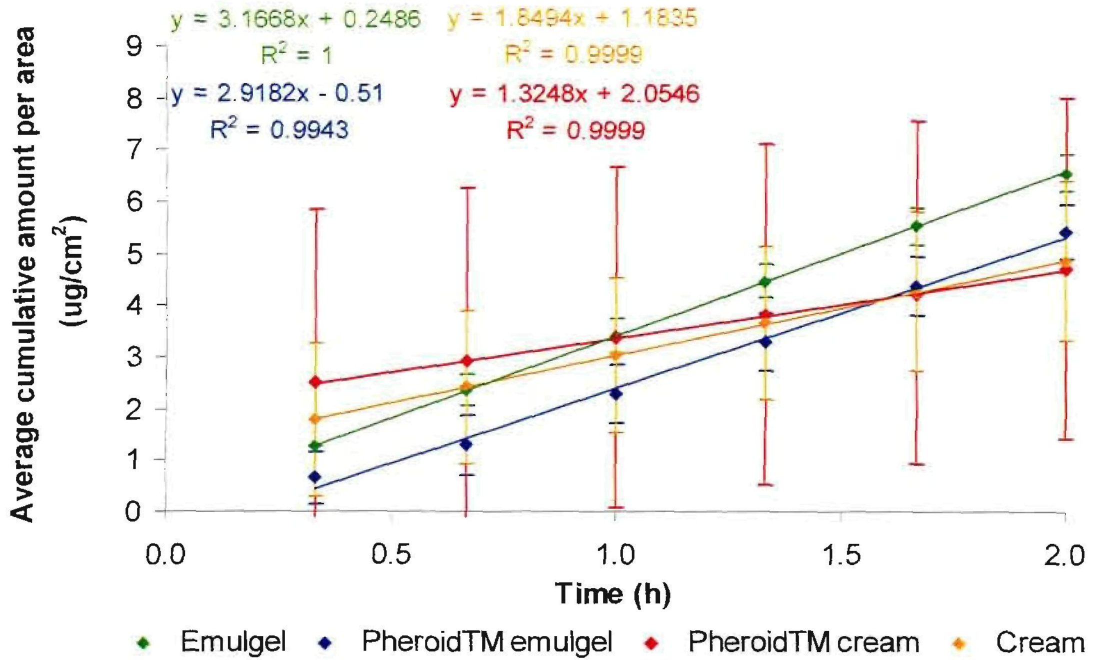


Figure 1: Average cumulative amount that penetrated through the skin as a function of time to illustrate the average flux (0-2 h) value in Pheroid™ cream, cream, Pheroid™ emulgel and emulgel

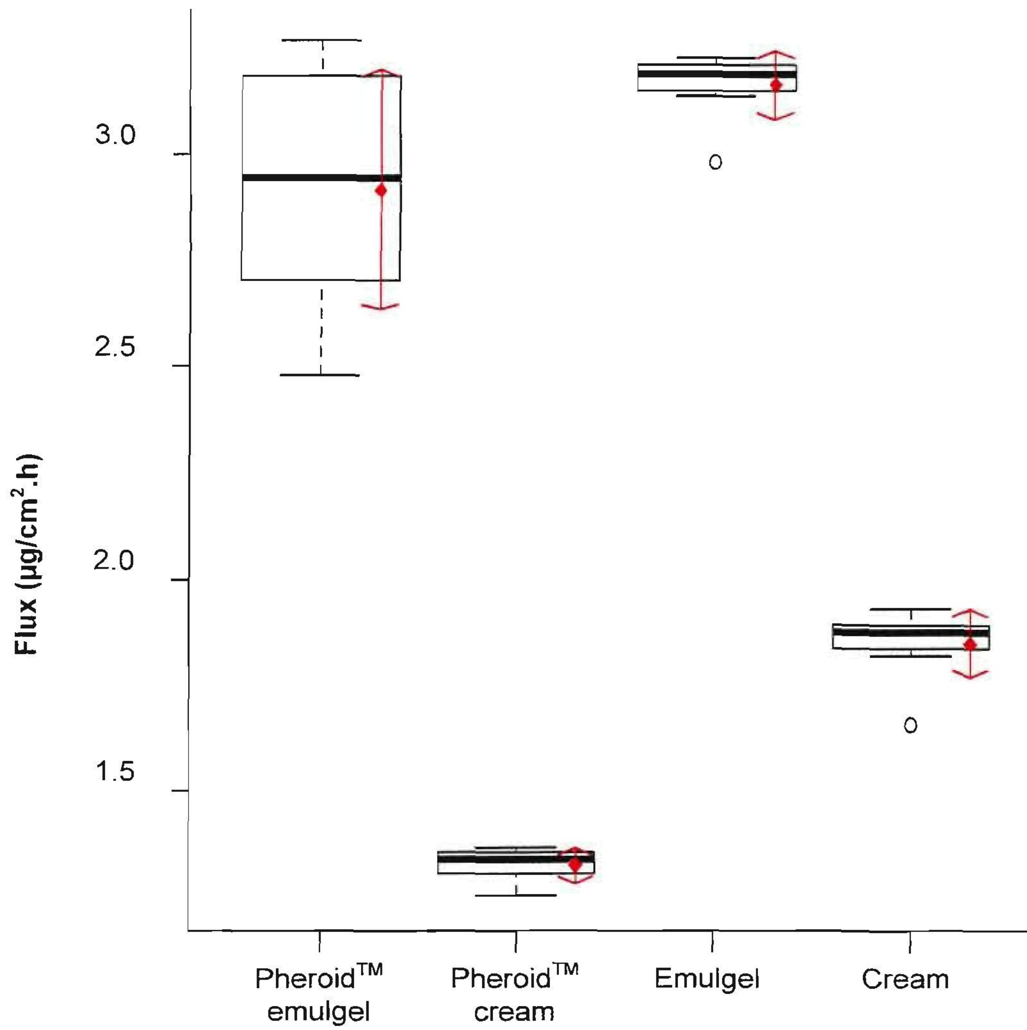


Figure 2: Box-plots and red lines of the flux values in Pheroid™ emulgel, Pheroid™ cream, emulgel and cream after application to the skin to illustrate median and average flux, respectively

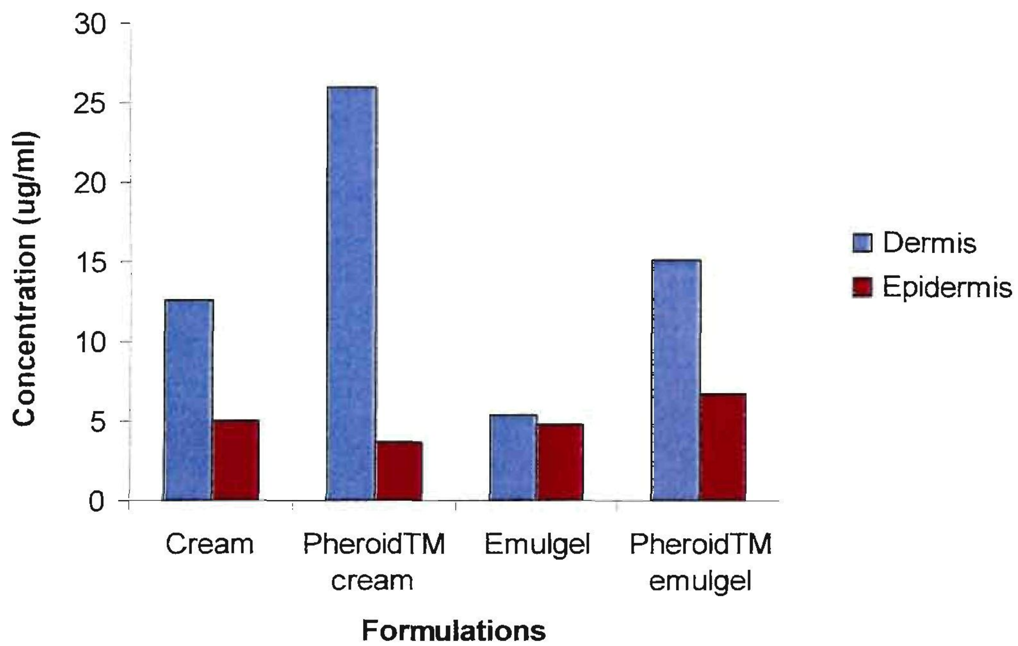


Figure 3: Comparison of the concentration (µg/ml) nicotinamide in the dermis and epidermis between the different formulations

CHAPTER 4

FINAL CONCLUSIONS AND FUTURE PROSPECTS

Acne is a very common skin disease and affects most adolescents and young adults (Gollnick & Cunliffe, 2003:1). It is characterised by comedones, papules, pustules and sometimes cysts and it commonly affects the face, chest and upper back (Barry, 1983:38). Because of the widespread use of topically applied antimicrobial agents for the treatment of acne, resistance of disease-related micro organisms developed. Therefore new strategies for the treatment of acne are necessary. Nicotinamide is a new approach to topical treatment of moderate inflammatory acne without the risk for developing resistance against micro-organisms (Otte *et al.*, 2005:257).

The aim of this study was:

- To formulate four different semi-solid formulations with nicotinamide as the active ingredient that can be used for the treatment of acne;
- To determine the stability of the formulated products over a 6 months period; and
- To determine the transdermal diffusion of nicotinamide in the different formulations.

A cream and emulgel with and without Pheroid™ vesicles were formulated. Stability tests were done on the different formulations over a 6 month period. The formulations were stored under different conditions namely, 25°C/60% RH, 30°C/60% RH and 40°C/70% RH. The pH, viscosity, mass loss, physical appearance and concentration were determined on months 0, 1, 2, 3 and 6. Unfortunately the change in colour, viscosity and concentration of the active ingredients in the formulations, indicated that the products were unstable over the six months period. The aqueous solubility and partition coefficient of nicotinamide were determined to see whether it was possible for nicotinamide to permeate the skin. With an aqueous solubility of 212.92 mg/ml, it was expected that nicotinamide would permeate excellently through the skin (Naik *et al.*, 2000:321). Unfortunately the log D value of -0.32 indicated that permeation may not be optimal (Roberts & Walters, 1998:34).

Membrane release studies were done to determine whether nicotinamide was released from the different formulations and the results confimed that nicotinamide was indeed released from all the formulations. The Pheroid™ emulgel released the highest percentage of nicotinamide (2.938%) after 6 hours. Skin diffusion studies were done to determine the concentration nicotinamide that permeated through the skin. Nicotinamide in the emulgel had the highest percentage of transdermal diffusion (0.069%) after 12 hours. The emulgel had the highest flux value of 3.166 µg/ml ± 0.079.

Tapestripping experiments were done to determine the concentration of nicotinamide that accumulated in the dermis and epidermis. Nicotinamide in the Pheroid™ emulgel had the highest concentration of 6.161 µg/ml in the epidermis. Nicotinamide in the Pheroid™ cream had the highest concentration of 16.047 µg/ml in the dermis.

Future prospects for further investigation include the following:

- The reason for the change in colour in the Pheroid™ formulations and an explanation for that;
- The reason for the significant change in viscosity in the formulated products should be determined. A higher concentration thickening agent should be added during the formulation of new products;
- Preservative testing of formulated products;
- A method to prevent nicotinamide from being absorbed systemically should be investigated. During the treatment of acne, nicotinamide should only reach the dermis and epidermis, where action is needed.

References

GOLLNICK, H. & CUNLIFFE, W. 2003. Management of acne – A report from a global alliance to improve outcomes in acne. *Journal of the American academy of dermatology,* 2003(49):1-38.

BARRY, B.W. 1983. Dermatological formulations. New York: Marcel Dekker. 480p.

OTTE, N., BORELLI, C. & KORTING, H.C. 2005. Nicotinamide – biologic action of an emerging cosmetic ingredient. *International journal of cosmetic science,* 2005(27):255-261.

NAIK, A., KALIA, Y.N. & GUY, R.H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical science & technology today,* 3(9):318-326.

ROBERTS, M.S. & WALTERS, K.A. 1998. The relationship between structure and barrier function of skin. (*In* Roberts, M.S. & Walters, K.A. *eds.* Dermal absorption and toxicity assessment. New York: Marcel Dekker. p. 1-42.)

APPENDIX A

VALIDATION OF THE HPLC ANALYTICAL METHOD FOR ASSAY ANALYSIS

A.1 Purpose of the validation

The purpose of this method validation was to ensure that the analytical method was sensitive and reliable in the determination of the amount of active ingredients in the different semi-solid formulations after each month of stability testing. The active ingredients include: nicotinamide, methyl paraben, propyl paraben, BHT (Butylated hydroxytoluene) and tocopherol.

A.2 Chromatographic conditions

Analytical instrument:	An Agilent® 1100 Series HPLC system was used for the analysis (Agilent Technologies, Palo Alto, CA). The instrument is designed with an Agilent® 1100 pump, diode array detector, autosampler injection mechanism and Chemstation Rev. A.06.02 software for data acquisition and analysis. Analysis was performed in a controlled laboratory environment at 25°C.
Column:	A high performance silica based, reversed phase Phenomenex® Luna C18 (2) column, (250 × 4.6 mm) with a 5µm particle size was used (Phenomenex®, Torrance, CA).
Mobile phase A:	A filtered and degassed mixture of 1g octanesulphonic acid in 1 L of HPLC water. The pH was set at 3.5 with 10% phosphoric acid.
Mobile phase B:	Methanol
Gradient table:	The gradient elution was employed starting at 50% octanesulphonic acid solution and 50% methanol for the first min, followed by a linear immerse to 100% methanol after 8 min. The composition was kept at 100% methanol until 25 min elapsed; thereafter the system was re-equilibrated at starting conditions for 5 min.
Solvent:	Methanol and HPLC water were used in the preparation of the standard solutions. For the preparation of samples, THF was

used for the preparation of cream samples and HPLC water was used for the preparation of gel samples.

Flow rate: 1.0 ml/min

Injection volume: 5 µl

Retention time: Nicotinamide eluted first after 2.5 min, followed by methyl paraben (6.8 min), propyl paraben (9.0 min), BHT (12.9 min) and tocopherol (19 min).

Run time: 25 minutes with a post runtime of 5 minutes.

In Figure A.1 the active ingredients with their retention times can be seen. Although BHA was not an active ingredient it was part of the Pheroid™ ingredients used in the formulation of the Pheroid™ cream and Pheroid™ emulgel.

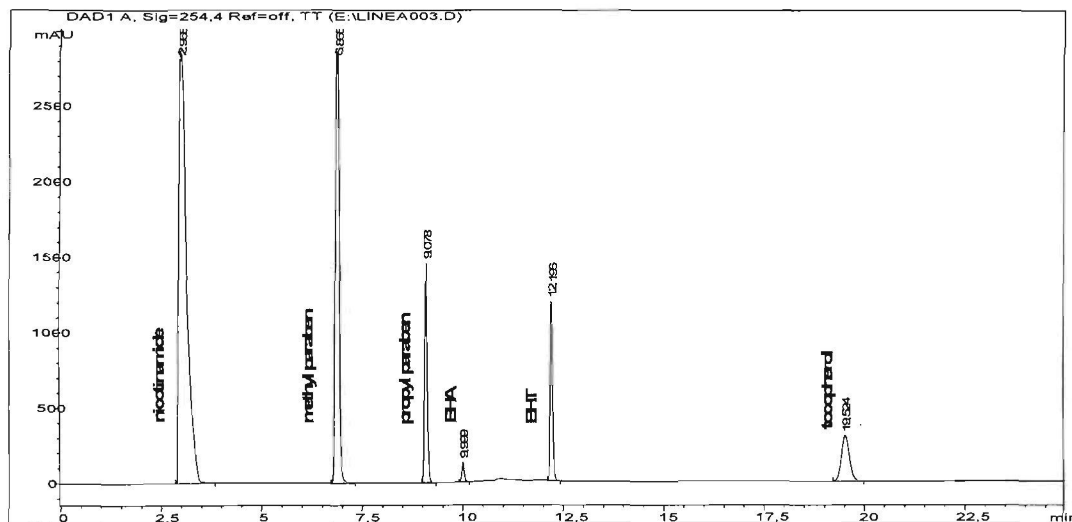


Figure A.1: Chromatogram of ingredients

A.3 Preparation of standard and samples

A.3.1 STANDARD PREPARATION

The following ingredients were weighed off to obtain a standard solution of 100% and were dissolved in a 50 ml volumetric flask. It was then made up to volume with methanol/HPLC water:

- Nicotinamide: 60 mg
- Methyl paraben: 8 mg
- Propyl paraben: 1.6 mg
- BHT: 4 mg
- Tocopherol: 4 mg

A.3.2 PLACEBO PREPARATION

To prepare a 100% placebo sample the following ingredients were weighed off and heated together:

- Vitamin F: 3.92 g
- Cremophor RH: 1.4 g
- Liquid paraffin: 24.92 g
- Span 60: 0.7 g
- Tween 80: 6.3 g
- Xantham gum: 0.7 g
- Cetyl alcohol: 12.46 g

A.3.3 SAMPLE PREPARATION

2 g cream was weighed off in a 50 ml volumetric flask and made up to volume with THF to obtain a 100% sample.

A.4 Validation parameters

A.4.1 LINEARITY

The linearity of an analytical method is described as its ability to obtain results that are directly proportional to the concentration of analyte in the sample.

Linear regression analysis was performed by injecting five different concentrations into the chromatograph. A 130% standard solution was prepared and dilutions were made to obtain concentrations between 5100 µg/ml and 9200 µg/ml.

The different concentration samples were injected in duplicate into the chromatograph.

A.4.1.1 LINEAR REGRESSION ANALYSIS

The linearity of the active ingredients were determined by performing linear regression analysis on the plot of the peak area ratios versus concentration (µg/ml).

The data is best described by a linear equation $y = mx + c$ where:

y = peak area ratios of the different active ingredients

m = slope

x = concentration of the different active ingredients in µg/ml

c = y-intercept

Nicotinamide

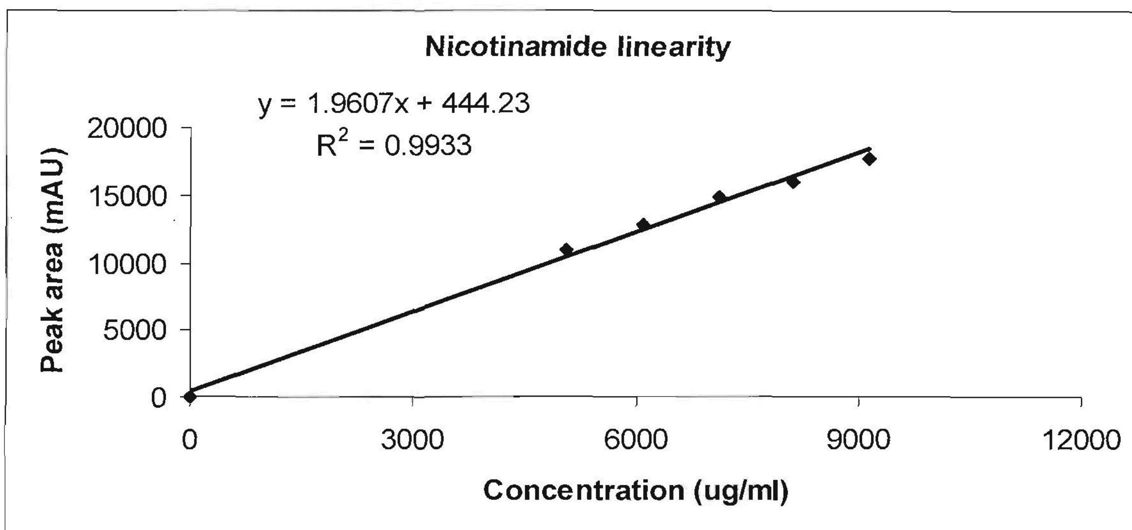


Figure A.2: Linear regression curve of nicotinamide standards

The regression value (r^2) obtained, indicates a high degree of linearity and therefore demonstrates the good stability of the analysis system.

Table A.1: Peak area ratio values of nicotinamide standards

Standard (µg/ml)	Peak area ratio
5076.5	10938.8
6091.8	12837.9
7107.1	14795.9
8122.4	16021.3
9137.7	17745.8
Slope	1.96
y-intercept	444.2
r^2	0.9933

Methyl paraben

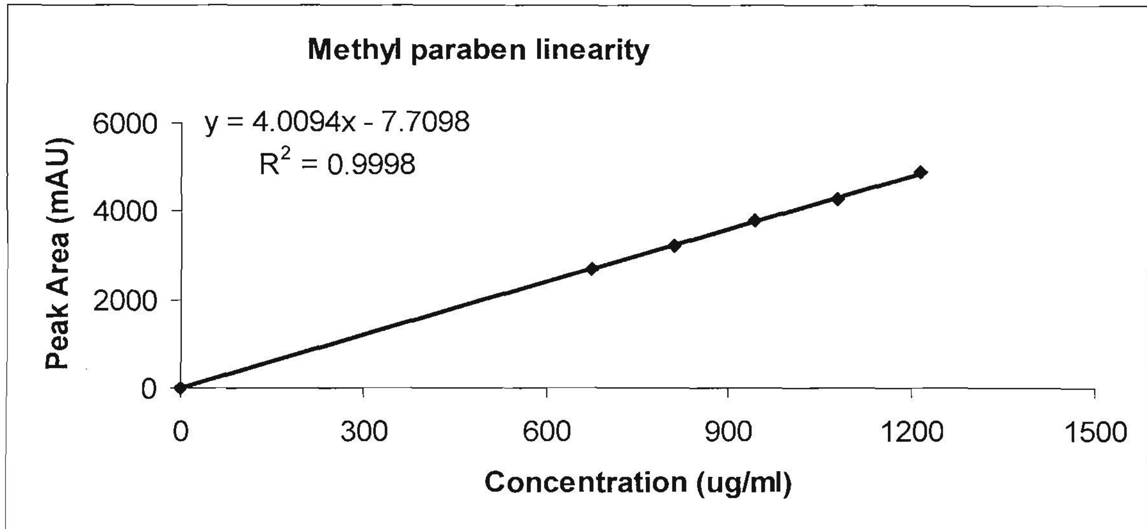


Figure A.3: Linear regression curve of methyl paraben standards

The regression value (r^2) obtained, indicates a high degree of linearity and therefore demonstrates the good stability of the analysis system.

