Formulation, *in vitro* release and transdermal diffusion of diclofenac salts by implementation of the delivery gap principle

H Smith
21184038

Dissertation submitted in fulfillment of the requirements for the degree *Magister Scientiae* in Pharmaceutics at the Potchefstroom Campus of the North-West University

Supervisor: Prof JL du Preez
Co-Supervisor: Prof J du Plessis
Assistant supervisor: Dr M Gerber
November 2013
# TABLE OF CONTENTS

LIST OF TABLES AND FIGURES ................................................................. viii  
ACKNOWLEDGEMENTS ................................................................. xiii  
ABSTRACT ........................................................................................... xv  
UITTREKSEL ........................................................................................ xvi  
REFERENCES ......................................................................................... xix  

## CHAPTER 1: INTRODUCTION AND PROBLEM STATEMENT  
1.1 INTRODUCTION ................................................................................. 1  
1.2 RESEARCH PROBLEM ................................................................. 5  
1.3 AIMS AND OBJECTIVES ............................................................... 5  
REFERENCES ......................................................................................... 7  

## CHAPTER 2: TRANSDERMAL DELIVERY OF DICLOFENAC USING THE SKIN DELIVERY GAP PRINCIPLE  
2.1 INTRODUCTION ................................................................................. 10  
2.2 PAIN AND INFLAMMATION .......................................................... 11  
2.2.1 PHYSIOLOGY AND PATHOLOGY .................................................. 11  
2.2.2 ROLE OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS IN PAIN AND INFLAMMATION TREATMENT ...................................................... 11  
2.3 DICLOFENAC ................................................................................... 12  
2.3.1 PHARMACOLOGY AND BIOPHARMACEUTICAL ASPECTS ......... 12  
2.3.2 PHYSICOCHEMICAL CHARACTERISTICS OF DICLOFENAC .......... 14  
2.4 TOPICAL APPLICATION OF DICLOFENAC ...................................... 16  
2.4.1 DRUG DELIVERY PATHWAYS VIA THE SKIN .......................... 16  
2.4.2 MORPHOLOGY OF THE SKIN ....................................................... 17  
2.4.2.1 Stratum corneum ................................................................... 17  
2.4.2.2 Viable epidermis ................................................................. 17
APPENDIX A: VALIDATION OF AN HPLC ANALYTICAL METHOD FOR THE DETERMINATION OF DICLOFENAC CONCENTRATION

A.1 PURPOSE OF THE VALIDATION ................................................................. 63
A.2 CHROMATOGRAPHIC CONDITIONS ....................................................... 63
A.3 PREPARATION OF SAMPLES AND STANDARD SOLUTION ...................... 64
A.4 VALIDATION PARAMETERS ........................................................................ 65
A.4.1 LINEARITY ............................................................................................... 65
A.4.2 ACCURACY ............................................................................................... 66
A.4.3 PRECISION ............................................................................................... 67
A.4.3.1 Repeatability (intra-day precision) ...................................................... 67
A.4.3.2 Intermediate precision ......................................................................... 68
A.4.3.3 Reproducibility (inter-day precision) .................................................. 69
A.4.4 SYSTEM REPEATABILITY (SUITABILITY) ............................................ 70
A.5 CONCLUSION ............................................................................................. 70
REFERENCES .................................................................................................... 71

APPENDIX B: FORMULATION OF AN EMULGEL WITH DICLOFENAC AS ACTIVE INGREDIENT

B.1 INTRODUCTION .......................................................................................... 72
B.2 FORMULATION OF AN EMULGEL ............................................................. 72
B.2.1 MAIN INGREDIENTS OF AN EMULGEL ............................................... 72
B.2.2 GENERAL METHOD FOR MANUFACTURING A GEL ......................... 72
B.3 METHODS AND MATERIALS ...................................................................... 73
B.3.1 DETERMINATION OF THE AMOUNTS OF DICLOFENAC SALTS EQUAL TO 1 g OF DICLOFENAC FREE ACID ......................................................... 73
B.3.2 INGREDIENTS FOR THE PREPARATION OF A FORMULATION OPTIMISED TOWARDS THE STRATUM CORNEUM CONTAINING DICLOFENAC ............ 73
B.3.3 INGREDIENTS FOR THE PREPARATION OF A MORE HYDROPHILIC FORMULATION CONTAINING DICLOFENAC ...................................................... 74
B.3.4 INGREDIENTS FOR THE PREPARATION OF A MORE LIPOPHILIC FORMULATION CONTAINING DICLOFENAC

B.3.5 METHOD FOR THE PREPARATION OF A FORMULATION CONTAINING DICLOFENAC

B.4 SUMMARY

REFERENCES

APPENDIX C: FRANZ CELL DIFFUSION STUDIES

C.1 INTRODUCTION

C.2 METHODS

C.2.1 HPLC ANALYSIS OF DICLOFENAC

C.2.2 AQUEOUS SOLUBILITY

C.2.3 OCTANOL BUFFER DISTRIBUTION COEFFICIENT (LOG D)

C.2.4 SKIN PREPARATION

C.2.5 PREPARATION OF PHOSPHATE BUFFER SOLUTION (pH 7.4)

C.2.6 MEMBRANE DIFFUSION STUDIES

C.2.7 TRANSDERMAL DIFFUSION STUDIES

C.2.8 TAPE STRIPPING

C.2.9 DATA ANALYSIS

C.3 RESULTS AND DISCUSSION

C.3.1 AQUEOUS SOLUBILITY

C.3.2 DISTRIBUTION COEFFICIENT (LOG D)