Table A.2: Peak area ratio values of methyl paraben standards

Standard (µg/ml)	Peak area ratio
673.4	2671.5
808.0	3228.7
942.8	3803.1
1077.4	4274.6
1212.1	4875.4
Slope	4.0
y-intercept	7.7
r^2	0.9998

Propyl paraben

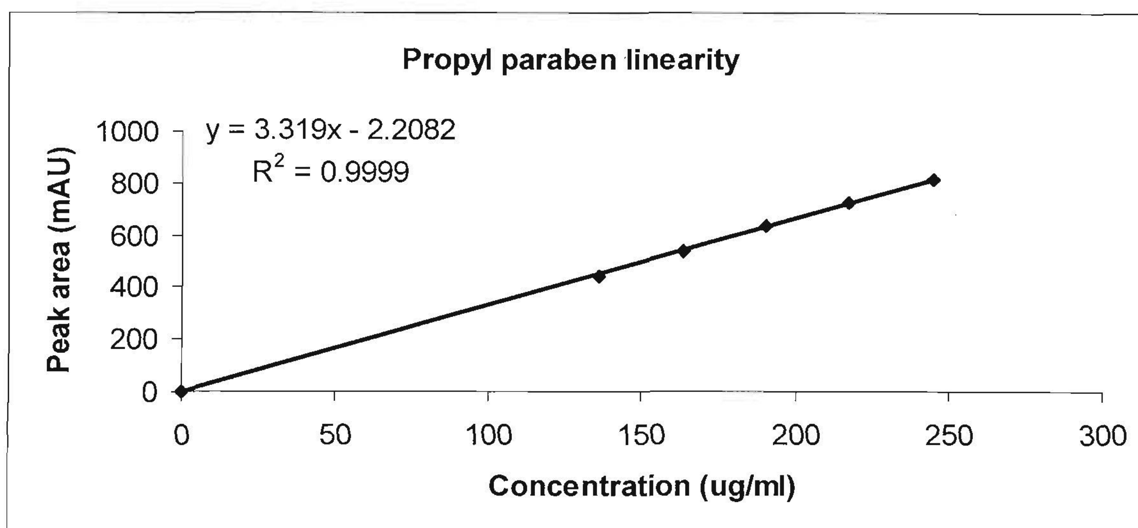


Figure A.4: Linear regression curve of propyl paraben standards

The regression value (r^2) obtained, indicates a high degree of linearity and therefore demonstrates the good stability of the analysis system.

Table A.3: Peak area ratio values of propyl paraben standards

Standard (µg/ml)	Peak area ratio
135.9	443.4
163.2	538.4
190.4	631.2
217.6	722.5
244.8	810.5
Slope	3.32
y-intercept	2.2
r^2	0.9999

BHT

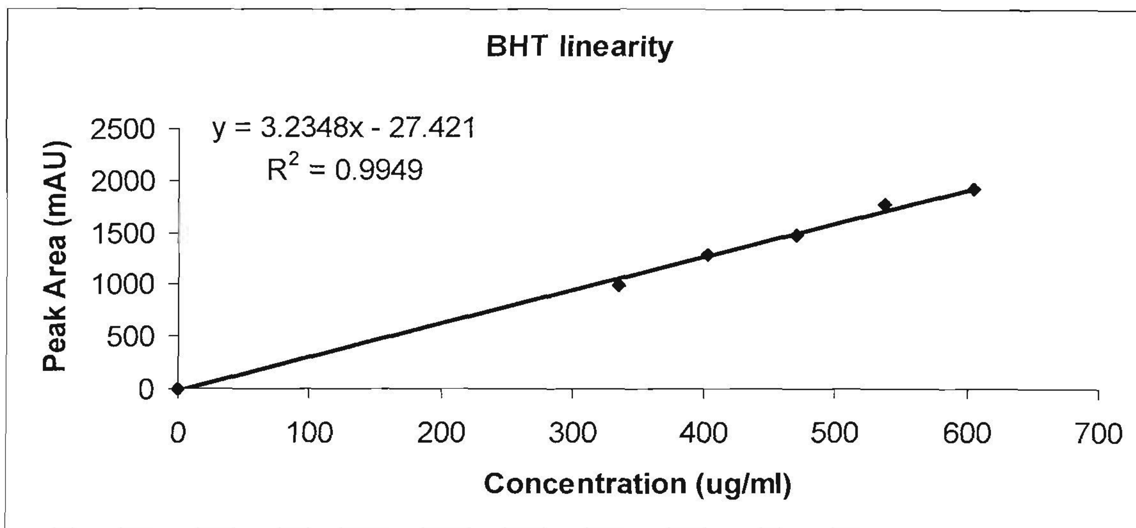


Figure A.5: Linear regression curve of BHT standards

The regression value (r^2) obtained, indicates a high degree of linearity and therefore demonstrates the good stability of the analysis system.

Table A.4: Peak area ratio values of BHT standards

Standard (µg/ml)	Peak area ratio
335.8	981.6
403.0	1292.2
470.2	1466.8
537.3	1778.6
604.5	1920.7
Slope	3.2
y-intercept	27.4
r^2	0.9949

Tocopherol

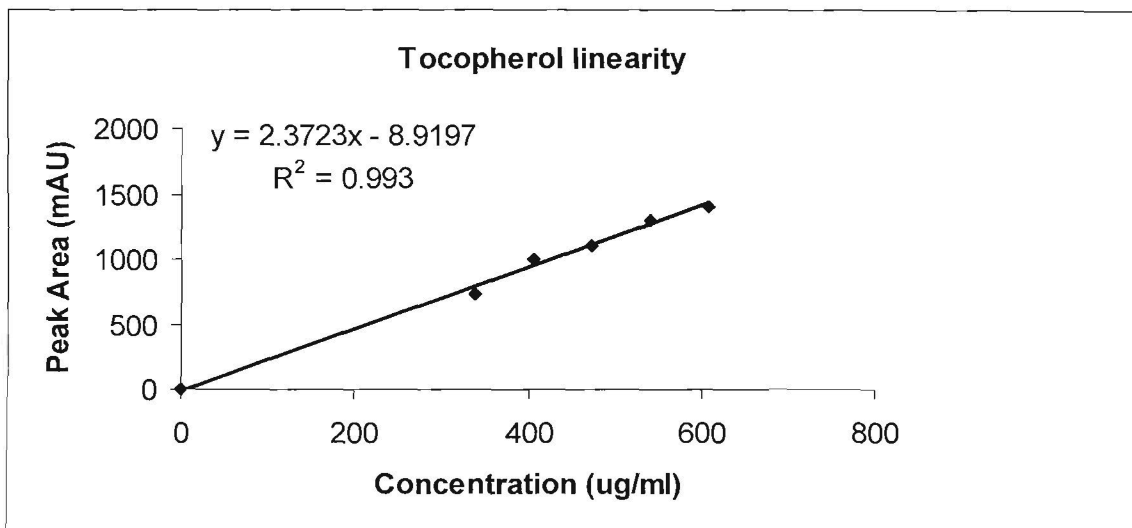


Figure A.6: Linear regression curve of tocopherol standards

The regression value (r^2) obtained, indicates a high degree of linearity and therefore demonstrates the good stability of the analysis system.

Table A.5: Peak area ratio values of tocopherol standards

Standard (μg/ml)	Peak area ratio
337.7	724.9
405.3	1006.3
472.8	1110.9
540.4	1304.7
607.9	1408.2
Slope	2.4
y-intercept	8.9
r^2	0.993

A.4.2 ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value, or an accepted reference and the value found.

A 100% standard solution was prepared as described in A.3.1.

A placebo solution was prepared (as described in A.3.2), and divided into nine 50 ml volumetric flasks. The first three flasks contained 1.6 g (80%) placebo solution, the next three flasks contained 2 g (100%) placebo solution and the last three flasks contained 2.4 g (120%) placebo solution.

The different placebo solutions were then spiked with the standard. The following procedure was then followed:

- 8 ml of standard was added to the 80% placebo solutions;
- 10 ml of standard was added to the 100% placebo solutions; and
- 12 ml of standard was added to the 120% placebo solutions

The placebo solutions with the spiked standard were made up to volume with methanol/HPLC water.

The different samples were injected in duplicate into the chromatograph.

A.4.2.1 ACCURACY ANALYSIS

Nicotinamide

Table A.6: Accuracy parameters of nicotinamide

Concentration spiked (μg/ml)	Peak area 1	Peak area 2	Mean	Recovery (μg/ml)	%
958.4	15719.4	15774.4	15746.9	978.3	102.1
958.4	15667.7	15807.1	15737.4	977.8	102.0
958.4	15715.8	15755.5	15735.7	977.6	102.0
1198	18620.5	18779.5	18700	1161.8	96.8
1198	18949.4	19020.8	18985.1	1179.5	98.5
1198	19493.2	18999.3	19246.3	1195.8	99.8
1437.6	21824.1	21771.6	21797.9	1354.3	94.2
1437.6	21999	21774.3	21886.7	1359.8	94.6
1437.6	22181.1	22181.1	22181.1	1378.1	95.9
				Mean	98.4
				SD*	3.0
				% RSD**	3.0

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The percentage nicotinamide recovery ranged between 94.2 % and 102.1% with an average recovery of 98.4%

Methyl paraben

Table A.7: Accuracy parameters of methyl paraben

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	%
131.2	1347.5	1363.2	1355.4	131.0	99.8
131.2	1365.4	1371.5	1368.5	132.2	100.8
131.2	1356.5	1353.9	1355.2	131.0	99.8
164	1700.2	1698.4	1699.3	164.2	100.1
164	1692.8	1703.3	1698.0	164.1	100.0
164	1690.3	1686.4	1688.4	163.1	99.5
196.8	2046.5	2033.2	2039.9	197.1	100.1
196.8	2046	2059.1	2052.6	198.3	100.8
196.8	2050.5	2050.5	2050.5	198.1	100.7
				Mean	100.2
				SD*	0.4
				% RSD**	0.4

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The percentage methyl paraben recovery ranged between 99.5 % and 100.8% with an average recovery of 100.2%

Propyl paraben

Table A.8: Accuracy parameters of propyl paraben

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	%
24	223.3	238.9	231.1	20.6	85.9
24	230.9	223.4	227.2	20.3	84.4
24	240.1	227.5	233.8	20.9	86.9
30	323.1	296.9	310.0	27.6	92.1
30	292	293.2	292.6	26.1	87.0
30	300.3	289.4	294.9	26.3	87.7
36	356.1	370.4	363.3	32.4	90.0
36	347.9	370.5	359.2	32.0	89.0
36	356.1	356.1	356.1	31.8	88.2
				Mean	87.9
				SD*	2.2
				% RSD**	2.5

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The percentage propyl paraben recovery ranged between 84.4 % and 92.1% with an average recovery of 87.9%

BHT

Table A.9: Accuracy parameters of BHT

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	%
67.2	742.1	679	710.6	69.6	103.6
67.2	758	709	733.5	71.8	106.9
67.2	730.5	716.3	723.4	70.8	105.4
84	938	884	911	89.2	106.2
84	899	887	893	87.5	104.1
84	969	933	951	93.1	110.9
100.8	927.5	966	946.8	92.7	92.0
100.8	1047.7	1015.3	1031.5	101.0	100.2
100.8	1114.5	1114.5	1114.5	109.1	108.3
				Mean	104.2
				SD*	5.2
				% RSD**	4.9

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The percentage BHT recovery ranged between 92.0 % and 108.3% with an average recovery of 104.2%

Tocopherol

Table A.10: Accuracy parameters of tocopherol

Concentration spiked (µg/ml)	Peak area 1	Peak area 2	Mean	Recovery (µg/ml)	%
62.4	373.2	343.3	358.3	53.1	85.1
62.4	435	388.7	411.9	61.0	97.8
62.4	411.3	400.1	405.7	60.1	96.4
78	530.2	474.9	502.6	74.5	95.5
78	514.7	498.5	506.6	75.1	96.3
78	556.1	531	543.6	80.6	103.3
93.6	483	531.3	507.2	75.2	80.3
93.6	626.6	579.6	603.1	89.4	95.5
93.6	636.8	636.8	636.8	94.4	100.8
				Mean	94.6
				SD*	6.9
				% RSD**	7.3

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The percentage tocopherol recovery ranged between 80.3 % and 103.3% with an average recovery of 94.6%

A.4.3 PRECISION

The precision of an analytical procedure expresses the proximity of agreement between a series of measurements obtained from multiple sampling of the same homogenous substance under the prescribed conditions. Precision was investigated in terms of intraday (repeatability) variation and interday (reproducibility) variation.

A.4.3.1 INTRADAY PRECISION (Repeatability)

The intraday precision was determined by performing HPLC analysis on three different samples of three different concentrations (80%, 100% and 120%) during the same day.

The different samples were injected in duplicate into the chromatograph.

Nicotinamide

Table A.11: Intraday precision parameters of nicotinamide

Mass (g)	Peak area 1	Peak area 2	Mean	Concentration spiked (µg/ml)	Concentration injected (µg/ml)	%
1.6	17328.2	17545.8	17437.0	1083.3	934.9	115.9
1.6	19651.2	19550.7	19601.0	1217.8	973.6	125.1
1.6	19233.1	19305.7	19269.4	1197.2	961.0	124.6
2.1	24704.7	24702.2	24703.5	1534.8	1288.6	119.1
1.9	22993.1	22985.8	22989.5	1428.3	1170.8	122.0
2.1	24628.3	24536.7	24582.5	1527.3	1264.6	120.8
2.5	27845.1	27895.1	27870.1	1731.6	1486.8	116.5
2.4	27552.0	27430.6	27491.3	1708.0	1467.1	116.4
2.5	27695.7	27669.6	27682.7	1719.9	1483.4	116.0
					Mean	119.6
					SD*	3.5
					% RSD**	2.9

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The requirements of the USP (United States Pharmacopeia) for acceptable Intraday repeatability is a % RSD of 2.

The % RSD of 2.9 is slightly higher than the requirements because of the possibility of homogeneity problems, but is still acceptable.

Methyl paraben

Table A.12: Intraday precision parameters of methyl paraben

Mass (g)	Peak area 1	Peak area 2	Mean	Concentration spiked (µg/ml)	Concentration injected (µg/ml)	%
1.6	1391.6	1393.1	1392.4	124.6	107.9	107.9
1.6	1568.1	1564.6	1566.4	129.8	116.6	116.6
1.6	1538.2	1537.6	1537.9	128.1	116.0	116.0
2.1	2027.2	2046.0	2036.6	171.8	114.5	114.5
2.0	1863.7	1872.0	1867.9	156.1	115.6	115.6
2.1	2027.8	2014.3	2021.1	168.6	115.8	115.8
2.5	2350.5	2351.3	2350.9	198.2	114.6	114.6
2.4	2310.4	2312.5	2311.5	195.6	114.2	114.2
2.5	2344.5	2338.6	2341.6	197.8	114.4	114.4
					Mean	114.4
					SD*	2.4
					% RSD**	2.1

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The requirements of the USP for acceptable Intraday repeatability is a % RSD of 2.

The % RSD of 2.1 is slightly higher than the requirements because of the possibility of homogeneity problems, but is still acceptable.

Propyl paraben

Table A.13: Intraday precision parameters of propyl paraben

Mass (g)	Peak area 1	Peak area 2	Mean	Concentration spiked (µg/ml)	Concentration injected (µg/ml)	%
1.6	235.6	235.7	235.7	21.0	24.9	84.3
1.6	273.4	267.4	270.4	24.1	26.0	92.9
1.6	264.8	262.4	262.6	23.5	25.6	91.7
2.1	359.1	355.6	357.4	31.9	34.4	92.8
1.9	322.2	321	321.6	28.7	31.2	91.9
2.1	346.4	351.4	348.9	31.1	33.7	92.3
2.5	408.4	409.2	408.8	36.5	39.6	92.0
2.4	400.9	400.6	400.8	35.7	39.1	91.4
2.5	408.1	405.7	406.9	36.3	39.6	91.7
					Mean	91.2
					SD*	2.5
					% RSD**	2.7

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The requirements of the USP for acceptable intraday repeatability are a % RSD of 2.

The % RSD of 2.7 is slightly higher than the requirements because of the possibility of homogeneity problems, but is still acceptable.

BHT

Table A.14: Intraday precision parameters of BHT

Mass (g)	Peak area 1	Peak area 2	Mean	Concentration spiked (µg/ml)	Concentration injected (µg/ml)	%
1.6	2480.7	2487.0	2483.9	50.5	62.3	80.9
1.6	2554.8	2556.8	2555.8	57.5	64.9	88.6
1.6	2538.8	2549.2	2544.0	56.3	64.1	87.9
2.1	2698.4	2707.9	2703.2	71.9	85.9	83.7
2.0	2656.8	2652.4	2654.6	67.2	78.1	86.0
2.1	2707.1	2692.2	2699.7	71.6	84.3	84.9
2.5	2833.7	2846.6	2840.2	85.4	99.1	86.1
2.4	2820.6	2823.1	2821.9	83.6	97.8	85.4
2.5	2827.8	2825.4	2826.6	84.0	98.9	84.9
					Mean	85.4
					SD*	2.1
					% RSD**	2.5

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The requirements of the USP for acceptable Intraday repeatability is a % RSD of 2.

The % RSD of 2.5 is slightly higher than the requirements because of the possibility of homogeneity problems and human error.

Tocopherol

Table A.15: Intraday precision parameters of tocopherol

Mass (g)	Peak area 1	Peak area 2	Mean	Concentration spiked (µg/ml)	Concentration injected (µg/ml)	%
1.6	373.2	379.2	376.2	55.8	62.3	89.5
1.6	423.6	424.1	423.9	62.8	64.9	96.8
1.6	421.1	419.5	420.3	62.3	64.1	97.2
2.1	558.5	560.4	559.5	82.9	85.9	96.5
2.0	510.2	510.7	510.5	75.7	78.1	96.9
2.1	550.3	547.4	548.9	81.3	84.3	96.5
2.5	650.2	653.9	652.1	96.6	99.1	97.5
2.4	639.8	638.4	639.1	94.7	97.8	96.8
2.5	645.5	643.3	644.4	95.5	98.9	96.6
					Mean	96.0
					SD*	2.3
					% RSD**	2.4

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The requirements of the USP for acceptable intraday repeatability are a % RSD of 2.

The % RSD of 2.4 is slightly higher than the requirements because of the possibility of homogeneity problems, but is still acceptable.

A.4.3.2 INTERDAY PRECISION (Reproducibility)

The interday precision was determined by performing HPLC analysis on three samples of the same known concentration on three consecutive days. Samples were injected in duplicate.

Nicotinamide

Table A.16: Interday precision parameters of nicotinamide

Mass (g)	Day 1	Day 2	Day 3	Between days
2.1	24703.5	22359.5	19955.5	
1.9	22989.5	20326	20186	
2.1	24582.5	21799.4	21179.3	
Mean	119.6	107.6	102.5	109.9
SD*	3.5	0.3	4.5	2.8
% RSD**	2.9	0.3	4.5	2.6

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The requirements of the USP for acceptable interday repeatability are a % RSD of 5. The % RSD of 2.6 complies with the pharmaceutical standards.

Methyl paraben

Table A.17: Interday precision parameters of methyl paraben

Mass (g)	Day 1	Day 2	Day 3	Between days
2.1	2036.6	2059.8	1757.8	
1.9	1867.9	1840.0	1800.5	
2.1	2021.1	1995.7	1906.6	
Mean	114.4	114.7	106.5	111.9
SD*	2.4	0.8	5.5	2.9
% RSD**	2.1	0.7	5.2	2.7

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The requirements of the USP for acceptable interday repeatability are a % RSD of 5. The % RSD of 2.7 complies with the pharmaceutical standards.

Propyl paraben

Table A.18: Interday precision parameters of propyl paraben

Mass (g)	Day 1	Day 2	Day 3	Between days
2.1	357.4	360.2	330.9	
1.9	321.6	328	336.4	
2.1	348.9	349.9	358.1	
Mean	91.2	93.2	92.2	92.2
SD*	2.5	0.5	4.5	2.5
% RSD**	2.7	0.5	4.9	2.7

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The requirements of the USP for acceptable interday repeatability are a % RSD of 5. The % RSD of 2.7 complies with the pharmaceutical standards.

BHT

Table A.19: Interday precision parameters of BHT

Mass (g)	Day 1	Day 2	Day 3	Between days
2.1	734.7	772.7	867.1	
1.9	686.1	548.6	715.7	
2.1	731.1	691.9	873.1	
Mean	84.8	79.1	96.7	86.9
SD*	2.1	7.9	4.9	4.9
% RSD**	2.4	10	5.1	17.5

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The requirements of the USP for acceptable interday repeatability are a % RSD of 5. The % RSD of 17.5 is higher than the requirements because of the possibility of homogeneity problems.

Tocopherol

Table A.20: Interday precision parameters of tocopherol

Mass (g)	Day 1	Day 2	Day 3	Between days
2.1	559.5	539.8	554.6	
1.9	510.5	481.7	528.0	
2.1	548.9	527.5	611.3	
Mean	96.0	92.4	104.6	97.7
SD*	2.3	0.7	6.4	3.1
% RSD**	2.4	0.8	6.1	3.1

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The requirements of the USP for acceptable interday repeatability are a % RSD of 5.

The % RSD of 3.1 complies with the pharmaceutical standards.

A.4.4 RUGGEDNESS

A.4.4.1 SAMPLE STABILITY

A 100% sample was left on the autosampler tray and analysed at hourly intervals for a period of 24 hours to determine the sample stability.