C.3.3 MEMBRANE DIFFUSION STUDIES

C.3.4 TRANSDERMAL DIFFUSION STUDIES

C.3.5 TAPE STRIPPING

C.4 CONCLUSION

REFERENCES
# APPENDIX D: AUTHOR’S GUIDE TO THE INTERNATIONAL JOURNAL OF PHARMACEUTICS

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.1</td>
<td>DESCRIPTION</td>
<td>108</td>
</tr>
<tr>
<td>D.1.1</td>
<td>EDITORIAL POLICY</td>
<td>108</td>
</tr>
<tr>
<td>D.2</td>
<td>GUIDE FOR AUTHORS</td>
<td>109</td>
</tr>
<tr>
<td>D.2.1</td>
<td>INTRODUCTION</td>
<td>109</td>
</tr>
<tr>
<td>D.2.1.1</td>
<td>Types of paper</td>
<td>109</td>
</tr>
<tr>
<td>D.2.2</td>
<td>BEFORE YOU BEGIN</td>
<td>110</td>
</tr>
<tr>
<td>D.2.2.1</td>
<td>Ethics in publishing</td>
<td>110</td>
</tr>
<tr>
<td>D.2.2.2</td>
<td>Human and animal rights</td>
<td>110</td>
</tr>
<tr>
<td>D.2.2.3</td>
<td>Conflict of interest</td>
<td>110</td>
</tr>
<tr>
<td>D.2.2.4</td>
<td>Submission declaration and verification</td>
<td>110</td>
</tr>
<tr>
<td>D.2.2.5</td>
<td>Contributors</td>
<td>111</td>
</tr>
<tr>
<td>D.2.2.6</td>
<td>Authorship</td>
<td>111</td>
</tr>
<tr>
<td>D.2.2.7</td>
<td>Changes to authorship</td>
<td>111</td>
</tr>
<tr>
<td>D.2.2.8</td>
<td>Article transfer service</td>
<td>112</td>
</tr>
<tr>
<td>D.2.2.9</td>
<td>Copyright</td>
<td>112</td>
</tr>
<tr>
<td>D.2.2.10</td>
<td>Role of the funding source</td>
<td>113</td>
</tr>
<tr>
<td>D.2.2.11</td>
<td>Funding body agreements and policies</td>
<td>113</td>
</tr>
<tr>
<td>D.2.2.12</td>
<td>Open access</td>
<td>113</td>
</tr>
<tr>
<td>D.2.2.13</td>
<td>Language (usage and editing services)</td>
<td>114</td>
</tr>
<tr>
<td>D.2.2.14</td>
<td>Submission</td>
<td>114</td>
</tr>
<tr>
<td>D.2.2.15</td>
<td>Referees</td>
<td>115</td>
</tr>
<tr>
<td>D.2.3</td>
<td>PREPARATION</td>
<td>115</td>
</tr>
<tr>
<td>D.2.3.1</td>
<td>Use of word processing software</td>
<td>115</td>
</tr>
<tr>
<td>D.2.3.2</td>
<td>Article structure</td>
<td>116</td>
</tr>
<tr>
<td>D.2.3.3</td>
<td>Essential title page information</td>
<td>117</td>
</tr>
<tr>
<td>D.2.3.4</td>
<td>Abstract</td>
<td>117</td>
</tr>
<tr>
<td>D.2.3.5</td>
<td>Graphical abstract</td>
<td>117</td>
</tr>
<tr>
<td>D.2.3.6</td>
<td>Keywords ................................................................................................................. 118</td>
<td></td>
</tr>
<tr>
<td>D.2.3.7</td>
<td>Chemical compounds .............................................................................................. 118</td>
<td></td>
</tr>
<tr>
<td>D.2.3.8</td>
<td>Abbreviations ....................................................................................................... 118</td>
<td></td>
</tr>
<tr>
<td>D.2.3.9</td>
<td>Acknowledgements ................................................................................................ 118</td>
<td></td>
</tr>
<tr>
<td>D.2.3.10</td>
<td>Units ....................................................................................................................... 119</td>
<td></td>
</tr>
<tr>
<td>D.2.3.11</td>
<td>Database linking .................................................................................................... 119</td>
<td></td>
</tr>
<tr>
<td>D.2.3.12</td>
<td>Math formulae ....................................................................................................... 119</td>
<td></td>
</tr>
<tr>
<td>D.2.3.13</td>
<td>Footnotes ............................................................................................................... 119</td>
<td></td>
</tr>
<tr>
<td>D.2.3.14</td>
<td>Tables .................................................................................................................... 121</td>
<td></td>
</tr>
<tr>
<td>D.2.3.15</td>
<td>References ............................................................................................................. 122</td>
<td></td>
</tr>
<tr>
<td>D.2.3.16</td>
<td>Video data ............................................................................................................. 124</td>
<td></td>
</tr>
<tr>
<td>D.2.3.17</td>
<td>AudioSlides .......................................................................................................... 124</td>
<td></td>
</tr>
<tr>
<td>D.2.3.18</td>
<td>Supplementary data ............................................................................................ 125</td>
<td></td>
</tr>
<tr>
<td>D.2.4</td>
<td>AFTER ACCEPTANCE ............................................................................................. 126</td>
<td></td>
</tr>
<tr>
<td>D.2.4.1</td>
<td>Use of the Digital Object Identifier .................................................................... 126</td>
<td></td>
</tr>
<tr>
<td>D.2.4.2</td>
<td>Online proof correction ....................................................................................... 126</td>
<td></td>
</tr>
<tr>
<td>D.2.4.3</td>
<td>Offprints ............................................................................................................... 127</td>
<td></td>
</tr>
<tr>
<td>D.2.5</td>
<td>AUTHOR INQUIRIES ............................................................................................. 127</td>
<td></td>
</tr>
</tbody>
</table>
LIST OF TABLES AND FIGURES

CHAPTER 1: INTRODUCTION AND PROBLEM STATEMENT

Figure 1.1: Schematic representation of optimal polarity of the formulation relative to the polarity of the penetrant to ensure 50% of the API penetrating the SC (Wiechers et al., 2004:177) .................................................................................................................................................. 4

CHAPTER 2: TRANSDERMAL DELIVERY OF DICLOFENAC USING THE SKIN DELIVERY GAP PRINCIPLE

Table 2.1: Biopharmaceutical aspects of diclofenac (Furst & Ulrich, 2007:576; Grosser et al., 2011:967) ................................................................................................................................. 13

Table 2.2: Chemical characteristics of diclofenac (Fini et al., 1999:164; NCBI, 2009d)........ 14

Table 2.3: Molecular structure and physical characteristics of diclofenac salts (Amoli Organics, 2008; BP, 2013a-b; NCBI, 2009a-c)................................................................. 15

Table 2.4: Vehicles for topical delivery (Kurian & Barankin, 2011:4)................................. 22

Figure 2.1: Illustration of the prostaglandin synthesis pathway, showing the roles of COX-1 and COX-2 in the production of prostaglandins (PGD₂, PGF₂, PGE₂) from arachidonic acid (Botting, 2006:210; Chell et al., 2006:109)................................................................. 12

Figure 2.2: Diagrammatic representation of skin layers, appendages, blood and lymphatic vessels (Jepps et al., 2013:154) ................................................................. 16

CHAPTER 3: ARTICLE FOR PUBLICATION IN THE INTERNATIONAL JOURNAL OF PHARMACEUTICS

Figure 1: Average cumulative amount of diclofenac per area (µg/cm²) of all nine formulations that permeated the skin between 4 - 12 hours as a function of time (n = 10 for all formulations, except DDEA-H en DHEP-L where n = 9).............. 36
Figure 2: Box plots and diamonds of the flux values of all nine formulations after topical application illustrating median (indicated by a horizontal line in the box) and average flux (indicated by diamond) ................................................................. 37

Figure 3: Comparison of the concentration (µg/ml) of diclofenac in the SCE and ED among different formulations ........................................................................................................... 38

APPENDIX A: VALIDATION OF AN HPLC ANALYTICAL METHOD FOR THE DETERMINATION OF DICLOFENAC CONCENTRATION

Table A.1: Peak area values of diclofenac .......................................................................................... 66
Table A.2: Accuracy parameters of diclofenac ............................................................................... 67
Table A.3: Repeatability parameters of diclofenac ......................................................................... 68
Table A.4: Sample stability parameters of diclofenac ................................................................. 69
Table A.5: Reproducibility parameters of diclofenac ............................................................... 70
Table A.6: Variations in response (%RSD) of the detection system with regard to peak area and retention time of diclofenac ................................................................. 70

Figure A.1: Linear regression curve of diclofenac standard solutions ........................................ 65

APPENDIX B: FORMULATION OF AN EMULGEL WITH DICLOFENAC AS ACTIVE INGREDIENT

Table B.1: Typical formula of an emulsion type gel, i.e. emulgel (Mitsui, 1997:353) ............ 72
Table B.2: Quantity of diclofenac salts equivalent to 1 g of diclofenac free acid ................. 73
Table B.3: Quantity of ingredients in emulgel formulations optimised towards the SC containing diclofenac ........................................................................................................ 73
Table B.4: Quantity of ingredients in more hydrophilic emulgel formulations containing diclofenac ........................................................................................................ 74
Table B.5: Quantity of ingredients in more lipophilic emulgel formulations containing diclofenac ........................................................................................................ 74
APPENDIX C: FRANZ CELL DIFFUSION STUDIES

Table C.1: Data generated during membrane diffusion studies ........................................ 81
Table C.2: Data generated during transdermal diffusion studies ........................................ 82
Table C.3: Data generated during tape stripping studies .................................................... 103

Figure C.1: Box plots of flux values of nine emulgel formulations after topical application, illustrating the median (indicated with a horizontal line in the box) and average flux (indicated with diamond) of diclofenac ................................................................. 84

Figure C.2: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DNa-O emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10) ......................................................................................................................... 85

Figure C.3: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DNa-O emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10) ......................................................................................................................... 86

Figure C.4: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DHEP-O emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10) ......................................................................................................................... 87

Figure C.5: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DHEP-O emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10) ......................................................................................................................... 88

Figure C.6: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DDEA-O emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10) ......................................................................................................................... 89

Figure C.7: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DDEA-O emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10) ......................................................................................................................... 90

Figure C.8: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DNa-H emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10) ......................................................................................................................... 91
Figure C.9: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DNa-H emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).................................................................................................................. 92

Figure C.10: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DHEP-H emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).................................................................................................................. 93

Figure C.11: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DHEP-H emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).................................................................................................................. 94

Figure C.12: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DDEA-H emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 9).................................................................................................................. 95

Figure C.13: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DDEA-H emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).................................................................................................................. 96

Figure C.14: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DNa-L emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).................................................................................................................. 97

Figure C.15: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DNa-L emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).................................................................................................................. 98

Figure C.16: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DHEP-L emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 9).................................................................................................................. 99

Figure C.17: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DHEP-L emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).................................................................................................................. 100

Figure C.18: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DDEA-L emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).................................................................................................................. 101
Figure C.19: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DDEA-L emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10) ................................................................. 102
ACKNOWLEDGEMENTS

Thank you, my Lord and Saviour, for blessing me more than I ever could have hoped for! Thank you for Your love and grace, for opportunities, talents and special people in my life.

Barry, my husband and best friend, thank you for your continuous encouragement, your support, for taking care of our beautiful son and for your love. Without you, I would not have been where I am today.

Thank you to my family for your confidence in me as well as your support during my studies. I am especially grateful to my mother who came from Cape Town to lend a hand at home.

To my “new” friends, with whom I shared many coffee breaks over the past two years and who made this time worthwhile. Johann, I thank you for all your help in the laboratory, the office and off-campus and for being available at almost any time. You truly are a special friend. Madél, I thank you for your friendship, chats and laughs, and your support. I will miss you dearly.

Prof. Jan du Preez, my supervisor, thank you for being approachable, willing to help and allowing your door to stand open – I always felt welcome in your office.

Dr. Anja Otto, thank you for sharing your time and vast knowledge with me when I needed it, even though I was not your student. Your mere presence made a difference.

Dr. Maides Malan, Mrs. Mariëtta Fourie, Dr. Joe Viljoen and Dr. Jan Steenekamp, you made it enjoyable to be a part of the pharmaceutics department.

Prof. Jeanetta du Plessis, thank you for being understanding and supportive especially towards the end of my study.

Dr. Minja Gerber, my co-supervisor, thank you for your contribution towards the formatting of my dissertation.

Prof. Faans Steyn, thank you for statistical analysis of my data in a very short period and for helping me to understand it.

Ms. Julia Handford, thank you for the language editing and formatting of my dissertation. You certainly made this task easier for me.

Ms. Anriëtte Pretorius, thank you for your kind help in the library and with the referencing in my dissertation.
The National Research Foundation (NRF) and the Centre of Excellence for Pharmaceutical Sciences (Pharmacen), North-West University, Potchefstroom Campus for funding this project.
ABSTRACT

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of inflammation and pain (Escribano et al., 2003:203). Diclofenac, a classical NSAID, is considerably more effective as an analgesic, antipyretic and anti-inflammatory drug than other traditional NSAIDs, like indomethacin and naproxen (Grosser et al., 2011:986). However, the use of diclofenac is known for its many side effects, such as gastric disorders, while fluid and sodium retention are also commonly observed (Rossiter, 2012:391).

Since topical diclofenac offers a more favourable safety profile, it is a valuable substitute for oral NSAID therapy in the treatment of osteoarthritis (Roth & Fuller, 2011:166). The benefits of topically applied NSAIDs, compared to oral administration and systemic delivery, include the easy cessation of treatment, should effects become troublesome (Brown et al., 2006:177), the avoidance of extensive, first-pass metabolism (Cleary, 1993:19; Kornick, 2003:953; Prausnitz & Langer, 2008:1261; Lionberger & Brennan, 2010:225), reduced systemic side effects (Colin Long, 2002:41), convenience of application and improved patient compliance (Cleary, 1993:19; Prausnitz & Langer, 2008:1261).

An approach that is often applied in optimising the solubility and dissolution rate of poorly water soluble, weak electrolytes is to prepare a salt of the active pharmaceutical ingredient (API) (Minghetti et al., 2007:815; O’Connor & Corrigan, 2001:281-282). Diclofenac is frequently administered as a salt, due to the high partition coefficient and very low water solubility of this molecule (Fini et al., 1999:164).

Formulating for efficacy (FFE™) is a software programme designed by JW Solutions to facilitate the formulation of cosmetic ingredients or solvents into a product that would optimally deliver active ingredients into the skin. The notion is built upon solubility, i.e. solubility of the active ingredient in the formulation and solubility of the formulation in the skin. This programme could also be employed to optimise amounts of predetermined ingredients, to propose formulations that would ensure optimal drug delivery, to calculate the skin delivery gap (SDG) and to demonstrate transdermal permeation of active ingredients and excipients (JW Solutions Software, 2013a). When the SDG is known, it mathematically indicates the optimal active ingredient and topical delivery vehicle to use (JW Solutions, 2013b).

In this study, diclofenac sodium (DNa), diclofenac diethylamine (DDEA) and diclofenac N-(2-hydroxyethyl) pyrrolidine (DHEP) were each formulated in the following emulgels:

- An emulgel optimised towards the stratum corneum (SC) (enhancing drug delivery into this layer and deeper tissues) (oily phase ~30%),
• A more hydrophilic emulgel (oily phase ~15%), and
• A more lipophilic emulgel (oily phase ~45%).

Components of the oily phase and its respective amounts, as well as the SDG of formulations were determined by utilising the FFE™ software of JW Solutions (2013a).

The aqueous solubilities of DNa, DDEA and DHEP were determined and their respective values were 11.4 mg/ml, 8.0 mg/ml and 11.9 mg/ml, all indicative of effortless percutaneous delivery (Naik et al., 2000:319). Log D (octanol-buffer distribution coefficient) (pH 7.4) determinations for DNa, DDEA and DHEP were performed and their values established at 1.270 (DNa), 1.291 (DDEA) and 1.285 (DHEP). According to these values, diclofenac, when topically applied as a salt in a suitable vehicle, should permeate transdermally without the aid of radical intervention (Naik et al., 2000:319; Walters, 2007:1312).

Membrane release studies were also carried out in order to determine the rate of API release from these new formulations. Results confirmed that diclofenac was indeed released from all nine of the formulated emulgels. The more hydrophilic DNa formulation released the highest average percentage of diclofenac (8.38%) after 6 hours. Subsequent transdermal diffusion studies were performed to determine the diclofenac concentration that permeated the skin. The more hydrophilic DNa emulgel showed the highest average percentage skin diffusion (0.09%) after 12 hours, as well as the highest average flux (1.42 ± 0.20 µg/cm².h).

The concentrations of diclofenac in the SC-epidermis (SCE) and epidermis-dermis (ED) were determined through tape stripping experiments. The more lipophilic DNa emulgel demonstrated the highest average concentration (0.27 µg/ml) in the ED, while the DNa emulgel that had been optimised towards the SC, had the highest concentration in the SCE (0.77 µg/ml).

Key words: diclofenac, FFE™, SDG, formulation, transdermal diffusion
UITTREKSEL

Nie-steroïdale anti-inflammatoriese middels (NSAIMs) word algemeen vir die behandeling van inflammasie en pyn gebruik (Escribano et al., 2003:203). Diklofenak, ’n klassieke NSAIM, is beduidend meer effektief as ’n analgetiese, antipiretiese en anti-inflammatoriese middel in vergelyking met ander tradisionele NSAIMs, soos indometasien en naprokseen (Grosser et al., 2011:986). Die gebruik van diklofenak is egter bekend vir sy verskeie newe-effekte, soos byvoorbeeld gastriese ongesteldhede, terwyl vog- en natriumterughouding algemeen voorkom (Rossiter, 2012:391).


Die bereiding van die sout van ’n aktiewe farmaseutiese bestanddeel (AFB) is ’n gewilde benadering in die optimalisering van die oplosbaarheid en dissolusietempo van swak wateroplosbare, swak elektroliete (Minghetti et al., 2007:815; O’Connor & Corrigan, 2001:281-282). Diklofenak word dikwels as ’n sout toegedien, vanwee die hoë verdelingskoëffisiënt en baie lae wateroplosbaarheid van hierdie molekuul (Fini et al., 1999:164).

“Formulating for efficacy” (FFE™) is ’n sagteware-program wat deur JW Solutions ontwikkel is om die formulering van kosmetiese bestanddele of oplosmiddels in ’n produk te fasiliteer, ten einde die optimale aflewering van die AFB in die vel te verseker. Hierdie konsep is op oplosbaarheid gebaseer, naalik oplosbaarheid van die AFB in die formulering, asook die oplosbaarheid van die formulering in die vel. Hierdie program kan ook aangewend word om die hoeveelheid voorafbepaalde bestanddele te optimaliseer, om aanbevelings ten opsigte van formulering met optimale geneesmiddelafluwering te maak, om die velafleweringsgaping (“skin delivery gap”, SDG) te bereken en om transdermale deursypeling van die aktiewe bestanddele en hulpmiddels te demonstreer (JW Solutions Software, 2013a). Wanneer die SDG bekend is, dui dit wiskundig aan watter aktiewe bestanddeel en topikale vervoersisteen gebruik kan word (JW Solutions, 2013b).
In hierdie studie is diklofenaknatrium (DNA), diklofenakdiëtielamien (DDEA) en diklofenak-N-(2-hidroksi-eti) pirrolidien (DHEP) elk in die volgende emulgels geformuleer:

- ‘n Emulgel geoptimaliseer tot die *stratum corneum* (SC) (wat geneesmiddelafl ewering in hierdie vellaag en dieperliggende weefsel bevorder) (olie fase ~30%),
- ‘n Meer hidrofiele emulgel (olie fase ~15%), en
- ‘n Meer lipofiele emulgel (olie fase ~45%).