Nicotinamide

Table A.21: Sample stability parameters for nicotinamide

Time (hours)	Peak area	%
0	18662	100.0
1	18663.2	100.0
2	18677.8	100.1
3	18584.1	99.6
4	18674.2	100.1
5	18682.5	100.1
6	18579.1	99.6
7	18582.0	99.6
8	18621.9	99.8
9	18617.7	99.8
10	18671.7	100.1
11	18615.8	99.8
12	18579.1	99.6
13	18649.2	99.9
14	18597.9	99.7
15	18657.2	100.0
16	18612.7	99.7
17	18619.4	99.8
18	18642.3	99.9
19	18615.9	99.8
20	18663.1	100.0
21	18573.8	99.5
22	18522.0	99.3
23	18583.5	99.6
24	18580.3	99.6
Mean	18621.1	99.8
SD*	41.2	0.2
% RSD**	0.2	0.2

*SD refers to standard deviation

**%RSD refers to relative standard deviation

Nicotinamide is stable after a period of 24 hours.

Methyl paraben

Table A.22: Sample stability parameters for methyl paraben

Time (hours)	Peak area	%
0	1694.5	100.0
1	1696.5	100.1
2	1711.0	101.0
3	1702.7	100.5
4	1701.1	100.4
5	1707.7	100.8
6	1694.9	100.0
7	1695.0	100.0
8	1703.3	100.5
9	1698.5	100.2
10	1688.6	99.7
11	1695.8	100.1
12	1689.0	99.7
13	1692.5	99.9
14	1685.0	99.4
15	1687.7	99.6
16	1685.7	99.5
17	1692.5	99.9
18	1686.8	99.5
19	1691.5	99.8
20	1687.5	99.6
21	1682.2	99.3
22	1683.7	99.4
23	1690.6	99.8
24	1695.0	100.0
Mean	1693.6	99.9
SD*	7.3	0.4
% RSD**	0.4	0.4

*SD refers to standard deviation

**%RSD refers to relative standard deviation

Methyl paraben is stable after a period of 24 hours.

Propyl paraben

Table A.23: Sample stability parameters for propyl paraben

Time (hours)	Peak area	%
0	285.3	100
1	281.9	98.8
2	280.0	98.1
3	283.9	99.5
4	288.0	100.9
5	276.7	96.9
6	283.6	99.4
7	278.3	97.5
8	279.0	98.0
9	278.7	97.7
10	279.5	98.0
11	280.2	98.2
12	283.7	99.4
13	282.3	98.9
14	276.1	96.8
15	278.6	97.7
16	279.7	98.0
17	280.6	98.4
18	280.1	98.2
19	280.2	98.2
20	276.3	96.8
21	274.2	96.1
22	276.3	96.8
23	282.0	98.8
24	273.8	96.0
Mean	280.0	98.1
SD*	3.4	1.2
% RSD**	1.2	1.2

*SD refers to standard deviation

**%RSD refers to relative standard deviation

Propyl paraben is stable after a period of 24 hours.

BHT

Table A.24: Sample stability parameters for BHT

Time (hours)	Peak area	%
0	762.9	100.0
1	762.8	100.0
2	762.1	99.9
3	761.9	99.9
4	760.1	99.6
5	762.2	99.9
6	759.1	99.5
7	758.2	99.4
8	806.6	105.7
9	757.3	99.3
10	755.5	99.0
11	756.1	99.1
12	753.4	98.8
13	753.4	98.8
14	752.1	98.6
15	750.8	98.4
16	752.3	98.6
17	753.9	98.8
18	753.1	98.7
19	751.0	98.4
20	750.8	98.4
21	751.6	98.5
22	751.9	98.6
23	754.2	98.9
24	751.5	98.5
Mean	757.8	99.3
SD*	10.8	1.4
% RSD**	1.4	1.4

*SD refers to standard deviation

**%RSD refers to relative standard deviation

BHT is stable after a period of 24 hours.

Tocopherol

Table A.25: Sample stability parameters for tocopherol

Time (hours)	Peak area	%
0	323.6	100.0
1	323.4	99.9
2	323.9	100.0
3	322.4	99.6
4	321.1	99.2
5	322.5	99.7
6	321.7	99.4
7	321.6	99.4
8	321.7	99.4
9	322.9	99.8
10	319.1	98.6
11	319.8	98.8
12	318.6	98.5
13	317.8	98.2
14	317.6	98.1
15	318.2	98.3
16	318.5	98.4
17	317.0	98.0
18	317.4	98.1
19	315.9	97.6
20	316.2	97.7
21	316.5	97.8
22	316.6	97.8
23	317.6	98.1
24	316.1	97.7
Mean	319.5	98.7
SD*	2.6	0.8
% RSD**	0.8	0.8

**SD refers to standard deviation*

**%RSD refers to relative standard deviation

Tocopherol is stable after a period of 24 hours.

A.4.4.2 SYSTEM REPEATABILITY

In order to evaluate the repeatability of the peak areas and retention times of the different actives, a 100% sample was injected 6 times. It was done on the same day and under the same conditions.

Nicotinamide

Table A.26: Variations in response (% RSD) of the detection system regarding peak area and retention time of nicotinamide

Injection	Peak area (mAU*s)	Retention time (min)
1	20986.6	3.3
2	20745.7	3.3
3	20646.7	3.3
4	20469.3	3.3
5	20571.3	3.3
6	20496.9	3.3
Mean	20652.8	3.3
SD*	175.5	0.009
% RSD**	0.8	0.3

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The variation in response (%RSD) proved to be excellent with a value of 0.8 for peak area and 0.3 for retention time

Methyl paraben

Table A.27: Variations in response (% RSD) of the detection system regarding peak area and retention time of methyl paraben

Injection	Peak area (mAU*s)	Retention time (min)
1	1914.8	6.7
2	1922.5	6.7
3	1923.7	6.6
4	1910.2	6.7
5	1926.6	6.6
6	1925.4	6.6
Mean	1920.5	6.6
SD*	6.0	0.01
% RSD**	0.3	0.2

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The variation in response (%RSD) proved to be excellent with a value of 0.3 for peak area and 0.2 for retention time

Propyl paraben

Table A.28: Variations in response (% RSD) of the detection system regarding peak area and retention time of propylparaben

Injection	Peak area (mAU*s)	Retention time (min)
1	351	8.9
2	349.9	8.9
3	352.4	8.9
4	350.4	8.9
5	352.1	8.9
6	354.5	8.9
Mean	351.7	8.9
SD*	1.5	0.007
% RSD**	0.4	0.08

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The variation in response (%RSD) proved to be excellent with a value of 0.4 for peak area and 0.08 for retention time

BHT

Table A.29: Variations in response (% RSD) of the detection system regarding peak area and retention time of BHT

Injection	Peak area (mAU*s)	Retention time (min)
1	2732.6	12.1
2	2747.2	12.1
3	2751.6	12.1
4	2750.3	12.1
5	2741.2	12.1
6	2757.3	12.1
Mean	2746.7	12.1
SD*	7.9	0.009
% RSD**	0.3	0.07

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The variation in response (%RSD) proved to be excellent with a value of 0.3 for peak area and 0.07 for retention time

Tocopherol

Table A.30: Variations in response (% RSD) of the detection system regarding peak area and retention time of tocopherol

Injection	Peak area (mAU*s)	Retention time (min)
1	656.8	18.7
2	657.0	18.7
3	657.8	18.7
4	656.1	18.7
5	660.0	18.7
6	660.6	18.7
Mean	658.1	18.7
SD*	1.7	0.03
% RSD**	0.3	0.1

*SD refers to standard deviation

**%RSD refers to relative standard deviation

The variation in response (%RSD) proved to be excellent with a value of 0.3 for peak area and 0.1 for retention time.

A.5 Conclusion

The HPLC method was found to be reliable and sensitive enough for the determination of the concentration of nicotinamide, methyl paraben, propyl paraben, BHT and tocopherol in semi-solid formulations.

APPENDIX B

FORMULATION OF A COSMECEUTICAL CREAM AND EMULGEL WITH NICOTINAMIDE AS THE ACTIVE INGREDIENT

B.1 Introduction

The definition of a drug is more or less equivocal in most countries. According to the Food, Drug, and Cosmetic Act (FDC), a drug is defined as an article intended for use in the diagnosis, mitigation, treatment, or prevention of a disease or intended to affect the structure or any function of the body. In the United States, according to the FDC, a cosmetic is defined as an article intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting structure or function. The cosmetic is not allowed to have any activity (i.e., without affecting structure of function) (Vermeer, 2000:9).

The term "cosmeceutical" is attributed to Dr. Albert Kligman who identified a hybrid category of products lying on the spectrum between drugs and cosmetics (Choi & Berson, 2006:163).

The term cosmeceutical is now commonly used to describe a cosmetic product that exerts a pharmaceutical therapeutic benefit, but not necessarily a biologic therapeutic benefit (Choi & Berson, 2006:163).

B.2 Development program for the formulation of cosmeceutical products

Efficiency of the formulation development process, depends on maximising forethought and planning before acting on ideas. In order to plan, product requirements must be defined before formulation begins, and modified when appropriate. Research regarding product ingredients and testing procedures, should minimise the amount of formulation modification prior to final formulation. Assessing the efficiency of the formulation process at the end of each project, will aid the efficiency of future projects (Radd, 1994:56).

B.2.1 FORMULATION OF COSMECEUTICAL PRODUCTS

According to Radd (2002:50), defining product requirements before development begins, minimises cost and time to market. These requirements set the direction for both development and testing efforts. Important areas to define include: product vision, product form, essential ingredients, critical ingredients, project cost, product performance and regulatory requirements.

A clear idea of the forthcoming product or line of products relative to other products in a category, must exist. The type of product will dictate how product development will be approached. Different forms require different key ingredients and have different processing needs. Essential ingredients represent the core technology around which the new product will be built. For example, special moisturisers or antioxidant ingredients are considered that are essential in cosmeceutical products. Critical ingredients are ingredients that must be used to create the products. Examples include penetration enhancers, buffers, emulsifiers and/or thickeners. It is also important to identify ingredients that should be avoided in the product. Critical cost and timing milestones must be established and drafted into a joint project timetable. A well constructed plan can enhance turnaround time for testing and purchasing, as well as minimise reformulation. Efficacy and sensory product attribute requirements should be specified, along with typical product use conditions. Regulatory requirements convey the ingredient restrictions, minimum / maximum active levels, minimum stability, expiry date and claim requirements (Radd, 1994:51).

B.2.2 PRE-FORMULATION

Pre-formulation include those studies that should be performed before commencement of formulation development. The primary goal of the pre-formulation process, it to permit the rational development of stable, safe, efficacious dosage forms, and it is mainly concerned with the characterisation of the physicochemical properties of the drug substance. In the pre-formulation stage, the final route of drug administration should be decided (Walters & Brain, 2002:321). According to Walters & Brain (2002:322), the pre-formulation study has several distinct phases, namely: general description of the compound, calorimetry, polymorphism, hygroscopicity, analytical development, intrinsic stability, solubility and partitioning characteristics and drug delivery characteristics.

B.2.3 EARLY FORMULATION

During early formulation a trial-and-error approach was used. Existing formulas were taken and changed as necessary.

B.2.4 FINAL FORMULATION

After the correct formula was formulated and the final formulation was chosen, the products were formulated in bulk, for storage and stability testing purposes. A stability programme according to the International Conference on Harmonisation (ICH, 2003:5) was followed at three different storage temperatures and humidities for six months.

B.2.5 PRESERVATION OF COSMECEUTICAL PRODUCTS

In addition to their principal ingredients of oil and water, cosmeceuticals often also contain such substances as glycerin and sorbitol which provide a source of carbon for micro organisms, and substances such as amino acid derivates and proteins, which provide a source of nitrogen. It is thus very easy for cosmeceutical products to be contaminated by fungi, bacteria and other micro-organisms. Therefore is it necessary to add preservatives to cosmeceuticals for their long-term protection against bacterial contamination and to prevent product deterioration. The micro-organisms that mostly contaminate cosmeceuticals and proliferate inside them, are mainly bacteria, but cosmeceuticals can also be contaminated by fungi and yeasts (Mitsui, 1997:199). Suppression of the proliferation of micro-organisms is called microbiostasis. Typical preservatives used in cosmecueticals are paraoxybenzoates, which are commonly known as parabens (Mitsui, 1997:201).

According to Mitsui (1997:202) characteristics of preservatives include the following:

- Efficacy against many species of micro organisms;
- Water solubility or easy dissolution in commonly used cosmetic ingredients;
- High safety;
- No irritation;
- Neutral with no effect on product pH;
- No reduction of product ingredient effectiveness;
- No adverse effect on product appearance;
- Stability over wide temperature and pH range;
- Readily available and stable supply; and
- Low in price and economical to use.

B.3 Formulation of a cream

According to Mitsui (1997:341) creams are skin care cosmetics which have been in general use since way back in history. Many different types of creams have been produced throughout history and the wide variety of creams that are being produced today is the result of advances in surface chemistry, higher levels of cosmetic production techniques and the development of new beauty treatments.

B.3.1 PURPOSE AND FUNCTION OF A CREAM

Creams are emulsions in which two liquids that are immiscible are made into a stable dispersion. This can be done by dispersing the one liquid as droplets, the dispersed phase, into the other liquid, the continuous phase or dispersion medium. Creams are stable over a broader range of conditions than lotions and oils, and therefore humectants and water can be included in higher concentrations. Because of this, they occupy a very important position among skin care products (Mitsui, 1997:341).

According to Mitsui (1997:342), the main functions of a cream are to maintain the moisture balance and to keep skin moist and supple through the supply of water, humectants and oils.

In addition to creams which only moisturise the skin, there are other products which have additional functions such as stimulating the circulation, cleansing the skin and treating a skin condition. Just by varying the amount of water, humectants and oil in the formulation, a cream can cater for different skin types, skin conditions, cosmetic routines and preferences (Mitsui, 1997:342).

B.3.2 MAIN INGREDIENTS OF A CREAM

According to Mitsui (1997:342), some of the main ingredients of a cream include oily ingredients, aqueous ingredients, surfactants, preservatives, chelating agents, perfumes and pharmaceutical agents.

Creams are either oil-in-water (O/W) or water-in-oil (W/O) emulsions. Special features of a cream are provided by the surfactants and oily ingredients in the formula. In the case of O/W emulsions, mainly hydrophilic surfactants are used. The oily ingredients used can also vary from those with no polarity, to those with very high polarity. In the case of W/O emulsions, lipophilic surfactants are mainly used. In the case where a cream with an oily nature is desired, a W/O emulsion is used. In the case where a cream with a light feeling is desired, an O/W emulsion is used (Mitsui, 1997:343).

B.3.3 GENERAL METHOD FOR MANUFACTURING A CREAM

- The humectants and other water phase ingredients are added to the purified water and this is heated to 70° C to make the water phase.
- The oil phase is prepared by making a solution of the solid oils, semi-solid oils, liquid oils, preservatives and antioxidants. The solution is heated to 70°C while stirring.
- The perfume is stirred into the oil phase just before emulsification.

- The oil phase is then gradually stirred into the water phase to carry out the preliminary emulsification.
- The mixture is processed in an emulsification apparatus to unite the emulsion particles, where after it is de-aired, filtered and cooled.
- It is then transferred to a storage tank and put into containers (Mitsui, 1997:343).

This is the general method of manufacturing an O/W emulsion type cream. In the case of a W/0 type cream, the water phase is gradually added to the oil phase to carry out the preliminary emulsification. The process from here on is the same as that for the O/W type (Mitsui, 1997:344).

In the manufacturing of a cream, the processes which affect the quality characteristic values the most are:

- The process for adjusting the emulsion particle size and
- The cooling process

The surface heat exchanger used in the cooling process, is very important for obtaining a cream with stable quality. When using this type of cooling equipment, it is important to pay attention to the setting of the cylinder rotation speed and the final temperature (Mitsui, 1997:345).

B.4 Formulation of a gel

Barry (1983:300) defines a gel as a two-compartment system of a semi-solid nature, rich in liquid with the presence of some form of continuous structure which provides solid-like properties. In a typical polar gel, a natural or synthetic polymer at a relatively low concentration builds a three-dimensional matrix throughout a hydrophilic liquid. The system may be clear or turbid, because the gelling agent does not fully dissolve or because it forms aggregates which disperse the light.

B.4.1 PURPOSE AND FUNCTION OF A GEL

Gels are a type of base which produce a uniform external appearance. It can range from transparent to semi-transparent and it gives a moist feeling. Aqueous gels are used in summer underneath make-up creams, because of their special feature of providing a moist and light feeling. In addition to gels which just give the skin a moist and light feeling, there are many others which have additional functions like stimulating the circulation, cleansing the skin and treating a skin condition. Aqueous gels contain a lot of moisture and are used as a base

material for moisturising and cooling effects, or as the base of cleansers to remove make-up. As they impart a moist, light and cool feeling, they are used in cosmetics during summer months and for oily skin. Oily gels are used to supply oil to the skin and are used as a winter product or for dry skin because of their moisturising properties (Mitsui, 1997:351).

B.4.2 MAIN INGREDIENTS OF A GEL

Water soluble polymer substances with gelling abilities, are much used in aqueous gels. Examples of this are carboxyvinyl polymer and methyl cellulose (Mitsui, 1997:351).

According to Mitsui (1997:351), some of the main ingredients of a gel include gel base, humectants, surfactants, preservatives, pharmaceutical agents, colouring agents and perfumes.

As gel products are usually transparent, it is important to ensure that the additives are uniformly dissolved and dispersed in them. An oil-containing gel which is transparent, or semi-transparent, can be made by adding a small amount of oil and a surfactant to an aqueous gel. Oily gels make use of the gelling ability and liquid crystal structure of surfactants. For oil gels, it is necessary to have a combination of oils with good compatibility, to select appropriate oil phase gelling agents and to use materials in combinations which maintain the stability of the gel. When making a gel formulation, the design must be based on a very broad understanding of the ingredients, considering factors like stability, safety, preservation and ease of use (Mitsui, 1997:352).

B.4.3 GENERAL METHOD FOR MANUFACTURING A GEL

Gels generally have high viscosities and it is necessary to select the equipment for making them in view of this feature. Mixing equipment should be capable of uniform mixing, there should be equipment for removing air bubbles and equipment suited for the transportation, filtering and cooling of high viscosity substances. As transparency is important in gels, attention should be paid to the dissolution and uniformity of the raw materials used (Mitsui, 1997:353).

In this study an emulgel was formulated. According to Lopez-Cervantez, *et al.* (2009:1), an emulgel is an oil-in-water emulsion that is incorporated into a gel.

B.5 Formulation of a nicotinamide containing cream, Pheroid™ cream, emulgel and Pheroid™ emulgel during this study

The purpose of this study was to formulate nicotinamide in a cream, Pheroid™ cream, emulgel and Pheroid™ emulgel. Several different formulations were prepared and the best formulations were selected on grounds of appearance, homogeneity and overnight stability.

The ingredients used in the formulations which were selected as candidates to continue with this study, are given in Table B.1. The supplier, as well as the batch number, is given.

Table B.1: Ingredients used in the selected formulations

Ingredient	Supplier	Batch number
Liquid paraffin	Merck Chemicals	1032980
Span 60	Fluka Analytical	423065/141002
Tween 80	Merck Chemicals	1032991
Methyl parabene	Merck Chemicals	GBGA0001371
Propyl parabene	Merck Chemicals	GBGA032949
BHT	Sigma-Aldrich	04416KD-076
Xanthan gum	Warren Chem Specialities	445090279C
Nicotinamide	Sigma-Aldrich	1335714
Cetyl alcohol	Merck Chemicals	S5183304904
Tocopherol	Chempure	UT0805099

B.6 Formulation of a nicotinamide containing cream during this study

B.6.1 FORMULA OF NICOTINAMIDE CREAM

The formula of the nicotinamide cream is given in table B.1

Table B.2: Formula of cream

Ingredients	% m/m	Activity
A: Cetyl alcohol	10%	Thickening agent
Liquid paraffin	20%	Oil phase of emulsion
Span 60	0.5%	Emulsifier
Tween 80	4.5%	Surface active agent
Methyl paraben	0.4%	Preservative
Propyl paraben	0.08%	Preservative
BHT	0.2%	Anti-oxidant
B: Xanthan gum	0.5%	Thickening agent
Nicotinamide	3%	Active ingredient
dH_2O	to 100%	Solvent

B.6.2 PROCEDURE TO PREPARE THE NICOTINAMIDE CREAM

- Dissolve nicotinamide in ±20% of the distilled water
- Heat the remaining water to 40°C
- Add xanthan gum slowly to heated water while homogenising at 777 rpm
- Add nicotinamide solution to Xanthan solution and heat to 80°C
- Mix A together and heat to 80°C
- Mix A and B while homogenising at 13500 rpm to exactly 40°C
- Cool down to room temperature

B.6.3 PROCEDURE TO PREPARE THE NICOTINAMIDE PHEROID™ CREAM

The same procedure was followed as mentioned above. The only difference was that tocopherol and the other Pheroid™ ingredients were added to B.

B.6.4 OUTCOMES

The cream applied easily and was not too oily. It had a homogeneous white texture. The Pheroid™ cream also applied easily and had a homogeneous white texture. It was a little bit oilier than the cream not containing Pheroid™.

B.7 Formulation of a nicotinamide containing emulgel during this study

B.7.1 FORMULA OF NICOTINAMIDE EMULGEL

The formula of the nicotinamide emulgel is given in table B.2

Table B.3: Formula of emulgel

Ingredients	% m/m	Activity
A: Liquid paraffin	20%	Oil phase of emulsion
Span 60	0.5%	Emulsifier
Tween 80	4.5%	Surface active agent
Methyl parabene	0.4%	Preservative
Propyl parabene	0.08%	Preservative
BHT	0.2%	Anti-oxidant
B: Xanthan gum	1.5%	Thickening / gel forming agent
Nicotinamide	3%	Active ingredient
dH_2O	to 100%	Solvent

B.7.2 PROCEDURE TO PREPARE THE NICOTINAMIDE EMULGEL

- Dissolve nicotinamide in ±20% of the distilled water
- Heat the remaining water to 40°C
- Add xanthan gum slowly to heated water while homogenising at 777 rpm
- Add nicotinamide solution to xanthan solution and heat to 80°C
- Mix A together and heat to 80°C
- Mix A and B while homogenising at 13500 rpm to exactly 40°C
- Cool down to room temperature

B.7.3 PROCEDURE TO PREPARE THE NICOTINAMIDE PHEROID™ EMULGEL

The same procedure was followed as mentioned above. The only difference was that tocopherol and the other Pheroid™ ingredients were added to B.