Die komponente van die oliefase en hul onderskeie hoeveelhede, asook die SDG van die formulering is met behulp van die FFE™-sagteware van JW Solutions (2013) bepaal.

Die wateroplosbaarheid van DNA, DDEA en DHEP is bepaal en die onderskeie waardes was 11.4 mg/ml, 8.0 mg/ml en 11.9 mg/ml, almal beduidend van onbelemmerde perkutane aflewering (Naik *et al.*, 2000:319). Log D (oktanol-buffer distribusie-koëffisiënt) (pH 7.4) bepalings vir DNA, DDEA en DHEP is uitgevoer en hulle waardes is op 1.270 (DNA), 1.291 (DDEA) en 1.285 (DHEP) vasgestel. Op grond hiervan is bevind dat diklofenak, wanneer topikaal toegedien as ‘n sout in ‘n geskikte verioorsisteem, sonder die hulp van radikale hulpsisteme behoort plaas te vind (Naik *et al.*, 2000:319; Walters, 2007:1312).

Membraanvrystellingstudies is voorts uitgevoer ten einde die tempo waarteen die AFB vanuit hierdie nuwe formuleringe vrygestel is, te bepaal. Resultate het bevestig dat diklofenak inderdaad uit al nege hierdie geformuleerde emulgels vrygestel is. Die meer hidrofiele DNA-formulerings het die hoogste gemiddelde persentasie diklofenakkrystelling (8,38%) na 6 ure getoon. Daaropvolgende transdermale diffusiestudies is uitgevoer om die konsentrasie diklofenak wat deur die vel gesypel het te bepaal. Die meer hidrofiele DNA-emulgel het die hoogste gemiddelde konsentrasie veldiffusie (0,09%) na 12 ure getoon, asook die hoogste gemiddelde vloed (1,42 ± 0,20 µg/cm².h).

Die konsentrasies diklofenak in die SC-epidermis (SCE) en epidermis-dermis (ED) is deur bandstropingseksperimente (“tape stripping”) bepaal. Die meer lipofiele DNA-emulgel het die hoogste gemiddelde konsentrasie (0,27 µg/ml) in die ED getoon, terwyl die DNA-formulerings, wat vir die SC geoptimaliseer was, die hoogste konsentrasie in die SCE (0,77 µg/ml) getoon het.

Sleutelwoorde: diklofenak, FFE™, SDG, formulering, transdermale diffusie
REFERENCES


CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

1.1 INTRODUCTION

The skin has a remarkably effective barrier function, particularly due to the unique lipid and corneocyte arrangement of its outermost layer, the lipophilic stratum corneum (SC) (Jepps et al., 2013:153-154; Naik et al., 2000:318). Permeants follow a convoluted route through intercellular lipid sheets, before reaching the viable dermis and ultimately the systemic circulation. Lipophilic compounds thus tend to permeate the SC with more ease. However, once an active pharmaceutical ingredient (API) has crossed the SC, it should then be able to partition into the more aqueous, viable epidermis. An API should therefore be sufficiently lipid soluble to penetrate the SC efficiently, but also adequately water soluble to leave the SC and enter the viable epidermis (Naik et al., 2000:319).

Methods that assist in the enhancement of transdermal drug delivery often induce alterations to the arrangement of the intercellular lipids (Hillery et al., 2001:211). The skin is well known for its metabolic activity and certain APIs, such as nitroglycerin, are partly metabolised transdermally before entering the circulation (Hillery et al., 2001:212). The distribution and location of the metabolising enzymes in the different layers of the skin are not yet precisely established (Steinsträsser & Merkle, 1994:20-21). Permeability on some areas of the body is notably higher than on others. Yet, for the larger part of the body surface, the variation in permeability from one region to another is not more than the average differences among individuals for a specific area (Hillery et al., 2001:211). Further considerations regarding absorption include application conditions (occluded or not), skin integrity (also affected by disease and age), API related factors (molecular size and vehicle) and formulation factors (Hillery et al., 2001:212-216; Morgan et al., 2003:441).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are generally used to manage osteoarthritis and related inflammatory disorders (NCCCC, 2008; Zhang et al., 2008:139; Zhang et al., 2010:478) and soft tissue injuries (Lionberger & Brennan, 2010:224). However, well known adverse effects associated with the use of NSAIDs, including diclofenac, are cardiovascular and gastrointestinal disturbances. Patients often experience abdominal pain, constipation, diarrhoea, dyspepsia, flatulence, abdominal pain, constipation, indigestion, nausea, vomiting and other complications, such as upper or lower gastrointestinal bleeding with the use of oral diclofenac (Novartis, 2013a; Novartis, 2013b; Rossiter, 2012:391).
During this study, diclofenac, chemically referred to as 2-[(2,6-dichlorophenyl)amino]-benzeneacetic acid (D), was utilised. It is an acidic compound (pKₐ 3.80 at 25 °C) with very low aqueous solubility in its unionised form (Kozakevych et al., 2013:70). It is an anti-inflammatory, analgesic and antipyretic drug that acts by inhibiting cyclo-oxygenase (COX) iso-enzymes (Grosser et al., 2011:986) and it is one of the most commonly prescribed NSAIDs worldwide (Cannon et al., 2006: 1771). Anti-platelet effects of low-dose aspirin are unaffected by diclofenac, as is generally observed with the concomitant use of other classic NSAIDs, such as ibufroen, naproxen and indomethacin (Cannon et al., 2006:1779; Catella-Lawson et al., 2001:1814-1815; Gladding et al., 2008:1061-1062).

According to Roth and Fuller (2011:166), topical diclofenac is a valuable substitute for oral NSAID therapy in the treatment of osteoarthritis, as it shows a more favourable safety profile. Topical application of NSAIDs is significantly restricted, due to the therapeutic effect being limited to the site of application. Prolonged contact of the formulation with the skin may further lead to skin reactions, including irritant dermatitis and erythema (Lionberger & Brennan, 2010:226).

Benefits of topically applied NSAIDs over oral administration and systemic delivery include:

- Adverse effects of topically applied NSAIDs are mostly limited to mild skin irritation that clears up upon termination of treatment. Topical application is therefore generally well tolerated, according to Dreiser (as quoted by Lionberger & Brennan, 2010:225).

- Should effects become troublesome, a topical dose can be easily discontinued (Brown et al., 2006:177).

- Extensive first-pass metabolism, as seen with oral diclofenac formulations, is circumvented by topical application (Cleary, 1993:19; Kornick, 2003:953; Prausnitz & Langer, 2008:1261; Lionberger & Brennan, 2010:225). This is true, despite the occurrence of dermal metabolism, since most APIs would probably not be degraded significantly by the limited area of viable epidermis underneath a transdermal patch, or topically applied NSAID (Hillery et al., 2001:212).

- The API has direct access, delivery and localisation at the site of action, hence avoiding or reducing systemic side effects (Colin Long, 2002:41).

- These systems are convenient and painless to apply and are normally modestly priced, which may result in improved patient compliance (Cleary, 1993:19; Prausnitz & Langer, 2008:1261).

- Topical application is a feasible option, should patients be unable to use oral medications (Brown et al., 2006:177).
The bioavailability of APIs is often enhanced by modulation of their physicochemical properties. An approach that is often applied to optimise the solubility and dissolution rate of poorly water soluble, weak electrolytes is to prepare a salt of the API. Diclofenac salts have therefore been made available as pharmaceutical products, including diclofenac N-(2-hydroxyethyl) pyrrolidine (also known as epolamine), diclofenac sodium, diclofenac diethylamine and diclofenac potassium (Minghetti et al., 2007:815; O’Connor & Corrigan, 2001:281-282).

The skin penetration process consists of a series of steps that are each potentially rate limiting. Firstly, the API diffuses from within the formulation to the skin surface. Thereafter it partitions into the skin, diffuses through the SC, partitions into the viable epidermis and diffuses through this layer to reach the dermis, into which the API subsequently partitions. After diffusion of the API through the dermis it partitions into fat deposits, or it is redistributed via the blood capillaries at the epidermal/dermal border (Barry, 1983:128). Following this course of events, it seems reasonable to conclude that each of the partition and diffusion stages plays a key role in the skin penetration process (Wiechers et al., 2004:174).

It should be possible to increase the amount of drug penetrating the SC by either increasing the solubility of the drug in the SC, or by decreasing its solubility in the formulation. To increase the flux or amount of penetration per unit area and time, however, the solubility in the formulation also needs to be increased (Wiechers et al., 2004:174). The larger the difference between the formulation and the deepest SC layers, therefore, the higher the flow of drug, down a concentration gradient, through the SC (Wiechers et al., 2004:175).

Attention to physicochemical properties of the formulated API is essential for enhancing delivery. Although it is possible to change the octanol/water partition coefficient (Log P) and the diffusivity of the penetrating drug, altering the SC/formulation partition coefficient requires much less effort, as this parameter is formulation dependent (Wiechers et al., 2004:175).

JW Solutions (2012) define the skin delivery gap (SDG) as the ratio of the minimum effective concentration (MEC) relative to the concentration reached at the target site. Drugs with an SDG of less than 1 are delivered readily, while more complex delivery systems are required as the SDG rises above 1. The MEC can be calculated based on molecular modelling of the skin and pharmacokinetic principles.

It has been concluded that the total amount of API dissolved in a formulation and available for skin penetration, as well as the polarity of the formulation relative to that of the SC, are determined by the formulation. The higher the amount of API in the formulation, the more will penetrate the skin until it is saturated. In this instance, a high API solubility in the formulation is thus desired. Should the API be more soluble in the SC than in the formulation, it would prefer leaving the formulation in order to enter the SC and a low solubility in the formulation relative to
that of the SC is therefore desirable (Wiechers et al., 2004:175). Simultaneous similarity and extreme difference in polarity of the formulation and the API are hence required to ensure that high concentrations of the API can be reached in the SC and subsequently at the site of action (Wiechers et al., 2004:178).

Clearly, it would be difficult to comply with both conditions concurrently. This challenge, however, can be dealt with by making use of the relative polarity index (RPI) (Wiechers et al., 2004:175). The polarity of each of the API, the SC and the emollient components of cosmetic formulations can be compared by utilising the RPI. This innovative method is applied by visualising a vertical line with a high polarity at the top and a high lipophilicity at the bottom. The octanol/water partitioning coefficient expresses the polarity of this system. Employment of this concept depends upon the numerical values (on a log<sub>10</sub> scale) of the polarities of the SC, the penetrating drug and the formulation (or the phase in which the API is dissolved) (Wiechers et al., 2004:176). These polarity values are placed on the RPI scale by plotting their positions on the vertical line. Three different scenarios can be visualised: (1) the polarity of the API phase equal to that of the SC, (2) the polarity of the API phase higher than that of the SC and (3) the polarity of the API phase lower than that of the SC (Wiechers et al., 2004:176).

In order to obtain an API concentration that is higher in the SC than in the formulation, the following equations are often used (Wiechers et al., 2004:177) where PPG is the penetrant polarity gap:

\[
\text{Polarity of formulation} > \text{Polarity of penetrant} + \text{PPG} \quad \text{Equation 1.1}
\]

\[
\text{Polarity of formulation} < \text{Polarity of penetrant} - \text{PPG} \quad \text{Equation 1.2}
\]

Equations 1.1 and 1.2 are schematically represented in Figure 1.1.

![Figure 1.1: Schematic representation of optimal polarity of the formulation relative to the polarity of the penetrant to ensure 50% of the API penetrating the SC (Wiechers et al., 2004:177).](image)

1.2 RESEARCH PROBLEM

The SDG principle, as postulated by Wiechers (2004:175) calls for a systematic investigation. According to this principle, transdermal delivery of APIs should be effective if the SDG of the API is below 1 (JW Solutions, 2012). The Formulating for Efficacy (FFE™) software makes use of this principle in calculating the composition of formulations containing specified APIs (Wiechers, 2004:175). Wiechers (2004:175) further states that the polarity of a formulation plays an important role in the transdermal delivery of an API. An inappropriate formulation polarity will reduce percutaneous delivery of the API. Accordingly, in this study, 1% diclofenac formulations, consisting of the same ingredients but in varying amounts, hence with different polarities and all with an SDG value below 1, were prepared by using the FFE™ software. Diclofenac release and transdermal delivery from each of these formulations were determined. The effect of formulation polarity on API delivery was subsequently determined.

Extensive research with regard to this theory and the implementation thereof could lead to the evolution of effective transdermal delivery systems.

1.3 AIMS AND OBJECTIVES

This project formed part of ongoing research being conducted at the Centre of Excellence for Pharmaceutical Sciences (Pharmacen) at the North-West University (NWU), on the optimisation of transdermal drug delivery. The objectives were the following:

- Determination of the aqueous solubility and distribution coefficients of diclofenac sodium (DNa), diclofenac diethylamine (DDEA) and diclofenac N-(2-hydroxyethyl) pyrrolidine (DHEP).
- Employment of the FFE™ software of JW Solutions in order to determine formulae for preparations containing diclofenac, according to particular polarity indexes.
- Preparation of 1% diclofenac formulations, consisting of the same ingredients in varying amounts, thus with different polarities and all with an SDG value below 1, based on calculations by the FFE™ software.
- Validation of a high performance liquid chromatographic (HPLC) method to determine concentrations of the different formulation ingredients, including that of the diclofenac.
- Development of emulgel formulations of the above diclofenac salts.
- Determination of API release by conducting membrane diffusion studies.
• Determination of transdermal and topical delivery by conducting diffusion studies by utilising excised human skin and subsequently employing the tape stripping method in order to determine the amount of API present in the different skin layers.
REFERENCES


CHAPTER 2

TRANSDERMAL DELIVERY OF DICLOFENAC
USING THE SKIN DELIVERY GAP PRINCIPLE

2.1 INTRODUCTION

Regardless of its intensity, pain is one of mankind’s most unpleasant sensations. It can be perceived as the body’s alarm to an anatomical or physiological disorder, or as an illness in itself. According to a 2011 report by the Global Industry Analysts, Inc., an estimated 1.5 billion people suffered from chronic pain. Global pain management markets would reach US $60 billion by 2015, driven by the continuous need for more effective pain interventions (AAPM, 2011).

The transdermal delivery of drugs (including those for the treatment of pain and inflammation) is an attractive route of administration, due to the wide range of benefits it offers, compared to other routes. Although transdermal delivery systems (TDSs) have already made a significant contribution to medical sciences and the cosmetic industry, research in this field is still expanding rapidly (Prausnitz & Langer, 2008:1261). Current transdermal research largely focuses on the enhancement of drug delivery from vehicles designed as part of the research process, or from existing systems.

Three generations of TDSs exist. The most commonly used first-generation TDSs act by disturbing the SC to enable transdermal delivery (e.g. patches, gels and sprays). The second-generation TDSs deliver APIs effectively by means of skin permeability enhancers (e.g. conventional chemical enhancers, iontophoresis, or noncavitational ultrasound). The third-generation TDSs disrupt the SC more extensively to more effectively promote drug delivery (e.g. novel chemical and biochemical enhancers, electrophoresis, cavitational ultrasound, microneedles, thermal ablation and microdermabrasion) (Prausnitz & Langer, 2008:1262-1266).

This chapter focuses on dermal anatomy, the pathophysiology of pain and inflammation, while anti-inflammatory drugs are discussed with emphasis on diclofenac, percutaneous absorption mechanisms, skin delivery vehicles and skin delivery by applying the FFE™ software and the SDG principle.
2.2 PAIN AND INFLAMMATION

2.2.1 PHYSIOLOGY AND PATHOLOGY

Although inflammation is essential for survival, diseases occur where a seemingly unnecessary and exaggerated response, occasionally with detrimental consequences, is sustained. Various harmful events and agents (including infections, antibodies and physical injuries) can elicit the inflammatory response. Typical symptoms of inflammation are calor (warmth), dolor (pain), rubor (redness) and tumour (swelling). These are introduced by local vasodilation that increases capillary permeability and infiltration of leukocytes and phagocytic cells, which may then further lead to tissue degeneration and fibrosis (Grosser et al., 2011:960).

Stimuli of nociceptors (peripheral terminals of primary afferent fibres that sense pain) include heat, acid, or pressure. The sensitivity of nociceptors is increased by inflammatory mediators that are released from non-neuronal cells during tissue injury, which aggravate pain perception. Infection, tissue damage, or inflammation often results in fever, due to an elevated body temperature set point, which is regulated by the hypothalamus (Grosser et al., 2011:961).