B.7.4 OUTCOMES

The emulgel had a clear white colour and applied easily. The Pheroid™ emulgel also applied easily and had the same colour and feel as the emulgel not containing Pheroid™.

B.8 Conclusion

Nicotinamide was formulated into the following different semi-solid formulations:

- 3% Cream;
- 3% Pheroid™ cream;
- 3% Emulgel; and
- 3% Pheroid™ emulgel

Each of the formulations was prepared in sufficient quantities and stored at different temperatures to perform stability tests. The appearance and texture of the formulated formulations were acceptable before storage at different temperatures.

The stability tests that were performed on the different formulations will be discussed In Appendix C.

B.9 References

BARRY, B.W. 1983. Dermatological formulations. New York: Marcel Dekker. 480p.

CHOI, C.M. & BERSON, D.S. 2006. Cosmeceuticals. *Seminars in cutaneous medicine and surgery,* 2006(25):163-168.

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE (ICH) Q1A(R2). 2003. Stability testing of new drug substances and products. p.1-22.

LOPEZ-CERVANTEZ, M., ESCOBAR-CHAVEZ, J.J., CASAS-ALANCASTER, N., QUINTANAR-GEURRERO, D. & GANEM-QUINTANAR, A. 2009. Development and characterization of a transdermal patch and an emulgel containing kanamycin intended to be used in the treatment of mycetoma. *Drug development and industrial pharmacy,* 1(2009):1-11.

MITSUI, T. *ed.* 1997. New cosmetic science. Amsterdam: Elsevier. 376p.

RADD, B. 1994. Efficient formulation of cosmeceutical products. *Cosmetics and toiletries,* 109(10):51-56.

VERMEER, B.J. 2000. Definition. (*In* ELSNER, P. & MAIBACH, H.I. *ed.* Cosmeceuticals: Drugs vs. Cosmetics. New York: Marcel Dekker. p.9-12.)

WALTERS, K.A. & BRAIN, K.R. 2002. Dermatological formulation and transdermal systems. (*In* SWARBRICK, J. *ed.* Dermatological and transdermal formulations. Vol.119. New York: Marcel Dekker. p.319-399.)

APPENDIX C

STABILITY TESTING OF SEMI-SOLID FORMULATIONS

C.1 Introduction

The successful formulation of cosmeceutical products requires that the products should be placed under certain conditions and tested to determine their stability over a certain period of time.

Like other products, the stability of cosmeceuticals must be matched to the expected period of usage as well as to the user's requirements. It is important to guarantee product quality by paying sufficient attention to the time required to distribute the product from the manufacturer to the consumer and the actual period the product will be used. It is not sufficient to simply guarantee the feeling on use and performance of a cosmeceutical. It's also important to consider the safety and stability in usage (Mitsui, 1997:191).

The first stage of stability testing is to observe whether or not there are any changes in the physicochemical properties of the semi-solid formulations. The following aspects should be considered:

- Chemical changes: colour change, colour fading, fragrance change, staining and crystallisation.
- Physical changes: separation, sedimentation, aggregation, blooming, sweating, gelling, unevenness, evaporation, solidification, softening and cracking (Mitsui, 1997:191).

If no changes in the physicochemical properties of the formulations are observed, the formulations are stored at different temperatures and humidities for 6 months and stability tests are done at fixed time periods.

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature and humidity (ICH, 2003:5).

In this study the four different semi-solid formulations that were formulated (as discussed in Appendix B) were stored at 25°C/60% RH (relative humidity), 30°C/60% RH and 40°C/70% RH. The following stability tests were done on month 0, 1, 2, 3 and 6:

- Concentration assay
- pH

- Viscosity
- Confocal laser scanning microscopy (CLSM)
- Visual appearance assessment
- Mass loss

According to the ICH (2003:13) a significant change for a drug product is defined as:

- a 5% change in assay from its initial value;
- any degradation products exceeding its acceptance criterion;
- failure to meet the acceptance criteria for appearance (e.g., colour, phase separation, caking, hardness); and
- failure to meet the acceptance criterion for pH.

C.2 Methods

C.2.1 CONCENTRATION ASSAY

The concentrations of the following ingredients in the different formulations were determined with HPLC analysis: nicotinamide, methylparaben, propylparaben, BHT and tocopherol. The validation of the HPLC analysis is discussed in Appendix A.

C.2.1.1 CHROMATOGRAPHIC CONDITIONS

Analytical instrument:	An Agilent® 1100 Series HPLC (High Performance Liquid Chromatograph) system was used for the analysis (Agilent Technologies, Palo Alto, CA). The instrument is designed with an Agilent® 1100 quaternary gradient pump, diode array detector, auto sampler injection mechanism and Chemstation Rev. A.06.02 software for data acquisition and analysis. Analysis was performed in a controlled laboratory environment at 25°C.
Column:	A high performance silica based, reversed phase Phenomenex® Luna C18 (2) column, (250 × 4.6 mm) with a 5 µm particle size was used (Phenomenex®, Torrance, CA).
Mobile phase A:	A filtered and degassed mixture of 1 g octanesulphonic acid in 1000 ml of HPLC water. The pH was set at 3.5 with 10% phosphoric acid.

Mobile phase B:	Methanol.
Solvent:	Methanol and HPLC water were used in the preparation of the standard solutions. For the preparation of samples, THF (tetrahydrofurane) was used for the preparation of cream samples and HPLC water was used for the preparation of gel samples.
Flow rate:	1.0 ml/min
Injection volume:	5 µl
Detection:	254 nm for the detection of nicotinamide, methyl paraben and propyl paraben, 220 nm for detection of BHT and tocopherol
Retention times:	The retention times of the ingredients were as follows: nicotinamide (2.5 min), methylparaben (6.8 min), propylparaben (9.0 min), BHT (12.9 min) and tocopherol (19.5 min).
Run time:	25 min with a post runtime of 5 min.

C.2.1.2 STANDARD PREPARATION

The following ingredients were weighed off and dissolved in a 100 ml volumetric flask. It was then made up to volume with methanol/HPLC water:

- Nicotinamide: 60.0 mg
- Methylparaben: 8.0 mg
- Propylparaben: 1.6 mg
- BHT: 4.0 mg
- Tocopherol: 4.0 mg

The standard solution was injected into the HPLC in triplicate.

C.2.1.3 SAMPLE PREPARATION

Each formulation (2 g) at each condition was weighed off in 100 ml volumetric flasks in duplicate. The cream and Pheroid™ cream samples were made up to volume with THF, and the emulgel and Pheroid™ emulgel samples were made up to volume with HPLC water. The solutions were filtered and injected into the HPLC in duplicate.

Figure C.1: Agilent® 1100 Series HPLC

C.2.2 pH

The apparatus that was used to measure the pH of the formulations was a Mettler Toledo pH meter (Switzerland). A Mettler Toledo Inlab® 410 electrode was used and the apparatus was calibrated every time before use. The pH of each formulation at each condition was measured in triplicate.

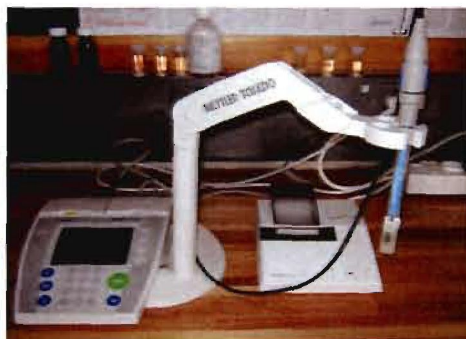


Figure C.2: Mettler Toledo pH meter

C.2.3 VISCOSITY

Rheology is the science of the flow of a product and its study begins with gathering data on the product's viscosity. Viscosity is the resistance to flow, caused by internal friction (Brookfield, 1998:2).

A viscometer is an instrument used to measure the viscosity of a fluid, semi-solid or solid suspension. It measures the viscosity by determining the resistance to a rotating spindle immersed in the sample medium. The spindle turns at a specific rate, measured in rpm. A Brookfield Viscometer (model DV II, Stoughton, Massachusetts, USA) was used. The

formulation was placed in the water bath to reach a temperature of 25°C. The spindle (Stoughton, MA) was placed in the formulation and the rate was specified. The viscosity reading was measured every 10 sec for 5 min. Approximately 32 readings were obtained and the average viscosity was determined.

Figure C.3: Brookfield Viscometer

C.2.4 CONFOCAL LASER SCANNING MICROSCOPY (CLSM)

Samples were prepared by weighing off 0.1 g of each formulation at each condition. Nile red (2 µl) and 1000 µl HPLC water were added. The samples were vortexed and placed in a dark cupboard. The solution (25 µl) was placed on a microscope plate and micrographs were taken with a Nikon PCM 2000 CLSM with a He/Ne laser-543 nm and an Argon ion laser with 457 – 517 nm.

C.2.5 VISUAL APPEARANCE ASSESSMENT

The visual appearance of each formulation was assessed by comparing the colour of the cream or emulgel to paint colour cards. Photos were taken with a camera (Canon E240) and compared to each other.

C.2.6 MASS LOSS

The apparatus that was used to determine the mass loss of each formulation was a Shimadzu (Japan) scale. The mass of each formulation at each condition was determined in triplicate. After the indicated time intervals the mass of each formulation was determined and was subtracted from the original mass to determine the mass loss.

Figure C.4: Shimadzu scale

C.3 Results and discussion

C.3.1 CONCENTRATION ASSAY

The percentage of the active ingredients was measured in the initial cream, Pheroid™ cream, emulgel and Pheroid™ emulgel. It was also measured at 25°C/60% RH, 30°C/60% RH and 40°C/70% RH on months 1, 2, 3 and 6 as described in Section C.2.1.

C.3.1.1 CREAM

Table C.1: Percentage of each active ingredient in cream at the different conditions after each time interval

	Initial	Month 1	Month 2	Month 3	Month 6
			25°C/60% RH		
Nicotinamide	108.766	103.733	103.743	102.779	99.800
Methyl paraben	107.278	103.430	102.210	102.326	100.534
Propyl paraben	99.853	96.511	95.572	95.465	93.449
BHT	109.400	100.295	98.710	98.609	92.921
			30°C/60% RH		
Nicotinamide	108.766	104.329	104.107	104.762	103.426
Methyl paraben	107.278	99.340	98.680	98.909	97.969
Propyl paraben	99.853	98.967	97.443	97.497	96.301
BHT	109.400	108.855	103.089	102.007	100.727
			40°C/70% RH		
Nicotinamide	108.766	107.466	106.266	104.596	101.674
Methyl paraben	107.278	107.686	104.290	105.577	101.235
Propyl paraben	99.853	95.598	93.514	92.099	91.958
BHT	109.400	104.291	104.898	102.741	98.477

C.3.1.2 PHEROID™ CREAM

Table C.2: Percentage of each active ingredient in Pheroid™ cream at the different condition after each time interval

	Initial	Month 1	Month 2	Month 3	Month 6
25°C/60% RH					
Nicotinamide	101.113	99.708	94.406	94.017	92.722
Methyl paraben	97.908	97.337	95.279	93.054	93.535
Propyl paraben	102.500	97.783	97.635	95.620	93.605
BHT	101.673	100.422	100.886	101.502	100.886
Tocopherol	105.218	103.877	103.837	103.694	103.162
30°C/60% RH					
Nicotinamide	101.113	101.750	101.429	97.464	95.874
Methyl paraben	102.908	101.485	101.917	101.265	100.550
Propyl paraben	102.500	94.390	93.371	91.180	95.064
BHT	101.673	84.974	77.734	87.358	87.471
Tocopherol	105.218	104.097	100.604	101.484	98.451
40°C/70% RH					
Nicotinamide	101.113	97.439	103.932	102.703	96.175
Methyl paraben	103.908	97.302	102.268	102.261	101.042
Propyl paraben	102.500	71.413	91.829	82.736	100.206
BHT	97.673	99.712	96.643	98.130	97.962
Tocopherol	101.218	101.921	101.656	97.377	80.588

C.3.1.3 EMULGEL

Table C.3: Percentage of each active ingredient in emulgel at the different conditions after each time interval

	Initial	Month 1	Month 2	Month 3	Month 6
			25°C/60% RH		
Nicotinamide	101.511	100.100	98.031	96.391	94.500
Methyl paraben	99.248	97.525	97.850	95.038	94.346
Propyl paraben	103.103	82.093	74.290	67.128	68.912
BHT	96.513	82.119	81.119	73.807	36.520
			30°C/60% RH		
Nicotinamide	108.511	107.576	106.717	107.724	102.946
Methyl paraben	99.248	100.639	99.240	97.099	97.004
Propyl paraben	103.103	92.049	90.977	90.027	89.335
BHT	96.513	81.655	77.683	53.123	40.309
			40°C/70% RH		
Nicotinamide	111.511	112.152	106.871	107.008	104.946
Methyl paraben	99.248	99.058	97.985	99.082	98.235
Propyl paraben	103.103	73.337	74.097	68.671	62.910
BHT	96.513	82.548	65.823	61.707	60.686

C.3.1.4 PHEROID™ EMULGEL

Table C.4: Percentage of each active ingredient in Pheroid™ emulgel at the different conditions after each time interval

	Initial	Month 1	Month 2	Month 3	Month 6
25°C/60% RH					
Nicotinamide	108.077	105.983	105.911	104.765	101.521
Methyl paraben	99.649	98.093	97.423	91.669	91.106
Propyl paraben	79.004	58.662	57.332	56.439	50.305
BHT	80.583	78.292	71.643	64.515	55.221
Tocopherol	121.777	69.742	68.457	67.784	62.343
30°C/60% RH					
Nicotinamide	108.077	108.871	107.647	105.831	103.237
Methyl paraben	99.649	96.029	95.222	89.259	89.795
Propyl paraben	89.004	87.837	84.623	80.101	83.912
BHT	61.583	57.671	58.491	52.450	51.941
Tocopherol	111.777	109.068	70.315	61.923	61.057
40°C/70% RH					
Nicotinamide	108.077	101.042	101.619	101.329	100.140
Methyl paraben	99.649	92.952	94.201	91.304	91.615
Propyl paraben	79.004	74.926	73.556	61.035	67.223
BHT	84.583	82.610	82.433	79.355	69.741
Tocopherol	81.777	71.094	72.152	66.179	64.562

The concentration nicotinamide in the cream, Pheroid™ cream, emulgel and Pheroid™ emulgel showed a significant change and didn't remain within the acceptable limits (5% change in initial value).

The concentration methylparaben in the Pheroid™ cream and emulgel did not show any significant change and remained within the acceptable limits of the ICH. In the cream and Pheroid™ emulgel the concentration methylparaben showed a significant change and did not remain within the acceptable limits of the ICH.

The concentration propylparaben in the cream and Pheroid™ cream did not show any significant change and remained within the acceptable limits of the ICH. In the emulgel and Pheroid™ emulgel propylparaben's concentration did show a significant change and didn't remain within the acceptable limits.

BHT's concentration in the Pheroid™ cream did not show any significant change and remained within the acceptable limits described in the ICH. The concentration BHT in the cream emulgel and Pheroid™ emulgel showed a significant change and didn't remain within the acceptable limits.

The concentration tocopherol in the Pheroid™ cream and Pheroid™ emulgel showed significant change and didn't remain within the acceptable limits.

The degradation of all the active ingredients can be due to the fact of insufficient protection by the anti-oxidants and preservatives.

C.3.2 pH

The pH was measured on the initial cream, Pheroid™ cream, emulgel and Pheroid™ emulgel. It was also measured at 25°C/60% RH, 30°C/60% RH and 40°C/70% RH on months 1, 2, 3 and 6 as described in Section C.2.2.

C.3.2.1 CREAM

Table C.5: pH of cream at the different conditions after each time interval

	Initial	Month 1	Month 2	Month 3	Month 6
			25°C/60% RH		
1	6.260	6.649	6.369	6.212	6.885
2	6.153	6.468	6.360	6.185	6.889
3	6.486	6.458	6.349	6.188	6.794
Average	6.300	6.525	6.359	6.195	6.856
SD	0.139	0.088	0.008	0.012	0.044
% RSD	2.203	1.345	0.129	0.195	0.640
			30°C/60% RH		
1	6.260	6.277	6.160	6.059	6.111
2	6.153	6.278	6.143	6.035	6.114
3	6.486	6.277	6.133	6.022	6.112
Average	6.300	6.277	6.145	6.039	6.112
SD	0.139	0.000	0.011	0.015	0.001
% RSD	2.203	0.008	0.181	0.254	0.020
			40°C/70% RH		
1	6.260	5.831	5.789	5.610	5.558
2	6.153	5.822	5.759	5.586	5.562
3	6.486	5.792	5.746	5.579	5.554
Average	6.300	5.815	5.765	5.592	5.558
SD	0.139	0.017	0.018	0.013	0.003
% RSD	2.203	0.287	0.312	0.237	0.059

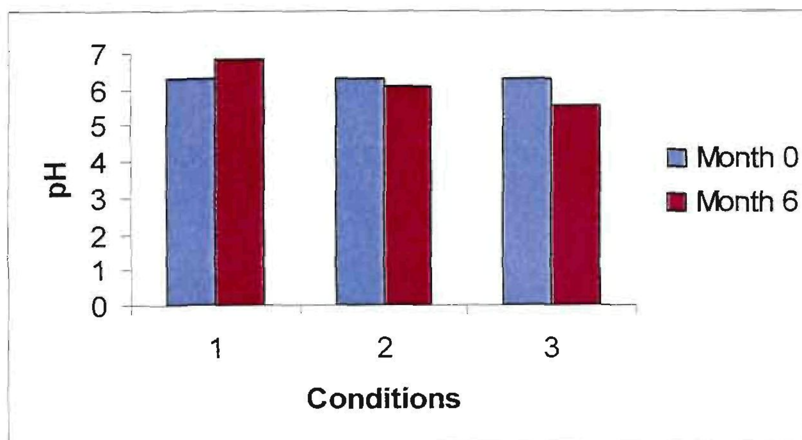


Figure C.5: The change in pH between months 0 and 6 for cream at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)

The pH of the cream remained relatively stable over the six months period. The biggest decrease in pH was the cream stored at 40°C/70% RH with a decrease of 11.8%.

C.3.2.2 PHEROID™ CREAM

Table C.6: pH of Pheroid™ cream at different conditions after each time interval

	Initial	Month 1	Month 2	Month 3	Month 6
25°C/60% RH					
1	6.414	6.341	6.127	6.435	5.782
2	6.409	6.322	6.102	6.205	5.776
3	6.387	6.308	6.091	6.191	5.779
Average	6.403	6.324	6.107	6.277	5.779
SD	0.012	0.014	0.015	0.112	0.002
% RSD	0.183	0.214	0.247	1.782	0.042
30°C/60% RH					
1	6.414	6.232	5.950	5.800	5.544
2	6.409	6.217	5.931	5.777	5.492
3	6.387	6.211	5.918	5.760	5.538
Average	6.403	6.220	5.933	5.779	5.525
SD	0.012	0.009	0.013	0.016	0.023
% RSD	0.183	0.142	0.221	0.284	0.420
40°C/70% RH					
1	6.414	5.847	5.706	5.438	5.240
2	6.409	5.821	5.689	5.414	5.238
3	6.387	5.812	5.675	5.406	5.241
Average	6.403	5.827	5.690	5.419	5.240
SD	0.012	0.015	0.013	0.014	0.001
% RSD	0.183	0.255	0.223	0.251	0.024

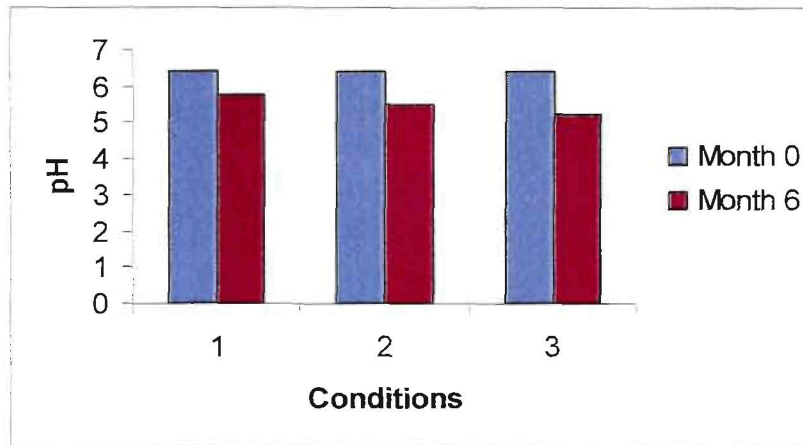


Figure C.6: The change in pH between months 0 and 6 for Pheroid™ cream at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)

The pH of the Pheroid cream remained relatively stable over the six months period. The biggest decrease in pH was the cream stored at 40°C/70% RH with a decrease of 18.2%.