2.2.2 ROLE OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS IN PAIN AND INFLAMMATION TREATMENT

The use of NSAIDs in the treatment of inflammation and pain is widespread. Most traditional NSAIDs act by inhibiting COX-2 at the inflammatory focus (Escribano et al., 2003:203) and are believed to counteract the sequelae of the inflammatory process, thereby reducing fever, pain and swelling (Grosser et al., 2011:959). Unfortunately, most NSAIDs also inhibit COX-1, an important enzyme in the production pathway of prostaglandins responsible for gastric mucosa protection in the stomach. COX-1 is also essential for appropriate platelet aggregation, the inhibition of thrombogenesis and in regulating adequate kidney function (Botting, 2006:210).

Figure 2.1 illustrates the prostaglandin synthesis pathway and the roles of COX-1 and COX-2 in producing the various prostaglandins (PGD$_2$, PGF$_2$, PGE$_2$) from arachidonic acid. COX-1 and COX-2 are essential in the production of PGH$_2$ from arachidonic acid. COX-1 is involved in the synthesis of all prostaglandins and TXA$_2$, whereas COX-2 is mainly involved in the synthesis of PGE$_2$ and PGI$_2$. From this illustration it is clear that COX-1 inhibition would cause a large variety of systemic side effects, compared to selective COX-2 inhibition (Botting, 2006:210; Chell et al., 2006:109).
**Figure 2.1:** Illustration of the prostaglandin synthesis pathway, showing the roles of COX-1 and COX-2 in the production of prostaglandins (PGD$_2$, PGF$_2$, PGE$_2$) from arachidonic acid (Botting, 2006:210; Chell et al., 2006:109).

### 2.3 DICLOFENAC

#### 2.3.1 PHARMACOLOGY AND BIOPHARMACEUTICAL ASPECTS

The mechanism of action of diclofenac is mainly the inhibition of COX-1 and COX-2, with selectivity for COX-2, resembling that of celecoxib, a selective inhibitor of COX-2 (Grosser et al., 2011:986). When tested *in vitro*, the non-selectivity of diclofenac is observed, while *ex vivo* studies show that it favours COX-2 over COX-1 inhibition to some degree (Giuliano & Warner, 1999:1828). Secondly, diclofenac decreases the intracellular concentration of free arachidonic acid in leukocytes, possibly due to interference with the availability and removal of arachidonic acid (Grosser et al., 2011:986).

Diclofenac is considerably more effective as an analgesic, antipyretic and anti-inflammatory drug than other traditional NSAIDs, such as indomethacin and naproxen (Grosser et al., 2011:986). Side effects that arise from using diclofenac such as gastric disorders, fluid and sodium retention are commonly observed. Less frequent effects include hypersensitivity reactions, nephrotoxicity, mild central nervous system effects (headache, dizziness, drowsiness,
and depression), hepatic dysfunction, haematological disturbances (like inhibition of platelet aggregation and occasionally pancytopenia), visual disorders and tinnitus (Rossiter, 2012:391). In the United States of America a black box warning was issued for its possible cardiovascular side effects (Grosser et al., 2011:987).

McCarty states (as quoted by Escribano et al., 2003:203) that gastropathies especially appear in individuals after chronic oral ingestion of diclofenac. These range from mild irritation of the gastric mucosa to erosion, peptic ulceration and bleeding (Rossiter, 2012:391). These side effects were observed in approximately 20% of patients of which around 2% ceased treatment (Grosser et al., 2011:987).

It is evident from the above negative side effects, caused by the oral administration of diclofenac, that a topical dosage form of this API would certainly be advantageous in the treatment of skin inflammation and the underlying tissues (Galer et al., 2000:293). A formula with advanced percutaneous delivery for localised conditions, like arthritis, arthralgia, myalgia, tendonitis and inflammatory disease of bone and ligaments would have the advantages of higher efficacy and less systemic side effects (Escribano et al., 2003:203). It is reported that topical applications with higher diclofenac concentrations, compared to standard dosage forms, were successfully designed for the treatment of osteoarthritis of the knee (Hewitt et al., 1998:988; Hui et al., 1998:1589). A similar therapeutic effect could be achieved, however, when using appropriate penetration enhancers with a typical 1% diclofenac concentration (Escribano et al., 2003:203).

**Table 2.1:** Biopharmaceutical aspects of diclofenac (Furst & Ulrich, 2007:576; Grosser et al., 2011:967)

<table>
<thead>
<tr>
<th>Description</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak concentration</td>
<td>2 - 3 hours</td>
</tr>
<tr>
<td>Protein binding</td>
<td>99%</td>
</tr>
<tr>
<td>Metabolites</td>
<td>Glucuronide and sulphide (renal 65%, bile 35%)</td>
</tr>
<tr>
<td>$T_{1/2}$ (oral preparations)</td>
<td>1 - 2 hours</td>
</tr>
<tr>
<td>Oral bioavailability (subject to first-pass effect)</td>
<td>50%</td>
</tr>
<tr>
<td>Urinary excretion of unchanged drug</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>
2.3.2 PHYSICOCHEMICAL CHARACTERISTICS OF DICLOFENAC

Table 2.2 summarises the physicochemical characteristics of diclofenac.

**Table 2.2:** Physicochemical characteristics of diclofenac (Fini *et al.*, 1999:164; NCBI, 2009d)

<table>
<thead>
<tr>
<th>Description</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>C₁₄H₁₁Cl₂NO₂</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>296.14864 [g/mol]</td>
</tr>
<tr>
<td>Dissociation constant (pKₐ)</td>
<td>4.15</td>
</tr>
<tr>
<td>Partition coefficient (LogP)</td>
<td>4.51</td>
</tr>
<tr>
<td>Solubility (mg/l)</td>
<td>17.8</td>
</tr>
</tbody>
</table>

Table 2.3 summarises the molecular structure and physical characteristics of diclofenac salts.
Table 2.3: Molecular structure and physical characteristics of diclofenac salts (Amoli Organics, 2008; BP, 2013a-b; NCBI, 2009a-c)

<table>
<thead>
<tr>
<th>Description</th>
<th>DNA</th>
<th>DDEA</th>
<th>DHEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular structure</td>
<td><img src="image1" alt="Molecular structure" /></td>
<td><img src="image2" alt="Molecular structure" /></td>
<td><img src="image3" alt="Molecular structure" /></td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C_{14}H_{10}Cl_{2}NNaO_{2}</td>
<td>C_{18}H_{22}Cl_{2}N_{2}O_{2}</td>
<td>C_{20}H_{24}Cl_{2}N_{2}O_{3}</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>318.130469 [g/mol]</td>
<td>369.28548 [g/mol]</td>
<td>411.32216 [g/mol]</td>
</tr>
<tr>
<td>Appearance</td>
<td>White or slightly yellowish, slightly hygroscopic, crystalline powder</td>
<td>White to light beige, crystalline powder</td>
<td>White to cream, crystalline powder</td>
</tr>
<tr>
<td>Melting point</td>
<td>~280 °C, with decomposition</td>
<td>~154 °C, with decomposition.</td>
<td>101 °C - 104 °C</td>
</tr>
</tbody>
</table>
2.4 TOPICAL APPLICATION OF DICLOFENAC

2.4.1 DRUG DELIVERY PATHWAYS VIA THE SKIN

There are two possible pathways through which topically applied drugs can cross the skin to ultimately result in systemic uptake (Barry, 2001:101; Hadgraft, 2001:1):

1. *Via* the SC and deeper skin layers, i.e. the viable epidermis, the dermis and deeper tissues. Systemic delivery relies on the vascular and lymphatic systems of the dermis.

2. *Via* appendages (hair follicles and eccrine sweat gland ducts) where systemic uptake is possible, due to the dense vascular supply of these structures.

![Diagram](image.png)

**Figure 2.2:** Diagrammatic representation of skin layers, appendages, blood and lymphatic vessels (Jepps *et al*., 2013:154).

Compared to the SC, the underlying skin layers and appendages, known for mainly causing hindrance to skin permeation, have not been investigated nearly as extensively with regards to drug penetration. Drug molecules predominantly move across the skin by diffusion, which is mediated either by active or convective transport. Active transport is described as the movement of particles through facilitation by protein transporters, whereas convective transport is the movement of particles due to the flow in the lymphatic or vascular networks or interstitial spaces. A drug can be effectively removed from the skin by means of distribution, clearance and skin metabolism (Jepps *et al*., 2013:153).
2.4.2 MORPHOLOGY OF THE SKIN

2.4.2.1 Stratum corneum

The SC is the outermost layer of the skin with an architecture often described as ‘bricks and mortar’ (Jepps et al., 2013:154; Michaels et al., 1975:989). As mentioned, the SC is the primary barrier to transdermal permeation, by limiting compounds from entering, but also from exiting the body through the skin (Jepps et al., 2013:154).