C.3.2.3 EMULGEL

Table C.7: pH of emulgel at different conditions after each time interval

	Initial	Month 1	Month 2	Month 3	Month 6
25°C/60% RH					
1	6.252	6.288	6.168	5.922	5.659
2	6.473	6.278	6.155	5.932	5.645
3	6.450	6.264	6.160	5.937	5.649
Average	6.392	6.277	6.161	5.930	5.651
SD	0.099	0.010	0.005	0.006	0.006
% RSD	1.552	0.157	0.087	0.105	0.104
30°C/60% RH					
1	6.252	6.189	6.096	5.849	5.796
2	6.473	6.183	6.078	5.846	5.776
3	6.450	6.180	6.071	5.843	5.792
Average	6.392	6.184	6.082	5.846	5.788
SD	0.099	0.004	0.011	0.002	0.009
% RSD	1.552	0.061	0.173	0.042	0.149
40°C/70% RH					
1	6.252	6.045	5.819	5.567	5.217
2	6.473	6.032	5.811	5.550	5.214
3	6.450	6.020	5.801	5.546	5.119
Average	6.392	6.032	5.810	5.554	5.183
SD	0.099	0.010	0.007	0.009	0.046
% RSD	1.552	0.169	0.127	0.164	0.878

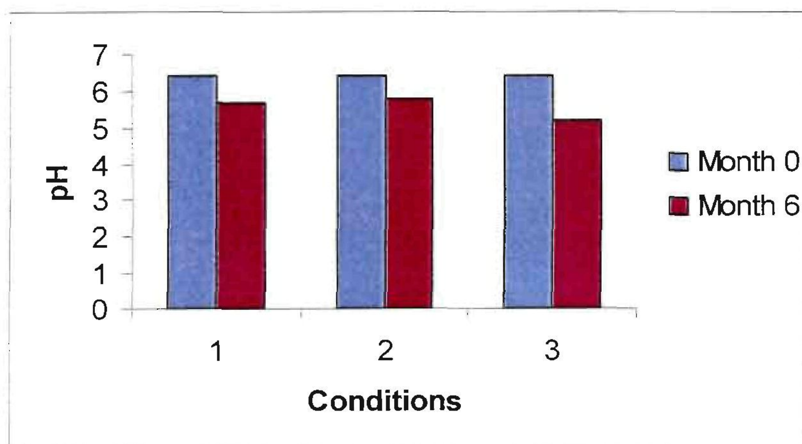


Figure C.7: The change in pH between months 0 and 6 for emulgel at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)

The pH of the emulgel remained relatively stable over the six months period. The biggest decrease in pH was the cream stored at 40°C/70% RH with a decrease of 19%.

C.3.2.4 PHEROID™ EMULGEL

Table C.8: pH of Pheroid™ emulgel at different conditions after each time interval

	Initial	Month 1	Month 2	Month 3	Month 6
25°C/60% RH					
1	6.348	6.220	6.086	5.819	5.596
2	6.349	6.211	6.070	5.816	5.587
3	6.364	6.209	6.064	5.817	5.596
Average	6.354	6.213	6.073	5.817	5.593
SD	0.007	0.005	0.009	0.001	0.004
% RSD	0.115	0.077	0.153	0.021	0.076
30°C/60% RH					
1	6.348	6.144	5.905	5.639	5.367
2	6.349	6.120	5.893	5.617	5.362
3	6.364	6.118	5.889	5.612	5.356
Average	6.354	6.127	5.896	5.623	5.362
SD	0.007	0.012	0.007	0.0117	0.005
% RSD	0.115	0.193	0.115	0.209	0.084
40°C/70% RH					
1	6.348	5.825	5.485	5.207	4.866
2	6.349	5.838	5.482	5.196	4.865
3	6.364	5.839	5.479	5.187	4.854
Average	6.354	5.834	5.482	5.197	4.862
SD	0.007	0.006	0.002	0.008	0.005
% RSD	0.115	0.109	0.045	0.157	0.112

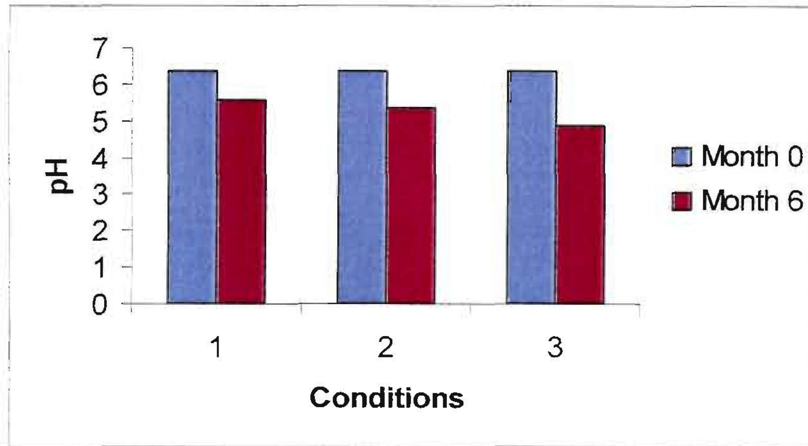


Figure C.8: The change in pH between months 0 and 6 for Pheroid™ emulgel at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)

The pH of the Pheroid emulgel showed a significant decrease over the six months period. The biggest decrease in pH was the cream stored at 40°C/70% RH with a decrease of 23.5%.

C.3.3 VISCOSITY

The viscosity was measured on the initial cream, Pheroid™ cream, emulgel and Pheroid™ emulgel. It was also measured at 25°C/60% RH on month 6 as described in Section C.2.3.

Table C.9: Viscosity of cream, Pheroid™ cream, emulgel and Pheroid™ emulgel at 25°C/60% RH after each time interval

	Initial	**Month 6**
	Cream	
Average	86179.420	22202.290
SD	7572.660	1974.750
% RSD	13.480	8.890
	Pheroid™ cream	
Average	225659.900	14594.050
SD	96835.290	1213.690
% RSD	42.910	8.320
	Emulgel	
Average	189274.100	40460.120
SD	19029.820	2792.580
% RSD	10.050	6.900
	Pheroid™ emulgel	
Average	163856.600	170105.300
SD	12779.030	14988.530
% RSD	7.800	8.810

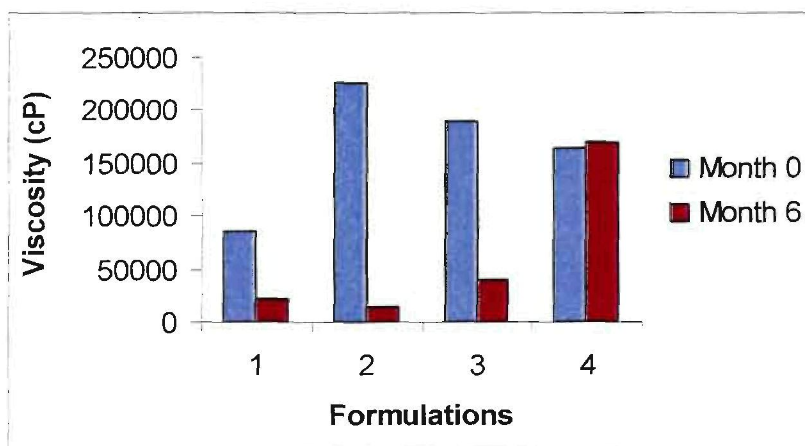


Figure C.9: The change in viscosity between month 0 and 6 for cream (1), Pheroid™ cream (2), emulgel (3) and Pheroid™ emulgel (4) at 25°C/60% RH

The viscosity of the cream, Pheroid™ cream, emulgel and Pheroid™ emulgel changed radically over the six months period. The cream showed a decrease in viscosity of 74.3%, the Pheroid™

emulgel a decrease of 93.5% and in the emulgel a decrease of 79% was observed. The decrease in viscosity can be explained as a reaction between some ingredients. The large decrease in viscosity is a warning of instability. In the Pheroid™ emulgel the viscosity relatively stayed the same.

C.3.4 CONFOCAL LASER SCANNING MICROSCOPY

CLSM was done on the initial cream, Pheroid™ cream, emulgel and Pheroid™ emulgel. It was also done at 25°C/60% RH, 30°C/60% RH and 40°C/70% RH on month 1, 2, 3 and 6 as described in Section C.2.4. Figure C.10 shows an overview to see the changes between month 0 and 6 of the different formulations.

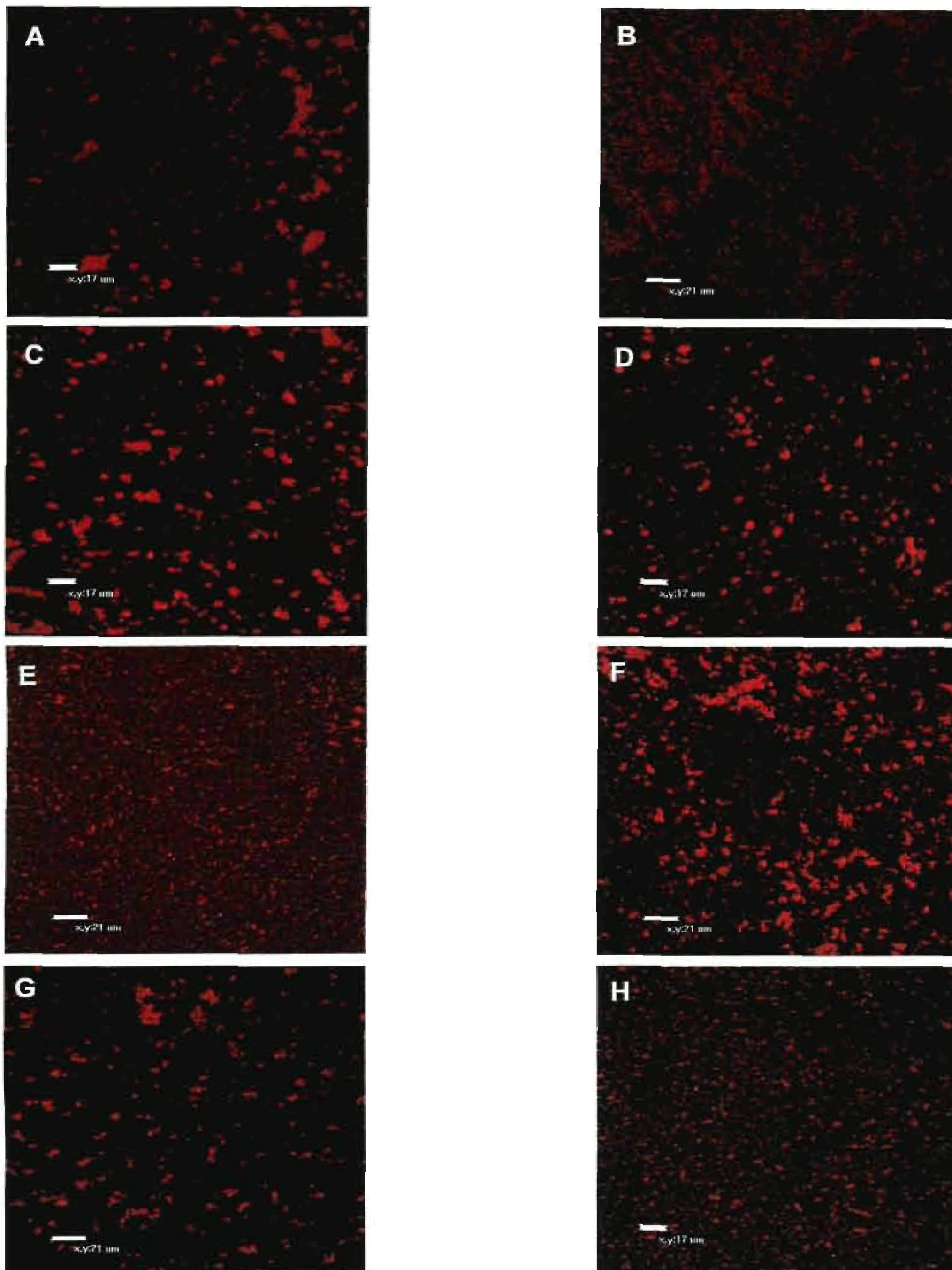


Figure C.10: Confocal image of cream on month 0 (A) and month 6 (B), Pheroid™ cream on month 0 (C) and month 6 (D), emulgel on month 0 (E) and month 6 (F) and Pheroid™ emulgel on month 0 (G) and month 6 (H)

There was no significant change in the formulations' particle sizes over the six month period. The particle sizes ranged between 1 and 10 µm. The homogeneity of the products also stayed the same.

C.3.5 VISUAL APPEARANCE ASSESSMENT

Visual appearance assessment was done on the initial cream, Pheroid™ cream, emulgel and Pheroid™ emulgel. It was also done at 25°C/60% RH, 30°C/60% RH and 40°C/70% RH on months 1, 2, 3 and 6 as described in Section C.2.5.

In Figure C.11 to Figure C.14 the change in colour during the six months period of each formulation is given.

C.3.5.1 CREAM

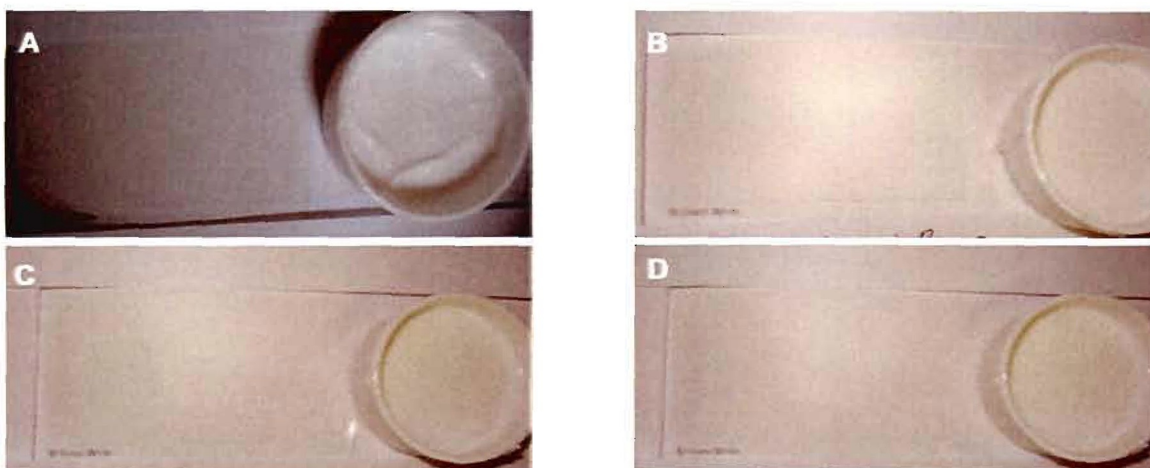


Figure C.11: Change in colour of cream from month 0 (A) to month 6 at 25°C/60% RH (B), 30°C/60% RH (C) and 40°C/70% RH (D)

C.3.5.2 PHEROID™ CREAM

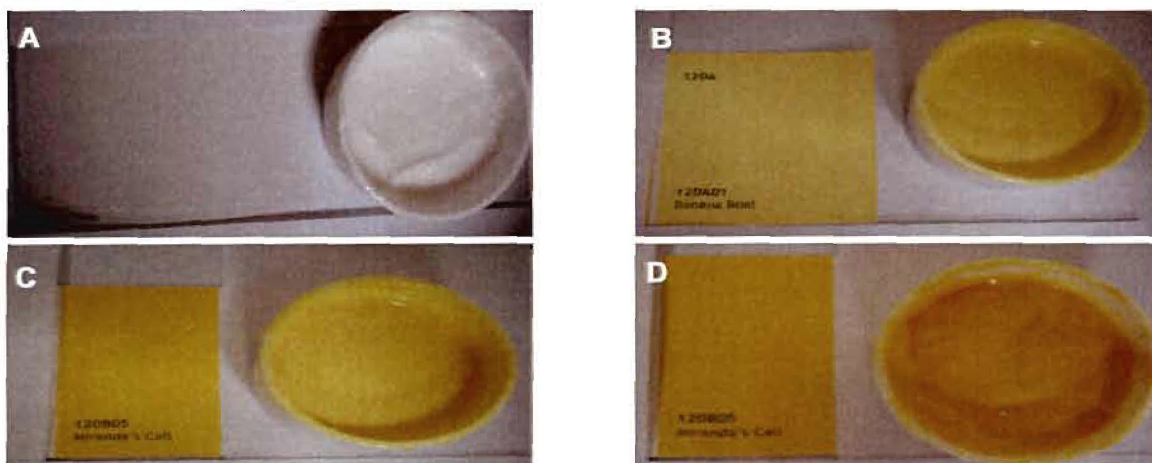


Figure C.12: Change in colour of Pheroid™ cream from month 0 (A) to month 6 at 25°C/60% RH (B), 30°C/60% RH (C) and 40°C/70% RH (D)

C.3.5.3 EMULGEL

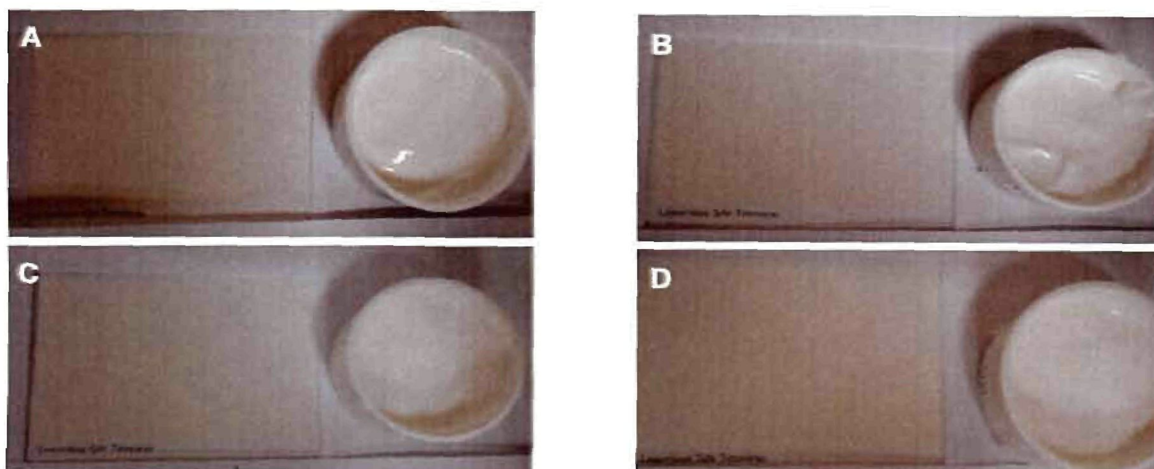


Figure C.13: Change in colour of emulgel from month 0 (A) to month 6 at 25°C/60% RH (B), 30°C/60% RH (C) and 40°C/70% RH (D)

C.3.5.4 PHEROID™ EMULGEL

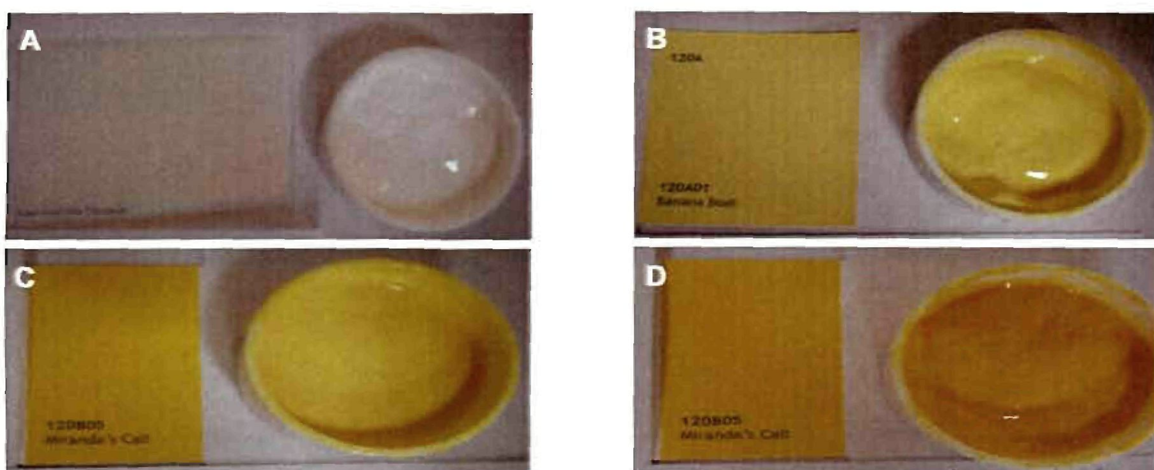


Figure C.14: Change in colour of Pheroid™ emulgel from month 0 (A) to month 6 at 25°C/60% RH (B), 30°C/60% RH (C) and 40°C/70% RH (D)

The cream's and emulgel's colour didn't show any significant change over the six month period. The colour of the Pheroid™ cream and Pheroid™ emulgel showed a radical change over the six months period. This indicated instability in the Pheroid™ products.

C.3.6 MASS LOSS

The mass loss was measured on the initial cream, Pheroid™ cream, emulgel and Pheroid™ emulgel. It was also measured at 25°C/60% RH, 30°C/60% RH and 40°C/70% RH on month 1, 2, 3 and 6 as described in Section C.2.6.

C.3.6.1 CREAM

Table C.10: Mass of cream at different conditions after each time interval

	Initial	Month 1	Month 2	Month 3	Month 6
			25°C/60% RH		
1	30.911	30.871	30.832	30.810	30.681
2	30.912	30.872	30.832	30.811	30.681
3	30.911	30.871	30.832	30.811	30.680
Average	30.911	30.871	30.832	30.810	30.680
			30°C/60% RH		
1	31.177	31.069	31.011	30.977	30.795
2	31.177	31.079	31.011	30.978	30.795
3	31.177	31.079	31.011	30.978	30.795
Average	31.177	31.075	31.011	30.977	30.795
			40°C/70% RH		
1	30.937	30.856	30.824	30.802	30.711
2	30.937	30.856	30.823	30.802	30.711
3	30.937	30.855	30.823	30.802	30.711
Average	30.937	30.855	30.823	30.802	30.711

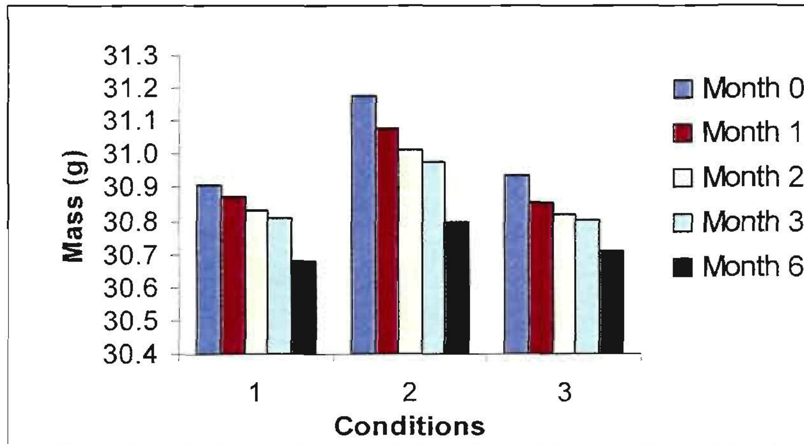


Figure C.15: The change in mass between months 0 and 6 for cream at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)

C.3.6.2 PHEROID™ CREAM

Table C.11: Mass of Pheroid™ cream at different conditions after each time interval

	Initial	Month 1	Month 2	Month 3	Month 6
25°C/60% RH					
1	31.045	31.037	31.027	31.025	30.996
2	31.047	31.037	31.027	31.025	30.996
3	31.047	31.037	31.027	31.025	30.996
Average	31.046	31.037	31.027	31.025	30.996
30°C/60% RH					
1	30.955	30.907	30.872	30.851	30.729
2	30.955	30.906	30.872	30.851	30.728
3	30.955	30.906	30.872	30.851	30.729
Average	30.955	30.906	30.872	30.851	30.728
40°C/70% RH					
1	31.059	30.960	30.915	30.883	30.709
2	31.059	30.960	30.915	30.884	30.708
3	31.059	30.960	30.915	30.883	30.708
Average	31.059	30.960	30.915	30.883	30.708

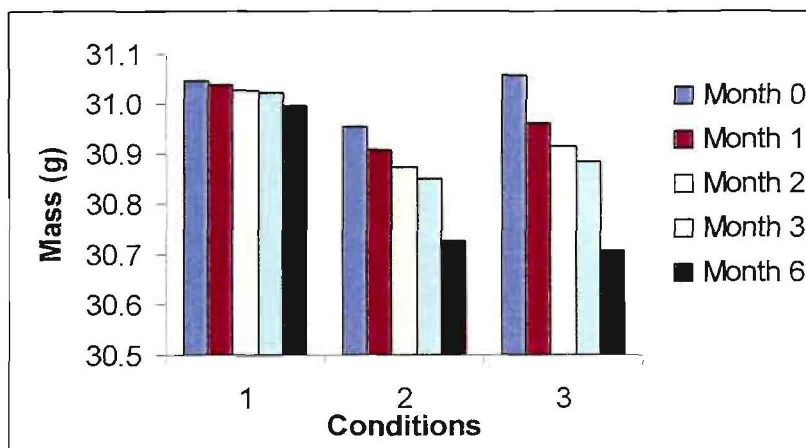


Figure C.16: The change in mass between months 0 and 6 for Pheroid™ cream at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)

C.3.6.3 EMULGEL

Table C.12: Mass of emulgel at different conditions after each time interval

	Initial	Month 1	Month 2	Month 3	Month 6
			25°C/60% RH		
1	30.943	30.933	30.917	30.912	30.873
2	30.943	30.933	30.917	30.912	30.873
3	30.943	30.933	30.917	30.912	30.872
Average	30.943	30.933	30.917	30.912	30.872
			30°C/60% RH		
1	31.016	30.970	30.873	30.846	30.643
2	31.016	30.970	30.873	30.846	30.643
3	31.016	30.970	30.873	30.846	30.643
Average	31.016	30.970	30.873	30.846	30.643
			40°C/70% RH		
1	31.049	30.901	30.747	30.624	30.175
2	31.049	30.901	30.747	30.624	30.175
3	31.049	30.900	30.747	30.624	30.175
Average	31.049	30.900	30.747	30.624	30.175

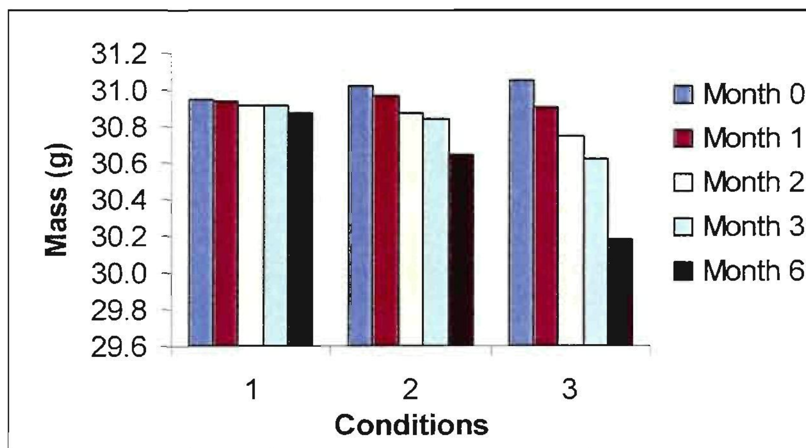


Figure C.17: The change in mass between months 0 and 6 for emulgel at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)

C.3.6.4 PHEROID™ EMULGEL

Table C.13: Mass of Pheroid™ emulgel at different conditions after each time interval

	Initial	Month 1	Month 2	Month 3	Month 6
25°C/60% RH					
1	30.941	30.937	30.929	30.927	30.909
2	30.941	30.937	30.929	30.927	30.909
3	30.941	30.936	30.929	30.927	30.909
Average	30.941	30.936	30.929	30.927	30.909
30°C/60% RH					
1	30.951	30.944	30.921	30.913	30.866
2	30.952	30.944	30.921	30.913	30.866
3	30.952	30.944	30.921	30.913	30.866
Average	30.951	30.944	30.921	30.913	30.866
40°C/70% RH					
1	30.933	30.752	30.688	30.640	30.484
2	30.933	30.752	30.688	30.639	30.485
3	30.933	30.752	30.688	30.639	30.484
Average	30.933	30.752	30.688	30.639	30.484

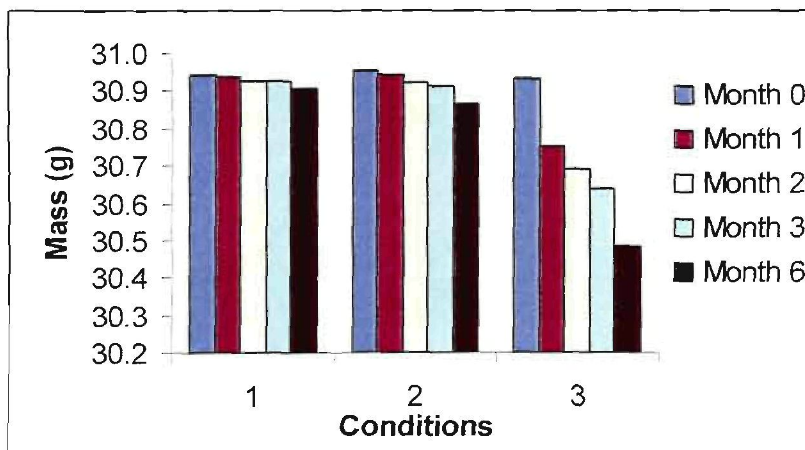


Figure C.18: The change in mass between months 0 and 6 for Pheroid™ emulgel at 25°C/60% RH (1), 30°C/60% RH (2) and 40°C/70% RH (3)

The mass of the cream, Pheroid™ cream, emulgel and Pheroid™ emulgel remained stable over the six months period. The biggest decrease in mass was the formulations stored at 40°C/70% RH. A decrease in mass was observed for cream (0.7%), Pheroid™ cream (1.1%), emulgel (2.8%) and Pheroid™ emulgel (1.5%). This shows that the containers used for storage of the products sealed well enough for limiting evaporation and preventing moisture absorption.

C.4 Conclusions

In the concentration assay no active ingredient showed stability over the six months period and didn't remain within the acceptable limits of the ICH standards (ICH, 2003:13). The concentration of nicotinamide in the products did show significant change over the six months period. The preservative and anti-oxidant ingredients showed also a significant change. Because preservative testing wasn't performed, it cannot be said that preservative action didn't take place. In future preservative testing may be considered. It can be concluded that the decrease in anti-oxidant concentration shows that nicotinamide was protected against degradation to a certain level.

The pH of the products showed relative stability over the six month period. In all the formulations the pH decreased. Although a change in pH was seen, the stratum corneum is remarkably resistant to alteration in pH, tolerating a range of 3 to 9 (Barry, 2002:511).

The viscosity of the cream, Pheroid™ cream, emulgel and Pheroid™ emulgel changed radically over the six month period. The cream showed a decrease in viscosity of 74.3 %, the Pheroid™ gel a decrease of 93.5% and in the emulgel a decrease of 79.0% was observed. The decrease in viscosity can be explained as a reaction between some ingredients. The large decrease in viscosity is a warning of instability.

There was no significant change in the formulations' particle sizes over the six month period. The homogeneity of the products also stayed the same. This is due to the fact that the homogenising in the formulation process was done at a speed fast enough to assure good homogeneity and small particle sizes.

The cream's and emulgel's colour didn't show any significant change over the six month period. The Pheroid™ cream and Pheroid™ emulgel showed a considerable colour change over the six month period. Degradation of the ingredients occurred and this showed that the Pheroid™ products were unstable. The reason for the change in colour and an explanation thereof must be further investigated.

Unfortunately the change in colour, viscosity and concentration of the active ingredients in the formulations showed instability of the products over the six month period. In future different anti-oxidants and preservatives should be considered and a higher concentration thickening agent should be used. The reason for the Pheroid™ formulations' change in colour should be investigated.

C.5 References

BARRY, B. 2002. Transdermal drug delivery. (*In* Aulton, M.E., *ed.* Pharmaceutics: The science of dosage form design. 2nd ed. London: Churchill Livingston. p. 499-533.)

BROOKFIELD. 1998. Brookfield viscometer/rheometers. Massachusetts: Brookfield engineering laboratories, Inc. p. 2-22.

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE (ICH) Q1A(R2). 2003. Stability testing of new drug substances and products. p. 1-22.

MITSUI, T. *ed.* 1997. New cosmetic science. Amsterdam: Elsevier. 376p.

APPENDIX D
FRANZ CELL DIFFUSION STUDIES

D.1 Introduction

The use of *in vitro* methods to assess the release of active ingredients from semi-solid dosage forms, is gaining increasing attention from both the pharmaceutical industry and regulatory authorities. The Franz diffusion cell is a popular method of studying the transdermal diffusion of drugs and is also used to assess drug release from semi-solid dosage forms (Chattaraj *et al.*, 1995:119). In Franz cell diffusion studies, a receptor solution is placed into the receptor compartment, which is maintained at 32°C. A membrane is placed over the diffusion cell opening. The formulation is applied to the membrane in the donor phase. The cells are stirred with a magnetic stir bar. Samples are then withdrawn from the receptor solution at regular time intervals. In this study, diffusion studies were done by using membranes, as well as full thickness human skin.

D.2 Methods

D.2.1 AQUEOUS SOLUBILITY

The aqueous solubility of nicotinamide was determined by preparing saturated solutions in PBS with a pH of 7.4. The solutions were stirred with magnetic bars in a 32°C water bath for 24 h. An excess amount of nicotinamide was inserted in order to keep the solution saturated at all times. The solutions were then filtered, diluted and analysed by HPLC. This experiment was performed in triplicate.

D.2.2 LOG D

The experimental *n*-octanol-PBS (phosphate buffered saline) partition coefficient (log D) was performed as follows: pre-saturated *n*-octanol and PBS (pH 7.4) were prepared by vigorously stirring equal amounts of each for 24 h, after which the two layers were separated. Nicotinamide (9.9 mg) was dissolved in 20 ml pre-saturated PBS. The nicotinamide pre-saturated PBS (3 ml) was placed in a test-tube and pre-saturated *n*-octanol (3 ml) was added. It was shaken for 3 h at 32°C and then centrifuged at 4500 rpm for 10 min. This experiment was performed in quaternary. The aqueous phase was analysed by HPLC. The logarithmic ratio of the concentration in the *n*-octanol phase to the concentration in the PBS was used to calculate the log D.

D.2.3 SKIN PREPARATION

Abdominal skin of Caucasian female patients was obtained after cosmetic abdominoplastic surgery. Ethical approval for obtaining and preparing the skin was provided by the Research Ethics Committee of the North-West University under the reference number 04D08. The surgeon's permission was received before asking consent from the patient. To ensure anonymity, the identity of the patient will not be published. The skin was frozen at -20°C within 24 h after it had been surgically removed. Before preparation, the skin was thawed to room temperature. The adipose layer was carefully removed with a scalpel. The skin was placed on Whatman® filter paper with the stratum corneum facing upwards. Circles with a diameter of ± 15 mm were punched into the skin. The skin circles were placed on aluminium foil before the foil was carefully closed and put into a Ziploc® bag. They were kept frozen at -20°C until they were used. Prior to the diffusion study, the skin circles were thawed to room temperature before mounting them in the diffusion apparatus.

D.2.4 PREPARATION OF PBS (pH 7.4)

- Potassium dihydrogen orthophosphate (13.620 g) was weighed off and dissolved in 500.0 ml HPLC water.
- Sodium hydroxide (3.147 g) was weighed off and dissolved in 786.8 ml HPLC water.
- The two solutions were mixed and the pH was set to 7.4 with 10% orthophosphoric acid.
- The solution was filtered and degassed.

D.2.5 DIFFUSION STUDIES

Vertical Franz diffusion cells with a receptor capacity of approximately 2 ml and a diffusion area of 1.075 cm^2 were used in the study. In each study 10 Franz cells were used. These cells consist of a donor (top) and receptor (bottom) compartment. The donor phase (semi-solid formulation with active) was placed in the donor compartment and the receptor phase (PBS, pH 7.4) was placed in the receptor compartment. A small magnetic stirring bar was placed in the receptor compartment of each Franz cell to maintain stirring throughout the experiment. The skin circles or membranes were mounted between the receptor and donor compartment with the stratum corneum facing upwards (towards the donor compartment). Dow Corning® high vacuum grease was used to seal the cells to prevent any leakage. The donor and receptor compartments were secured with a horseshoe clamp. The receptor compartments were filled with PBS (pH 7.4) while securing that no air bubbles had formed. The donor compartments were filled with enough of the semi-solid formulation to keep the skin saturated. It was then covered with Parafilm® to prevent evaporation. The Franz cells were placed in a 37°C water

bath in order to attain a skin temperature of 32°C. The entire content of the receptor phases were withdrawn on specific time intervals and replaced with fresh PBS (pH 7.4) that was pre-heated to 32°C. HPLC vials were then filled with the withdrawn PBS (receptor phase). The concentration of nicotinamide was immediately analysed by HPLC.

D.2.6 SKIN DIFFUSION

The method as discussed in D.2.5 was used for the skin diffusion studies. The entire content of the receptor phases was withdrawn and replaced with fresh PBS (pH 7.4) after 20, 40, 60, 80, and 100 min, as well as 2, 4, 6, 8, 10 and 12 h. Tape stripping was performed after the 12 h withdrawal (see section D.2.8).

D.2.7 MEMBRANE DIFFUSION

The method as discussed in D.2.5 was used for the membrane diffusion studies. The entire content of the receptor phases was withdrawn and replaced with fresh PBS (pH 7.4) after 30, 60, and 90 min, as well as 2, 4 and 6 h. The aim of the membrane studies was to determine whether nicotinamide is released from the formulations. Therefore there is a difference in withdrawal times from the diffusion studies.

D.2.8 TAPE STRIPPING

According to Lademann *et al.* (2009:317) tape stripping is a simple and efficient method for the assessment of quality and efficacy of cosmetical and dermatological formulation. After topical application and penetration of formulations, the cell layers of the stratum corneum are successively removed from the same skin area, using adhesive films. The tape strips contain the amount of penetrated formulation, which can be analysed by chemical methods (Lademann *et al.*, 2009:317). At the end of the diffusion study the diffusion cells were carefully seperated and the pieces of skin were pinned onto a piece of Parafilm®, stapled to a solid surface. The diffused area could clearly be seen. The pieces of skin were dabbed dry with tissue. Pieces of 3M Scotch® Magic™ Tape were cut into sizes big enough to cover the diffused area, and small enough not to overlap the areas outside the diffused area. The first tape strip was discarded, as it is seen as part of the cleaning procedure. The next 15 strips (epidermis) were placed in a vial filled with enough PBS (pH 7.4) to cover the strips. An indication of the complete removal of the stratum corneum is when the viable epidermal layer glistens. The vials were kept overnight at 4°C. The remaining skin was cut into pieces to enlarge the surface area. It was placed in vials filled with enoughf PBS (pH 7.4) to cover the skin pieces and were kept overnight at 4°C. The tape samples were filtered and analysed by HPLC. The skin samples were homogenised and filtered in turn to be analysed by HPLC.

D.2.9 HPLC ANALYSIS OF NICOTINAMIDE

A HPLC method had already been developed and validated in conjunction with Prof Jan du Preez from the Analytical Technology Laboratory at the North-West University, Potchefstroom Campus, South Africa. An Agilent 1200 Series HPLC equipped with an Agilent 1200 pump, auto sampler injection mechanism and UV-detector was used (Agilent Technologies, Palo Alto, CA). The apparatus was interfaced with Chemstation Rev. A.06.02 data acquisition and analysis software. High performance silica based, reversed phase Phenomenex Luna C18 (2) column (250 × 4.6 mm) with a 5 µm particle size was used (Phenomenex, Torrance, CA). The mobile phase consisted of octanesulphonic acid. The operating flow rate was 1.0 ml/min and the injection volume was 50 µl. The UV-detector was set at 220 nm for the detection of nicotinamide. The retention time of nicotinamide was between 2 and 3 min, and the runtime was 8 min. Analysis was performed in a controlled laboratory environment at 25°C. The solvent used was PBS. The PBS was prepared by accurately weighing and dissolving potassium dihydrogen orthophosphate (2.7 g) and potassium hydroxide (0.95 g) in 1000 ml HPLC water. The pH of the PBS was established to 7.4 by 10% orthophosphoric acid. Analyses were performed in a controlled laboratory environment at 25°C.

D.2.10 DATA ANALYSIS

For the diffusion studies the cumulative concentration of the drug that permeated the skin, was plotted against time. The linear portion of the graph represented the flux of nicotinamide. Average flux values were obtained by the slope of the straight line. The profiles exhibited character with a clear steady-state flux between 0 and 2 h. The yield of each cell was expressed as a percentage of the applied concentration. The percentage yield after 12 h was also determined.

For the membrane studies the percentage yield after 6 h was determined.

For statistical analysis the median (statistically calculated centre of a set of data) of the flux and cumulative concentration values after 12 h, were examined using Statsoft (2008) and SAS Institute (2005). If there is a huge variation in the experimental flux values, the median flux is a more exact method to determine flux (Gerber *et al.*, 2008). A Kruskal-Wallis test was performed to determine a p-value. A p-value less than 0.05 would indicate a statistical significant difference between the data in the different groups (Steyn *et al.*, 1994:604-606).

D.3 Results and discussion

D.3.1 AQUEOUS SOLUBILITY

The aqueous solubility for a drug to ideally permeate the skin is more than 1 mg/ml (Naik *et al.*, 2000). The solubility of nicotinamide was determined to be 212.92 mg/ml in PBS (pH 7.4) at a temperature of 32°C.

When looking at the solubility of nicotinamide (212.92 mg/ml), it was expected that nicotinamide would permeate excellently through the skin.

D.3.2 OCTANOL-BUFFER PARTITION COEFFICIENT (LOG D)

A log P (octanol-water partition coefficient) of between 1 and 3 indicates the ability of a drug to dissolve both in water and oil, ensuring that the compound would permeate the skin comparatively fast (Roberts & Walters, 1998). The log D for nicotinamide was determined to be -0.32. In contrast to the aqueous solubility value that predicted good permeation through the skin, the log D value predicted that permeation might not be optimal.

D.3.3 MEMBRANE RELEASE STUDIES

Table D.1: Data obtained from membrane release studies

	Average % released after 6 h	Average cumulative concentration after 6 h (μg/cm^2)	Median cumulative concentration after 6 h (μg/ml)
Cream	0.578	259.634	273.670
Pheroid™ cream	0.636	307.318	312.420
Emulgel	1.208	589.855	515.900
Pheroid™ emulgel	2.938	1790.280	1696.080

The Pheroid™ emulgel released the highest % nicotinamide after 6 h, followed by the emulgel, Pheroid™ cream and cream. The Pheroid™ emulgel released more than twice the amount of the emulgel and five times more than the Pheroid™ cream and cream. This could be because of the emulgel's higher water content that makes it more water soluble and increases the permeability (Bouwstra *et al.*, 2003:22). There was a slight difference between the % nicotinamide released from the Pheroid™ cream and cream. The Pheroid™ cream released 10% more nicotinamide than the cream. This could be because of the penetration enhancing effect of the Pheroid™ vesicles (Grobler *et al.*, 2008:284).

There was not a significant difference between the average cumulative concentration values after 6 h and the median cumulative concentration values after 6 h of the cream and Pheroid cream. This could be because of the small amount of outliers in the data. Average values were determined by the sum of the data, divided by the number of data points. The median values determine the centre point of the data points. There was a significant difference between the average cumulative concentration values after 6 h and the median cumulative concentration values after 6 h of the emulgel and Pheroid emulgel. This was because of the many outliers in the data, as well as the large variation in the cumulative concentration against time data of each individual Franz cell in each study.

When the data was statistically analysed and compared in the Kruskal-Wallis test, certain p-values were obtained to indicate whether there was a significant difference or not between the different groups. When the average cumulative concentration of nicotinamide after 6 h of each formulation was compared to each other, the p-value was 0.0001. This means that there was a significant difference between the different formulations. There was a significant difference between the cream and Pheroid™ gel with a p-value of 0.000035. There is also a significant difference between the gel and Pheroid™ gel with a p-value of 0.022 and between the Pheroid™ cream and Pheroid™ gel with a p-value of 0.018.

D.3.4 DIFFUSION STUDIES

Table D.2: Data obtained from skin diffusion studies

	Average % diffused after 12 h	Average flux (μg/cm^2.h)	Average cumulative [ ] after 12 h (μg/ml)	Median flux (μg/cm^2.h)	Median cumulative [ ] after 12 h (μg/ml)
Cream	0.025	1.849 ± 0.080	13.913	1.874	11.186
Pheroid™ cream	0.038	1.324 ± 0.040	21.409	1.337	21.030
Emulgel	0.069	3.166 ± 0.079	29.068	3.191	30.770
Pheroid™ emulgel	0.048	2.918 ± 0.282	23.604	2.945	25.865

The emulgel showed the highest average % nicotinamide diffused after 12 h followed by the Pheroid™ emulgel, Pheroid™ cream and cream. There was not a significant difference in the average % nicotinamide of each formulation diffused after 12 h.