Desquamation is a process by which epidermal keratinocytes travel from the dermal-epidermal junction to the SC (Walters & Roberts, 2002:15), where they then differentiate into enucleated corneocytes and are cast off (Jepps et al., 2013:154; Madison, 2003:234-235). The corneocytes are flat, interconnected cells that are densely packed in a lipid matrix, predominantly comprising ceramides, cholesterol, triglycerides and fatty acids, of which the respective amounts and exact structures are yet to be determined (Jepps et al., 2013:154; McGrath & Uitto, 2010:3.1; Norlén, 2008:59).

The corneocytes have a structure that is supposedly backed by a closely packed arrangement of keratin filaments and lipophilic cell walls, comprised of cross-linked proteins. Natural moisturising factor (a variety of hygroscopic compounds) within the corneocyte network assists in the hydration of the SC (Jepps et al., 2013:154). Any defects in the SC are thus expected to influence the ability of this layer to hinder skin permeation (Jepps et al., 2013:154).

2.4.2.2 Viable epidermis

The main component of the avascular viable epidermis is keratinocytes (McGrath & Uitto, 2010:3.7-3.8). The epidermis is connected to the underlying dermis by means of papillae that extend into the dermis at the epidermal-dermal junction (McGrath & Uitto, 2010:3.25). It is at this junction that cells start to undergo desquamation (Jepps et al., 2013:155). In the absence of the SC, the viable epidermis significantly limits percutaneous delivery (Andrews et al., 2013:1108).

2.4.2.3 Dermis and deeper layers

The dermis, the thickest dermal layer (~4mm), contains the vascular, lymphatic and nervous systems of the skin. Skin appendages also originate in this component. The dermis can be divided into two layers, namely the vascularised upper papillary dermis (100 - 200 µm) and the thicker, reticular dermis. Both layers are made up of several components of connective tissue, including collagen and elastin fibres (Jepps et al., 2013:156; Young & Heath, 2000:157). A ground substance, consisting of water, plasma proteins and polysaccharide-polypeptide complexes are found in the dermis (McGrath & Uitto, 2010:3.2).
The underlying fat microlobules and fibrous collagen constitute the hypodermis (Jepps et al., 2013:156). Dermal insulation, storage of energy and fixing of the overlying skin to deeper musculoskeletal structures are functions of the hypodermis (McGrath & Uitto, 2010:3.3).

2.4.2.4 Appendages

The opening to each hair follicle (called the infundibulum) stretches from the skin surface to a sebaceous duct, about 500 µm deep, where it is connected to a sebaceous gland (Jepps et al., 2013:159-160). The epithelial cells of the SC taper down the infundibulum, forming its wall (McGrath & Uitto, 2010:3.1). Beyond the sebaceous duct the viable epidermis progresses into the outer root sheath of the follicle. The inner root sheath lines the hair shaft (Lauer, 1999:429) that ends in the hair bulb at the base of the follicle (Jepps et al., 2013:160; McGrath & Uitto, 2010:3.13).

Sebaceous glands are found in all skin areas, especially on the face and scalp, but not on the palms and foot soles. In areas around body openings, such as the lips, eyelids and nipples, however, glands are not connected to hair follicles (McGrath & Uitto, 2010:3.3; Young & Heath, 2000:164). Among other functions, the oily secretion of the glands, known as sebum, has antibacterial activity, limits heat loss, acts as delivery system for antioxidants and helps to maintain skin integrity and hydration (Hunter et al., 1995:17; McGrath & Uitto, 2010:3.3; Young & Heath, 2000:164; Zouboulis, 2003:xiv).

Eccrine sweat glands are found in the hypodermis. Sweat, secreted by these glands, travels through the dermis and epidermis towards the skin surface via ducts and plays a role in dermal sensitisation and cooling of the body. Apocrine and apo-eccrine glands also appear in the skin (Jepps et al., 2013:160; McGrath & Uitto, 2010:3.3; Young & Heath, 2000:169).

2.4.2.5 Skin circulation

The vascular circulation of the skin facilitates the nutritional supply of this organ and its appendages, as well as thermoregulation of the body, by increasing or decreasing blood flow according to requirements. Vascular and lymphatic structures are housed in the hypodermis. Arteries and veins form branches that pass upwards through the skin to supply the hypodermis, dermis and capillary networks surrounding appendages (Young & Heath, 2000:171).
2.4.3 PERCUTANEOUS ABSORPTION MECHANISMS

2.4.3.1 Stratum corneum

The lipid solubility or polarity of a drug, its ability to cross the corneocyte cell wall and its affinity for the intracellular status of the corneocytes are all factors that determine the route and effectiveness by which a drug permeates the SC (Jepps et al., 2013:154; Scheuplein, 1965:342-345).

The predominant mechanism that drives drugs across the SC is passive diffusion, subdivided into the transcellular route and the seemingly more important, intercellular route (Bodde et al., 1991:236; Jepps et al., 2013:154). Transcellular transport involves a drug to cross both the lipid matrix and corneocytes, whereas intercellular transport requires movement via the lipid matrix alone (Jepps et al., 2013:154).

2.4.3.2 Viable epidermis

Scheuplein (as quoted by Jepps, et al., 2013:155) points out that due to the aqueous nature of the viable epidermis, lipophilic or non-polar drugs permeate this layer with extreme difficulty by diffusion. Following an investigation of the relationships between transdermal delivery and octanol-vehicle and phosphate buffer solution-vehicle partition coefficients, it was concluded that the viable epidermis is the primary barrier against the permeation of NSAIDs from a non-polar carrier (Wenkers & Lippold, 1999:1330). Keratin in the viable epidermis may disturb the diffusion, or partition of drug into and across this skin component (Heard et al., 2003:243). The amount of free drug that can penetrate skin regions beyond the viable epidermis is often also influenced by binding and sequestration, active transport and metabolism of the compound (Jepps et al., 2013:155) in the basal layer (Hikima et al., 2003:159; Liu et al., 1991:872).

2.4.3.3 Dermis and deeper layers

The eminent blood supply and lymphatics of the dermis play an important role in the dermal permeation and distribution of drugs from this mostly acellular and polar layer (Jepps et al., 2013:156).

Numerous trials (Boutsiouki et al., 2001:326-327; Higaki et al., 2002:139; Morgan et al., 2003:441; Riviere et al., 1991:617-619; Riviere et al., 1992:211; Sugibayashi, 1999:199) had demonstrated that by administering a drug in conjunction with a vasodilator, drug clearance via the blood vessels is higher so that decreased steady-state concentrations and an increased distribution at opposing sites take place. Administration of a vasoconstrictor, instead of a vasodilator has the opposite effect (Borg et al., 1999:277; Higaki et al., 2005:229-233) and enhances the penetration of drugs to deeper tissues, like the muscles (Higaki, et al., 2002:139).
Factors that may also enhance the systemic delivery of a compound include the arrangement of blood vessels in a certain direction (Cross & Roberts, 1999:311-312), the transport of drugs to a binding site due to lymphatic clearance (Cross & Roberts, 1993:606; Jepps et al., 2013; Supersaxo et al., 1990:167; Yoshikawa, 1992:1195) and dermal carrier proteins (influenced by the lipophilicity of the compound) (Jepps et al., 2013:157).

2.4.3.4 Appendages

A drug may be delivered dermally without penetrating the limiting SC by means of hair follicles, sebaceous glands and eccrine or apocrine ducts (Jepps et al., 2013:160).

Experiments have indicated that lipophilic drugs are readily transported by oily sebum into hair follicles and sebaceous glands, although the extent of permeation into deeper follicular or glandular parts, and the surrounding blood vessels, or skin remains unclear (Jepps et al., 2013:160; Lauer, 1999:430). According to Wilke et al. (1999:48) delivery via the eccrine or apocrine ducts is a feasible concept, based on recent investigation. Enhancing and inhibiting factors of appendageal transport act in a manner that is still unclear (Jepps et al., 2013:160).

2.4.4 SALT FORMATION OF DICLOFENAC

Compared to the unionised form of an API, the solubility and dissolution rate of the salt form are usually much more favourable (Fini et al., 1999:164; Wells & Aulton, 2007:340-341). These APIs in salt form can also continue to partition to a certain extent, for as long as the anion and cation can couple as ion-pairs (Fini et al., 1999:171).