The emulgel showed the highest average cumulative concentration of nicotinamide after 12 h, followed by the Pheroid™ emulgel, cream and Pheroid™ cream. There was a significant difference between the cream and the rest of the formulations. The Pheroid™ cream showed

very poor diffusion over the 12 h period. This could be because of the high oil content in the cream formulations. Absorption is related to molecular weight, where small molecules penetrate faster than larger ones. Therefore oil molecules, which are larger than water molecules, will penetrate slower. The emulgel formulation with the lowest oil content showed the best diffusion. With an increase in the oil content a decrease in diffusion was observed (Barry, 2002:513).

There was not a significant difference between the average cumulative concentration of nicotinamide diffused after 12 h and the median cumulative concentration of nicotinamide diffused after 12 h. This was because of the small variation in data points and therefore a small amount of outliers in the data. Average values were determined by the sum of the data divided by the number of data points. The median values determined the centre point of the data points.

In all the formulations a definite flux between 0 and 2 h could be seen.

The average flux values didn't differ much from the median flux values. This was because of the small variation in data points due to a small amount of outliers in the data. The median flux values gave a more accurate representation of the true flux since it took all the data into consideration and was not affected by a distortion in the spread of the data, as in the case with average flux values (Gerber, 2008:402). The flux values of each formulation are given in Figure D.1 - D.5.

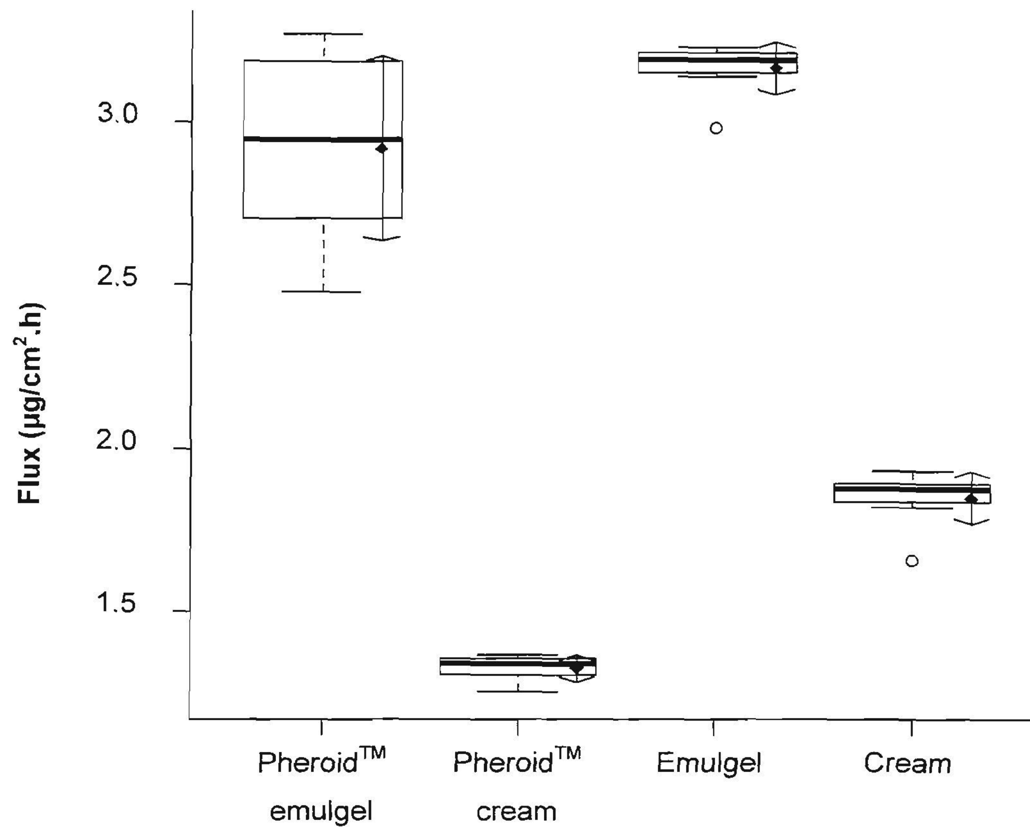


Figure D.1: Box-plots and red lines of the flux values in Pheroid™ emulgel, Pheroid™ cream, emulgel and cream after application to the skin to illustrate median and average flux, respectively

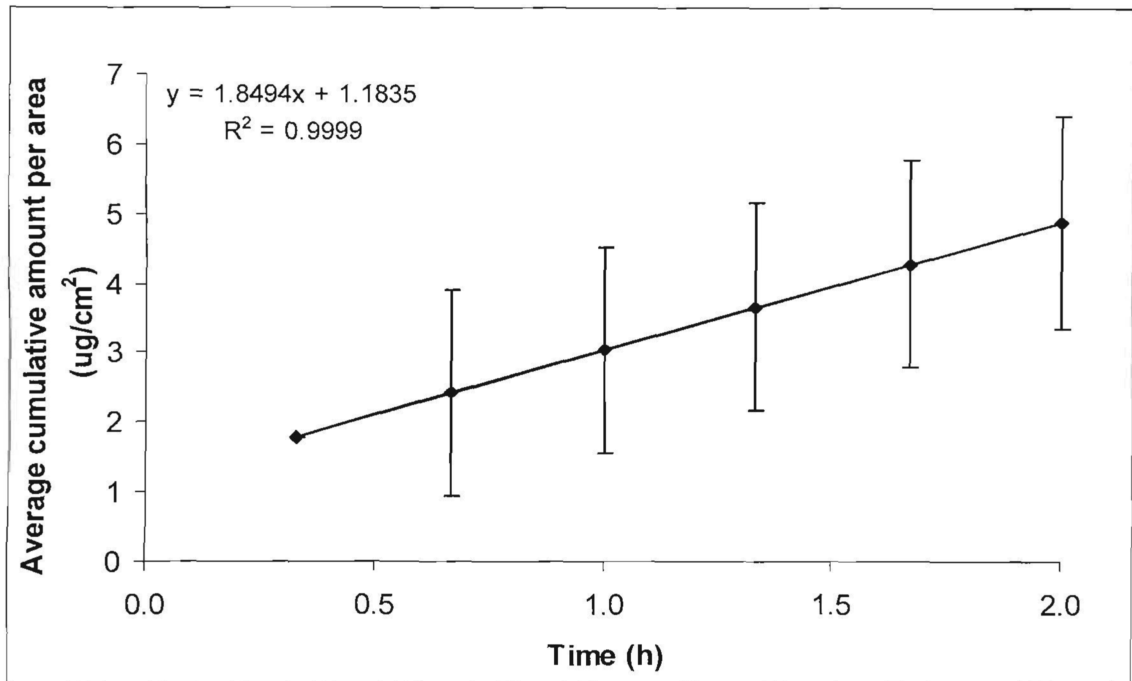


Figure D.2: **Average cumulative amount that penetrated through the skin as a function of time to illustrate the average flux (0-2 h) value in cream**

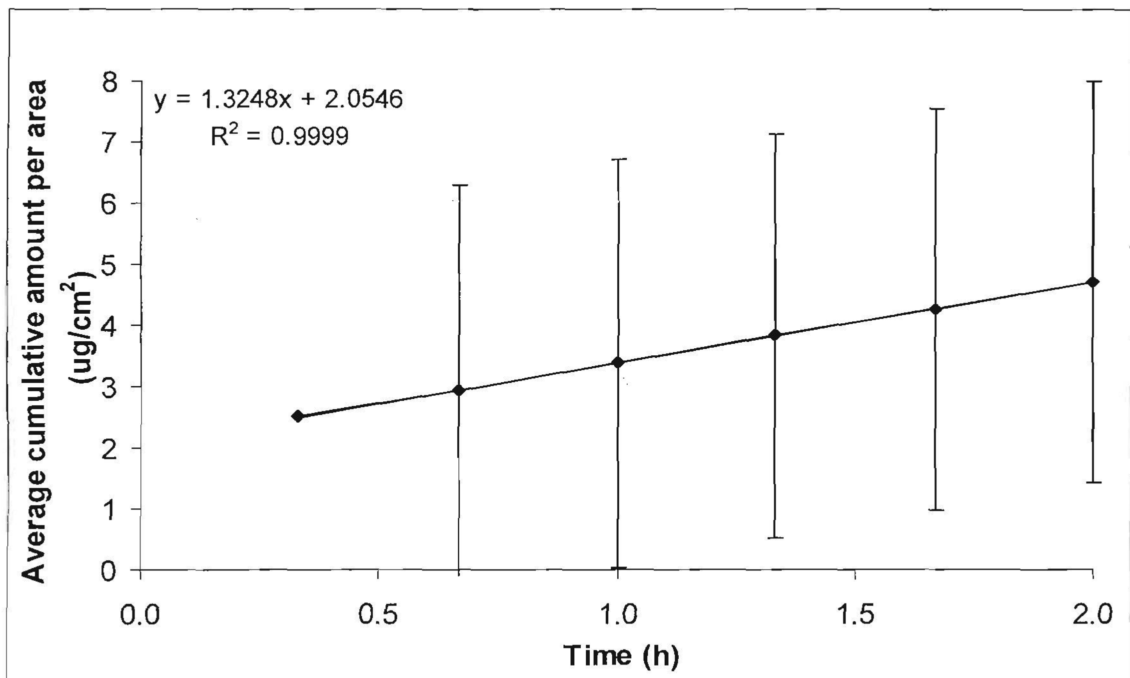


Figure D.3: **Average cumulative amount that penetrated through the skin as a function of time to illustrate the average flux (0-2 h) value in Pheroid™ cream**

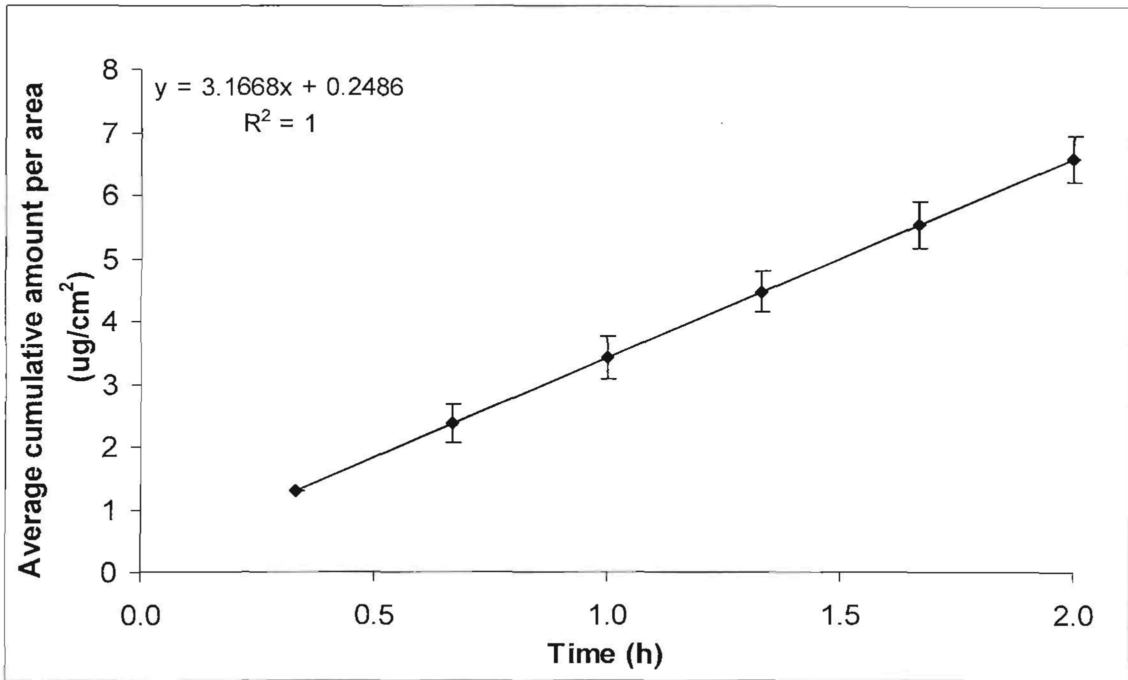


Figure D.4: **Average cumulative amount that penetrated through the skin as a function of time to illustrate the average flux (0-2 h) value in emulgel**

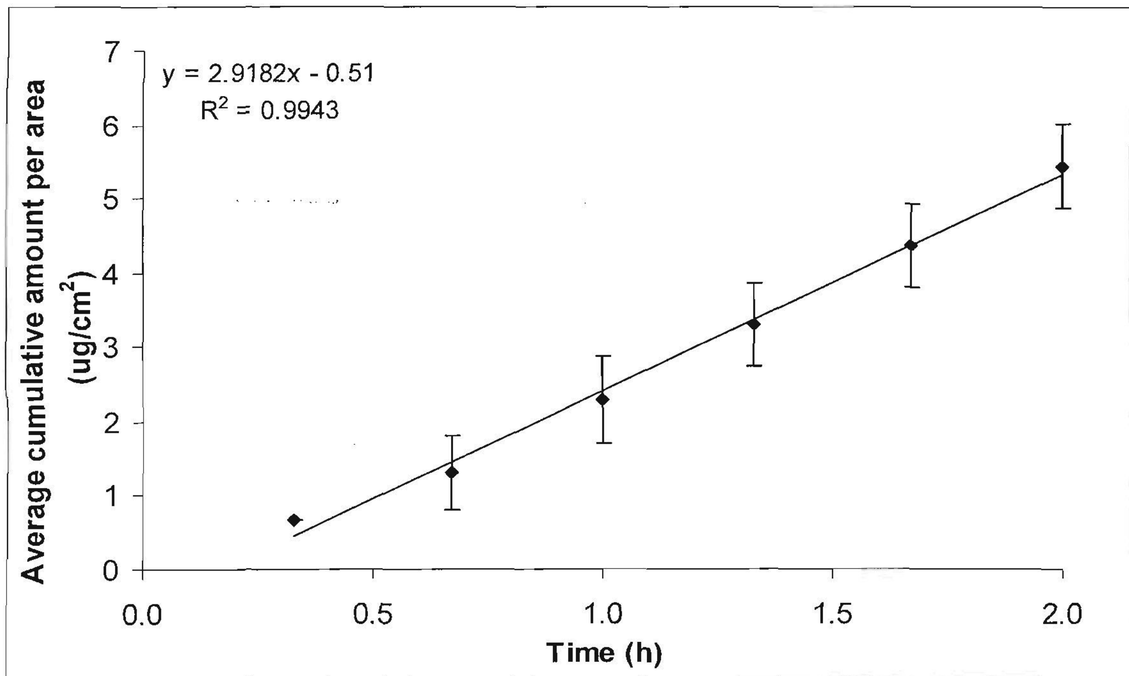


Figure D.5: **Average cumulative amount that penetrated through the skin as a function of time to illustrate the average flux (0-2 h) value in Pheroid™ emulgel**

The median flux is the 50th percentile or the centre of all the flux data and was found to be higher for the emulgel and Pheroid™ emulgel. It can be seen from Figure D.2 - D.5 that the distribution for the emulgel and Pheroid™ emulgel is more symmetrical than the distribution for the cream and Pheroid™ cream. From the box-plot it is clear that the emulgel gave the best flux value of 3.166 µg/cm^2.h, followed by the Pheroid™ emulgel with 2.918 µg/cm^2.h. This can be because of rapid penetration into the skin via the hydrophilic transappendageal route (Dayan, 200732). The cream with a flux value of 1.849 µg/cm^2.h and Pheroid™ cream with a flux value of 1.324 µg/cm^2.h gave the smallest flux values. This could be because of the big oil molecules that madee it difficult for permeation through the skin (Barry, 2002:513).

When the data was statistically analysed and compared in the Kruskal-Wallis test, certain p-values were obtained to indicate whether there was a significant difference or not between the different groups. When the median cumulative concentration of nicotinamide after 12 h of each formulation was compared to each other the p-value is 0.0164. This means that there was a significant difference between the different formulations. There was a significant difference between the cream and gel with a p-value of 0.010795.

D.3.5 TAPE STRIPPING

Table D.3: Data obtained from tape stripping studies

	Average epidermis (µg/ml)	Average dermis (µg/ml)
Cream	5.044	12.616
Pheroid™ cream	3.655	26.029
Emulgel	4.816	5.393
Pheroid™ emulgel	6.767	15.147

Nicotinamide in the Pheroid™ emulgel had the greatest concentration in the epidermis followed by the cream, emulgel and Pheroid™ cream that had the lowest concentration nicotinamide in the epidermis. This shows that the Pheroid™ vesicles in the Pheroid™ emulgel increased the penetration into the epidermis. The Pheroid™ vesicles in the Pheroid™ cream on the other hand didn't show any increase in penetration into the epidermis.

Nicotinamide in the Pheroid™ cream had the highest concentration in the dermis, followed by the Pheroid™ emulgel, cream and emulgel that had the lowest concentration nicotinamide in the dermis. This shows that the Pheroid™ vesicles in the Pheroid™ cream increased the penetration into the dermis (Grobler *et al.*, 2008:293).

The concentration nicotinamide in the dermis was considerably higher than the concentration nicotinamide in the epidermis in all the formulations. This shows that nicotinamide penetrated deeper than just the top layer of the skin. It penetrated into the dermis. For the treatment of acne this is exactly where the action of treatment is needed, because acne develops from the sebaceous glands and follicles which are situated in the dermis (Barry, 1983:38).

When the data was statistically analysed and compared to each other in the Kruskal-Wallis test, certain p-values were obtained to indicate whether there was a significant difference or not between the different groups. When the median concentration of nicotinamide in the epidermis of each formulation was compared to each other in the Kruskal-Wallis test, the p-value was 0.3368. This means that there was no significant difference between the different formulations.

When the median concentration of nicotinamide in the dermis of each formulation was compared to each other, the p-value was 0.0130. This indicated that there was a significant difference between the different formulations. There was a significant difference between the gel and Pheroid™ cream ($p = 0.0292$) and between the gel and Pheroid™ gel ($p = 0.0265$).

When the median concentration of nicotinamide in the epidermis of all the formulations was compared to the average flux value, the p-value was 0.4377. This showed that there was no significant difference between the average concentration in the epidermis and the average flux value.

When the median concentration of nicotinamide in the dermis of all the formulations was compared to the average flux value, the p-value was 0.0796. This means that there was no significant difference between the average concentration in the dermis and the average flux value.

The concentration nicotinamide in the epidermis was considerably lower than the average cumulative concentration. This also shows a deeper penetration of nicotinamide, although it is not necessary for nicotinamide in the treatment of acne to be systemically absorbed (Barry, 1983:38). Nicotinamide in the Pheroid™ emulgel showed the highest average concentration in the epidermis followed by the cream, emulgel and Pheroid™ cream. The Pheroid™ emulgel had 25% more nicotinamide in the epidermis than the cream, 29% more than the emulgel and 46% more than in the Pheroid™ cream. The Pheroid™ cream showed almost twice the average concentration nicotinamide in the dermis than the Pheroid™ emulgel, cream and five times more than in the emulgel. The cream didn't show a big difference in the average concentration nicotinamide in the dermis and the average cumulative concentration after 12 h, but the Pheroid™ cream showed 18% more nicotinamide in the dermis than the cumulative concentration after 12 h. The emulgel showed 81% less nicotinamide in the dermis and the

Pheroid^{TM} emulgel 26% less nicotinamide in the dermis than the cumulative concentration after 12 h.

D.4 Conclusion

Nicotinamide's aqueous solubility value of 212.92 mg/ml predicted good permeation through the skin in contrast to the log D value of -0.32 that predicted that permeation might not be optimal.

In the membrane release studies the Pheroid^{TM} emulgel released the highest % nicotinamide of 2.938% after 6 h, followed by the emulgel, Pheroid^{TM} cream and cream. This could be because of the emulgel's greater water content that makes it more water soluble and increases permeation. When comparing the % nicotinamide released from the Pheroid^{TM} formulations (cream (0.636%) and Pheroid^{TM} emulgel (2.938%)) to those without Pheroid^{TM} (cream (0.578%) and emulgel (1.208%)), it showed that the Pheroid^{TM} vesicles enhanced the penetration of. nicotinamide.

In the skin diffusion studies, the emulgel showed the highest average cumulative concentration of nicotinamide, as well as the highest average % nicotinamide diffused after 12 h, followed by the Pheroid^{TM} emulgel, cream and Pheroid^{TM} cream. The cream formulations showed very poor diffusion over the 12 h period. The high oil content in the cream formulations could be the reason for this problem. With an increase in the oil content a decrease in diffusion was observed.

The emulgel (3.166 μg/cm^2.h) had the highest average flux value which might be attributed to rapid penetration into the skin via the hydrophilic transappendageal route. The Pheroid^{TM} cream (1.324 μg/cm^2.h) had the smallest average flux value and could be attributed to the large molecules that made it difficult for permeation through the skin.

According to Barry (2002:507), the eventual aim in dermatological biopharmaceutics is to design drugs with selective penetrability for incorporation into vehicles or devices that deliver the medicaments to the active site, at a controlled rate and concentration, for the necessary time. Therefore the aim was to deliver nicotinamide to the dermis where the drug action is most needed for the treatment of acne.

The Pheroid^{TM} vesicles in the Pheroid^{TM} emulgel showed an increase in the penetration to the epidermis while the Pheroid^{TM} vesicles in the Pheroid^{TM} cream didn't show any increase. Although nicotinamide in the Pheroid^{TM} cream showed the lowest concentration in the epidermis, it showed the highest concentration in the dermis. This shows that although Pheroid^{TM} technology has great advantages in enhancing skin penetration, it differs from

formulation to formulation. Pheroid^{TM} technology has advantages, but unfortunately can't be set as a rule to all formulations.

The concentration nicotinamide in the dermis was considerably higher than the concentration nicotinamide in the epidermis in all the formulations. This shows that nicotinamide penetrated deeper than just the top layer of the skin. It penetrated into the dermis where the action of treatment for acne is needed.

The concentration nicotinamide in the epidermis was considerably lower than the average cumulative concentration that diffused the skin after 12 h. This also showed a deeper penetration of nicotinamide.

The concentration nicotinamide in the dermis didn't differ much from the average cumulative concentration that diffused the skin after 12 h. As it is not necessary for nicotinamide to be systemically absorbed in the treatment of acne, it will be best to find a way to prevent systemical absorption and rather try to get a greater concentration nicotinamide to the dermis, where treatment is needed most.

Thus the following can be concluded:

- The emulgel is the best formulation to use when transdermal delivery and systemical absorption of nicotinamide is required;
- The emulgel is the best formulation to use when a quick delivery of nicotinamide is wanted;
- The best formulation to use when nicotinamide must be delivered to the epidermis is the Pheroid^{TM} emulgel; and
- The Pheroid^{TM} cream is the best formulation to use when nicotinamide must be delivered to the dermis like in the treatment of acne.

D.5 Photos of apparatus used during Franz cell diffusion studies

Figure D.6: A) Vertical Franz diffusion cell with donor and receptor compartments and

B) Horseshoe clamps

Figure D.7: A) Dow Corning vacuum grease and

B) Assembled Franz diffusion cells

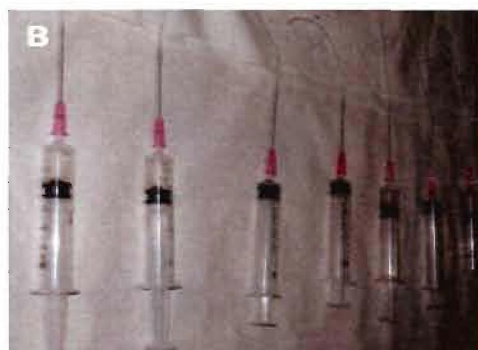


Figure D.8: A) Grant water bath and

B) Syringes used to withdraw samples from receptor compartments

Figure D.9: A) HPLC vials where samples were placed in for analyses and

B) Eppendorf Centrifuge system

Figure D.10: A) Milli-Q water purifying system and

B) Agilent® 1200 Series HPLC

Figure D.11: A) Reversed phase Phenomenex® Luna C18 (2) column and

B) Variomag® magnetic stirring plate

D.6 References

BARRY, B.W. 1983. *Dermatological formulations*. New York: Marcel Dekker. 480p.

BARRY, B. 2002. Transdermal drug delivery. (*In* Aulton, M.E., *ed.* Pharmaceutics: The science of dosage form design. 2nd ed. London: Churchill Livingston. p. 499-533.)

BOUWSTRA, J.A., HONEYWELL-NGUYEN, P.L., GOORIS, G.S. & PONEC, M. 2003. Structure of the skin barrier and its modulation by vesicular formulations. *Progress in lipid research,* 42(1):1-36.

CHATTARAJ, S.C., SWARBRICK, J. & KANFER, I. 1995. A simple diffusion cell to monitor drug release from semi-solid dosage forms. *International journal of pharmaceutics,* 1995(120):119-124.

DAYAN, N. 2007. Pathways for Skin Penetration. (*In* Kozlowski, A. Biotechnology in Cosmetics: Concepts, Tools and Techniques. USA: Allured Publishing Corporation. p. 29-42.)

GERBER, M., BREYTENBACH, J.C., DU PLESSIS, J. 2008. Transdermal penetration of zalcitabine, lamivudine and synthesized N-acyl lamivudine esters. *International journal of pharmaceutics,* 351:186-193.

GROBLER, A., KOTZE, A. & DU PLESSIS, J. 2008. The design of a skin-friendly carrier for cosmetic compounds using Pheroid™ technology. (*In* Wiechers, J. *ed.* Science and applications of skin delivery systems. Wheaton, IL: Allured Publishing. p. 283-311.)

LADEMANN, J., JACOBI, U., SURBER, C. WEIGMANN, H.J. & FLUHR, J.W. 2009. The tape stripping procedure – evaluation of some critical parameter. *European journal of pharmaceutics and biopharmaceutics,* 2009(72):317-323.

NAIK, A., KALIA, Y.N., GUY, R.H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical science technology today*, 3(2000):318-325.

ROBERTS, M.S. & WALTERS, K.A. 1998. The relationship between structure and barrier function of skin. (*In* Roberts, M.S. & Walters, K.A. *eds.* Dermal absorption and toxicity assessment. New York: Marcel Dekker. p. 1-42.)

SAS Institute. 2005. SAS OnlineDoc, version 9.1. http://www.sas.com Date of access: 10 Nov. 2009.

STATSOFT. 2008. Statistica, version 8.0. http://www.statsoft.co.za Date of access: 10 Nov. 2009.

STEYN, A.G.W., SMIT, C.F., DU TOIT, S.H.C. & STRASHEIM, C. 1994. Modern Statistics in Practice. Pretoria: J.L. van Schaik. 640p.

APPENDIX E

INTERNATIONAL JOURNAL OF PHARMACEUTICS: GUIDE FOR AUTHORS

E.1 Scope of the journal

The International Journal of Pharmaceutics publishes innovative papers, reviews, mini-reviews, rapid communications and notes dealing with physical, chemical, biological, microbiological and engineering studies related to the conception, design, production, characterisation and evaluation of drug delivery systems in vitro and in vivo. "Drug" is defined as any therapeutic or diagnostic entity, including oligonucleotides, gene constructs and radiopharmaceuticals.

Areas of particular interest include: pharmaceutical nanotechnology; physical pharmacy; polymer chemistry and physical chemistry as applied to pharmaceutics; excipient function and characterisation; biopharmaceutics; absorption mechanisms; membrane function and transport; novel routes and modes of delivery; responsive delivery systems, feedback and control mechanisms including biosensors; applications of cell and molecular biology to drug delivery; prodrug design; bioadhesion (carrier-ligand interactions); and biotechnology (protein and peptide formulation and delivery).

Note: In 2004, a new section was started on pharmaceutical nanotechnology. For more details, see Editorials in 279/1-2, 281/1, and 288/1.

E.2 Editorial Policy

The over-riding criteria for publication are originality, high scientific quality and interest to a multidisciplinary audience. Papers not sufficiently substantiated by experimental detail will not be published. Any technical queries will be referred back to the author, although the Editors reserve the right to make alterations in the text without altering the technical content. Manuscripts submitted under multiple authorship are reviewed on the assumption that all listed authors concur with the submission and that a copy of the final manuscript has been approved by all authors and tacitly or explicitly by the responsible authorities in the laboratories where the work was carried out. If accepted, the manuscript shall not be published elsewhere in the same form, in either the same or another language, without the consent of the Editors and Publisher.

Authors must state in a covering letter when submitting papers for publication the novelty embodied in their work or in the approach taken in their research. Routine bioequivalence studies are unlikely to find favour. No paper will be published which does not disclose fully the

nature of the formulation used or details of materials which are key to the performance of a product, drug or excipient. Work which is predictable in outcome, for example the inclusion of another drug in a cyclodextrin to yield enhanced dissolution, will not be published unless it provides new insight into fundamental principles.

E.3 Submission of Manuscripts

Authors are strongly encouraged to submit their manuscript electronically by using the Elsevier submission site at (http://www.elsevier.com/journals).

After registration, authors will be asked to upload their manuscript and associated artwork. Full instructions on how to use the online submission tool are available at the web address listed above.

If an author cannot submit their manuscript electronically, then for the initial submission of manuscripts for consideration, hardcopies are sufficient. The original plus two copies, complete with two sets of figures (including originals or duplicates of sufficient quality for clarity of reproduction) and tables, must be submitted in English. All data that would help referees to evaluate the paper should also be supplied. Manuscripts should be typewritten with double spacing and adequate margins on one side of the sheet only (not more than 26 lines per page). All pages should be numbered sequentially. Manuscripts should be sent to one of the following Editors-in-Chief according to the geographical origin of the author. Please include full contact information - corresponding author name, e-mail address, telephone and fax numbers, and full postal address.

After final acceptance for publication, your revised manuscript on disk together with two printed hard copies should be submitted to the accepting editor. It is important that the file on disk and the printout are identical. Both will then be forwarded by the editor to Elsevier. In-depth guidelines for submitting artwork/illustrations can be found at: http://www.elsevier.com/artworkinstructions.

When the paper is to be published as a Rapid Communication, this should be clearly indicated to the Editor-in-Chief.

E.3.1 EUROPE, AFRICA, NEAR EAST

Prof A.T. Florence, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WCIN 1AX, U.K., Fax: +44 20 7837 5092; E-mail: ijp@pharmacy.ac.uk

E.3.2 THE AMERICAS, AUSTRALIA AND NEW ZEALAND

Prof J.H. Rytting, Pharmaceutical Chemistry Dept, University of Kansas, 2095 Constant Avenue, Lawrence, KS 66047, U.S.A., Fax: +1 785 864 5736; E-mail: ijp@ku.edu

E.3.3 JAPAN AND FAR EAST

Prof. T. Sonobe, Department of Pharmaceutical Engineering, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka-shi 422-8526, Japan. Fax: +81-54-264-5614; E-mail: sonobe@gakushikai.jp

E.4 Manuscript Types

E.4.1 FULL LENGTH MANUSCRIPTS

The arrangement of full length papers should accord with the following:

E.4.1.1 TITLE

The full title should not exceed 85 characters including spaces between words.

E.4.1.2 LIST OF AUTHORS

Initial(s) (one given name may be used) followed by the surname of author(s) together with their affiliations. When the work has been carried out at more than one address, the affiliation of each author should be clearly indicated using superscript, lower-case letters. The author to whom correspondence should be directed must be indicated with an asterisk.

E.4.1.3 Affiliation(s) Name(s) and address(es) of the establishment(s) where the work was done, designated by superscript, lower-case letters where appropriate.

E.4.1.4 ABSTRACT

An Abstract not exceeding 200 words (a single paragraph) should be provided typed on a separate sheet.

E.4.1.5 KEYWORDS

A maximum of 6 keywords or short phrases suitable for indexing should be supplied. If possible keywords should be selected from Index Medicus or Excerpta Medica Index. Authors may also wish to refer to the Subject Index published in International Journal of Pharmaceutics, for example, Vol. 287/1-2, pp. 205-219.

E.4.1.6 CORRESPONDING AUTHOR

The author to whom correspondence should be directed should be designated with an asterisk (do not include the address unless different from that indicated by the author's affiliation). Telephone, fax and e-mail address of the corresponding author must be provided.

E.4.1.7 TEXT

The text should be divided into main sections, such as the following: 1. Introduction. 2. Materials and methods. 3. Results. 4. Discussion. Acknowledgements. References. Figure legends. Tables and Figures. These sections must be numbered consecutively as indicated. Subdivisions of a section should also be numbered within that section, for example, 2.1. Materials, 2.2. Relative humidity measurement, 2.3. Sample preparation, etc.

E.4.1.8 NOMENCLATURE

Standard nomenclature should be used throughout; unfamiliar or new terms and arbitrary abbreviations should be defined when first used. Unnecessary or ambiguous abbreviations and symbols are to be avoided. Data should be expressed in SI units.

E.4.1.9 FIGURE LEGENDS, TABLE LEGENDS, FOOTNOTES

Figure legends, tables and footnotes should be typed on separate sheets, lines double spaced. Footnotes, to be numbered consecutively in superscript throughout the text, should be used as little as possible.

E.4.1.10 REFERENCES

See below for full details.

E.4.2 RAPID COMMUNICATIONS

- These articles should not exceed 1500 words or equivalent space.
- Figures should not be included otherwise delay in publication will be incurred.
- Do not subdivide the text into sections. An Abstract should be included as well as a full reference list.
- No proofs will be sent to the author(s).

E.4.3 NOTES

Should be prepared as described for full length manuscripts, except for the following:

- The maximum length should be 1500 words, including figures and tables.
- Do not subdivide the text into sections. An Abstract and reference list should be included.

E.4.4 REVIEWS AND MINI-REVIEWS

Suggestions for review articles will be considered by the Editors-in-Chief. "Mini-reviews" of a topic are especially welcome.

E.5 References

E.5.1 TEXT CITATION

The Harvard system of citation must be used. References should be cited in the text within parentheses: where several citations are given within a single set of parentheses, they should be arranged in ascending order of year of publication; where more than one reference with the same year of publication is cited, they should be arranged in alphabetical order of the first authors' names. When referring to a work of more than two authors, the name of the first author should be given, followed by et al.

Examples of text citations:

(Gesztes et al., 1988; Chestnut et al., 1989; Legros et al., 1990; Mhando and Li Wan Po, 1990; Korsten et al., 1991; Langerman et al., 1991, 1992a,b; Masters et al., 1991; Bonhomme et al., 1992; Kolli et al., 1992).

(Shaw et al., 1978; Nakano and Arita 1990b; Nakano et al., 1990a,b; Bone et al., 1992)

E.5.2 REFERENCE LIST

All references cited in the text should be listed at the end of the paper (typed with double spacing) and assembled alphabetically. More than one paper from the same author(s) in the same year must be identified by the letters a b c, etc. placed after the year of publication.

References must consist of names and initials of all authors, year, title of paper, abbreviated title of periodical, and volume and first and last page numbers. 'Personal communication' and 'unpublished data' should be cited in the text only. Papers referred to as 'submitted for

publication' must include the name of the journal to which submission has been made. Journal titles should be abbreviated according to the 'List of Serial Title Word Abbreviations' (available from International Serials Data System, 20, rue Bachaumont, 75002 Paris, France. ISBN 2-904939-02-8).

Example of arrangement in the reference list:

Crowe, J.H., Crowe, L.M., Chapman, D., 1984a. Infrared spectroscopic studies on interactions of water and carbohydrates with a biological membrane. Arch Biochem. Biophys., 232, 400-407.

Crowe, J.H., Crowe, L.M., Hoekstra, F.A., 1989. Phase transitions and permeability changes in dry membranes during rehydration. J. Bioenerg. Biomembr., 21, 77-92.

Crowe, J.H., Crowe, L.M., Carpenter, J.F., Aurell Wistrom, C., 1987. Stabilization of dry phospholipid bilayers and proteins by sugars. Biochem. J., 242, 1-10.

Crowe, J.H., Crowe, L.M., Carpenter, J.F., Rudolph, A.S., Wistrom, C.A., Spargo, B.J., Anchordoguy, T.J., 1988. Interactions of sugars with membranes. Biochim. Biophys. Acta, 947, 367-384.

Crowe, L.M., Crowe, J.H., Womersley, C., Reid, D., Appel, L., Rudolph, A., 1986. Prevention of fusion and leakage in freeze-dried liposomes by carbohydrates. Biochim. Biophys. Acta, 861, 131-140.

Crowe, L.M., Mouradian, R., Crowe, J.H., Jackson, S.A., Womersley, C., 1984b. Effects of carbohydrates on membrane stability at low water activities. Biochim. Biophys. Acta, 769, 141-150.

Examples of presentation for various types of publication

Langerman, L., Chaimsky, G., Golomb, E., Tverskoy, M., Kook, A.I., Benita, S., 1990. A rabbit model for evaluation of spinal anesthesia: chronic cannulation of the subarachnoid space. Anesth. Analg., 71, 529-535.

Timsina, M.P., Martin, G.P., Marriott, C., Ganderton, D., Yianneskis, M., 1994. Drug delivery to the respiratory tract using dry powder inhalers. Int. J. Pharm., 101, 1-13.

Gibaldi, M. and Perrier, D., 1982. Pharmacokinetics, 2nd Ed., Dekker, New York.

Deppeler, H.P., 1981. Hydrochlorothiazide. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances, Vol. 10, Academic Press, New York, pp. 405-441.

US Pharmacopeia XXII, 1990. US Pharmacopeial Convention, Rockville, MD, pp. 1434-1435.

Mueller, L.G., 1988. Novel anti-inflammatory esters, pharmaceutical compositions and methods for reducing inflammation. UK Patent GB 2 204 869 A, 23 Nov.

Du Plessis, J., 1992. Topical liposomal delivery of biologically active peptides. Ph.D Thesis, Potchefstroom University for CHE, South Africa.

E.5.3 USE OF DIGITAL OBJECT IDENTIFIER (DOI)

The digital object identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document particularly "Articles in press" because they have not yet received their full bibliographic information.

The correct format for citing a DOI is shown as follows: doi:10.1016/j.ijpharm.2005.01.041

E.6 Articles in special issues

Please ensure that the words 'this issue' are added (in the list and text) to any references to other articles in this Special Issue.

E.7 Figures and Tables

E.7.1 FIGURES

Line drawings (including graphs) should be drawn in black ink on white paper or on tracing paper with blue or faint grey rulings; graduation will not be reproduced. Lettering should be large enough to permit photographic reduction. If figures are not to be reduced, their format should not exceed 16 x 20 cm. Photographs (or half-tone illustrations) must be of good quality, submitted as black and white prints on glossy paper, and have as much contrast as possible. The magnification of micrographs should be indicated by a scale bar in the figure. Figures should be clearly marked on the reverse side with the number, orientation (top) and author's name; a soft pencil or a felt-tipped pen should be used for marking photographs. The illustrations should be numbered with Arabic numerals. The legends should be typed separately with double spacing.

Colour illustrations should be submitted as original photographs, high-quality computer prints or transparencies, close to the size expected in publication, or as 35 mm slides. Polaroid colour prints are not suitable. If, together with your accepted article, you submit usable colour figures then Elsevier will ensure, at no additional charge that these figures will appear in colour on the web (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in colour in the printed version. For colour reproduction in print, you will receive information regarding the total cost from Elsevier after receipt of your accepted article. The 2008 price for colour figures is EUR 285 for the first page and EUR 191 for subsequent pages. For further information on the preparation of electronic artwork, please see http://www.elsevier.com/artworkinstructions.

Please note: Because of technical complications which can arise by converting colour figures to 'grey scale' (for the printed version should you not opt for colour in print) please submit in addition usable black and white prints corresponding to all the colour illustrations.

E.7.2 TABLES

All tables must be numbered consecutively (with Arabic numerals) and be cited in the text. Titles should be short but descriptive. Tables should be compiled on separate sheets, together with a legend and/or footnotes identified by superscripts a.b.c, etc. Do not use vertical lines and keep horizontal rules to a minimum.

E.7.3 DNA SEQUENCES AND GENBANK ACCESSION NUMBERS

Many Elsevier journals cite "gene accession numbers" in their running text and footnotes. Gene accession numbers refer to genes or DNA sequences about which further information can be found in the databases at the National Centre for Biotechnical Information (NCBI) at the National Library of Medicine.

Authors are encouraged to check accession numbers used very carefully. An error in a letter or number can result in a dead link. Note that in the final version of the electronic copy, the *accession num*ber text will be linked to the appropriate source in the NCBI databases enabling readers to go directly to that source from the article.

E.8 Copyright guidelines for authors

Upon acceptance of an article, authors will be asked to sign a 'Journal Publishing Agreement' (for more information on this and copyright see http://www.elsevier.com/copyright). Acceptance of the agreement will ensure the widest possible dissemination of information. An e-mail (or

letter) will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations (please consult http://www.elsevier.com/permissions).

If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has pre-printed forms for use by authors in these cases: please consult http://www.elsevier.com/permissions.

E.9 Authors' rights

As an author you (or your employer or institution) retain certain rights; for details you are referred to http://www.elsevier.com/authorsrights.

E.10 Proofs, Offprints and Page Charges

One set of page proofs in PDF format will be sent by e-mail to the corresponding author (if we do not have an e-mail address then paper proofs will be sent by post). Elsevier now sends PDF proofs which can be annotated; for this you will need to download Adobe Reader version 7 or higher available free from http://www.adobe.com/products/acrobat/readstep2.html. Instructions on how to annotate PDF files will accompany the proofs. The exact system requirements are given at the Adobe site http://www.adobe.com/products/acrobat/acrrsystemreqs.html#70win.

If you do not wish to use the PDF annotations function, you may list the corrections (including replies to the Query Form) and return to Elsevier in an e-mail. Please list your corrections quoting line number. If, for any reason, this is not possible, then mark the corrections and any other comments (including replies to the Query Form) on a printout of your proof and return by fax, or scan the pages and e-mail, or by post.

Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. We will do everything possible to get your article published quickly and accurately. Therefore, it is important to ensure that all of your corrections are sent back to us in one communication: please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is

solely your responsibility. Note that Elsevier may proceed with the publication of your article if no response is received.

The corresponding author, at no cost, will be provided with a PDF file of the article via e-mail or, alternatively, 25 free paper offprints. The PDF file is a watermarked version of the published article and includes a cover sheet with the journal cover image and a disclaimer outlining the terms and conditions of use. Additional paper offprints can be ordered by the authors. An order form with prices will be sent to the corresponding author.

There are no page charges.

E.11 Language Services

Authors who require information about language editing and copyediting services pre- and post-submission please visit http://www.elsevier.com/locate/languagepolishing or contact authorsupport@elsevier.com for more information. Please note Elsevier neither endorses nor takes responsibility for any products, goods or services offered by outside vendors through our services or in any advertising. For more information please refer to our Terms & Conditions http://www.elsevier.com/termsandconditions.

E.12 Funding body agreements and policies

Elsevier has established agreements and developed policies to allow authors who publish in Elsevier journals to comply with potential manuscript archiving requirements as specified as conditions of their grant awards. To learn more about existing agreements and policies please visit http://www.elsevier.com/fundingbodies.

E.13 Author enquiries

For enquiries relating to the submission of articles (including electronic submission where available) please visit this journal's homepage at http://www.elsevier.com/locate/ijpharm. You can track accepted articles at http://www.elsevier.com/trackarticle and set up e-mail alerts to inform you of when an article's status has changed. Also accessible from here is information on copyright, frequently asked questions and more.

Contact details for questions arising after acceptance of an article, especially those relating to proofs, are provided after registration of an article for publication. No responsibility is assumed by the Publisher for any use or operation of any methods, products, instructions or ideas contained in the material herein. Because of the rapid advances made in the medical sciences, independent verification of diagnoses and drug dosages should be made.