According to Fini et al. (1999:171), the pairing behaviour of organic cations, such as diethylamine and hydroxyethyl pyrrolidine, is more favourable than that of inorganic ones, like sodium. The hydrophobic natures of organic counter-ions also lead to an agreeable increase in the partition coefficient of the ion-pairs. The high lipid solubility of DHEP can be attributed to tight ion-pairs that form, while DDEA displays a higher partition coefficient of the ion-pair.

Due to the water solubility of a salt, an API can be formulated as such in a hydrophilic dosage form, like a gel, be deposited on the skin and travel across the lipophilic SC as ion-pair. Because pH changes are slow and limited, the transdermal delivery route prevents breakdown of a salt form before its dermal absorption, thereby allowing the API to maintain its partitioning abilities (Fini et al., 1999:171). The dissociation of salts and the subsequent formation of ion-pairs improve transdermal API absorption, as it also offers alternative percutaneous pathways (lipophilic and hydrophilic routes) (Fini et al., 1999:171).

Diclofenac is frequently administered as a salt, due to the high partition coefficient and very low water solubility of the molecule. Salt formation of diclofenac, however, does not completely
eliminate the high lipophilicity of the parent molecule and some salts retain a moderate affinity towards an oily environment (Fini et al., 1999:164; Maitani et al., 1994:1300). Hydrophilic diclofenac salts can cross the skin via the lipid pathway or hydrophilic pores. It most likely permeates lipophilic membranes as ion-pairs, although diffusion of diclofenac as a free acid is possible (Maitani et al., 1994:1300).

2.5 TRANSDERMAL DELIVERY

2.5.1 DELIVERY VEHICLES

Topical delivery vehicles transport active compounds and prime the skin for drug penetration (Foldvari, 2000:423). They enter the skin and cause dermal swelling, which increases the extent of diffusion of the drug into underlying skin structures (Abbott, 2012:218). The type of vehicle is determined by the solubility, size and structure of the active ingredient (Foldvari, 2000:423).

Traditional delivery vehicles, such as creams and ointments are often associated with poor patient adherence, due to their oily character. For this reason (as well as to minimise adverse effects and improve clinical efficacy, patient compliance and control over skin conditions), more acceptable vehicles, such as gels, foams and sprays are being developed. These vehicles may also restrict disease processes of dermatological conditions progressing towards enteral therapy, thus avoiding unnecessary systemic side effects associated with these drugs (Kurian & Barankin, 2011:4).

2.5.1.1 Selecting a vehicle

A variety of vehicles are available for the topical delivery of active ingredients (Table 2.4). Skin type, aim and target site of the drug, formulation aspects and patient preference are amongst the many factors to consider when determining the desirable vehicle to employ (Kurian & Barankin, 2011:4). Creams are usually preferred by patients with dry skin, while gels or solutions are often the vehicle of choice for patients with oily skins. Foams are especially useful when application to hairy or large areas is required (Kurian & Barankin, 2011:4-5).
<table>
<thead>
<tr>
<th>Topical delivery vehicle</th>
<th>Favourable characteristics</th>
<th>Undesirable effects</th>
</tr>
</thead>
</table>
| Cream                    | • Suitable for use on most skin areas due to oil and aqueous base  
                           • Likely to be less irritating  
                           • Emollient properties  
                           • Most appropriate vehicle for dry or sensitive skin | • Thicker consistency often causes an oily feel |
| Foam                     | • Minimal residue on skin surface after application  
                           • Quick drying, convenience of application, no fragrance  
                           • Easy to spread, particularly useful when applying to larger area | • Uncommon effects at site of application include burning, stinging and itching |
| Gel                      | • High water content  
                           • Cooling effect upon application  
                           • Better long-term efficacy compared to other treatments  
                           • Quick onset of action, good safety profile, high patient satisfaction | • Possible burning, pruritis, dryness, irritation, peeling or redness of skin (<1% of patients) |
| Lotion                   | • Aqueous or alcohol base  
                           • Most versatile vehicle  
                           • Suitable for all skin types  
                           • Lighter feel  
                           • Most appropriate vehicle for application to large or hairy areas or skin sites subject to chafing | • Can cause burning and dryness |
| Ointment                 | • Effective on very dry skin and thickened skin lesions  
                           • Often contains no preservatives  
                           • High potency and active ingredient penetration | • Difficult to wash off due to water-insolubility  
                           • Application often results in a greasy feel |
| Shampoo                  | • Short contact application (about 15 minutes)  
                           • Reduced side effects  
                           • Can be used for extended periods of time  
                           • High patient satisfaction, which may increase adherence and treatment efficacy | • Uncommon effects include burning, skin atrophy and telangiectasia |
| Solution                 | • Easy to spread  
                           • Minimal residue after application | • Alcohol base can cause stinging or exacerbate dryness and irritation |
| Spray                    | • Can apply to large areas of skin | • Uncommon effects include erythema, scaling, dryness, burning and lack of smoothness |
2.5.1.2 Emulgel as vehicle

Emulsions are heterogeneous systems, consisting of two immiscible liquid phases (in pharmaceutics normally water and an oil). The dispersed phase is dispersed as droplets in the other, i.e. the continuous phase. An aqueous, continuous phase is called an oil-in-water emulsion, whereas a water-in-oil emulsion denotes an aqueous dispersed phase (Attwood, 2007:92; Otto et al., 2009:5). Emulsions differ in consistency and vary from liquid lotions to semisolid creams (Otto et al., 2009:5). As transdermal delivery vehicles, emulsions are considered fairly refined and easily removable (Akram et al., 2013:323). Both oil-in-water and water-in-oil emulsions are widely used as transdermal delivery vehicles (Eccleston, 1992:1548).

In addition to the above properties that make gel formulations favourable delivery vehicles (Table 2.4), they also exhibit thixotropic behaviour, have an emollient character, are easily washable and miscible with many other ingredients (Offner & Klech-Gelotte, 2007:1882).

By combining an emulsion and a gel, a dosage form known as an emulgel is formed. Considering the respective characteristics of emulsions and gels, it is evident that emulgels offer very attractive alternatives as topical delivery vehicles (Akram et al., 2013:323).

2.5.2 FORMULATING FOR EFFICACY

FFE™ is a software programme, designed by JW Solutions, to facilitate the formulation of cosmetic ingredients or solvents into a product that would optimally deliver active ingredients into the skin (JW Solutions, 2013a).

The notion is built upon solubility, specifically the solubility of the active ingredient in the formulation and the solubility of the formulation in the skin. For optimal results and delivery, good solubility in both instances is imperative, although a certain balance between the two needs to be established. This balance, as well as the approximate solubilities can be calculated utilising the Hansen solubility parameters (HSP). A formulation that is soluble in the skin is often said to open up the skin in order for active ingredients to permeate into it more readily (JW Solutions, 2013a).

The programme can also be employed to optimise amounts of predetermined ingredients, to propose formulations that would ensure optimal drug delivery, to calculate the SDG and to demonstrate transdermal permeation of active ingredients and excipients (JW Solutions, 2013a).

When optimising the formulation, there are three possibilities to choose from (JW Solutions, 2013b):
1. Optimise towards the active ingredient and obtain the maximum amount of dissolved active in the formulation.

2. Optimise towards the SC and enhance drug delivery into this layer and subsequently into deeper tissues (most preferred option).

3. Optimise towards the target concentration, thereby attain the highest possible driving force to push the active ingredient from the formulation into the skin.

2.5.2.1 Hansen solubility parameters

The three-dimensional HSP are used to determine the solubilities of the ingredients in a formulation and the extent to which they would partition into the skin. The essence of these parameters is that ‘like dissolves like’. The three parameters that describe the character of the skin and each of the formulation components include $\delta D$ (the dispersion force amongst molecules), $\delta P$ (the dipolar force amongst molecules) and $\delta H$ (the hydrogen bonds amongst molecules). By employing these parameters when characterising molecules, different numerical values (instead of vague descriptive terms) are linked to components, hence resulting in more meaningful depictions of each. Compounds can be polar or hydrophilic by either having a high $\delta P$ or $\delta H$ value, but the behaviour of such compounds would differ. Contrary, non-polar or lipophilic compounds are distinguished from each other through the difference in $\delta D$ values, i.e. the higher the $\delta D$, the more polarisable the aromatic electrons (Abbott et al., 2012:218).

The HSP distance determines the extent to which compounds are alike and is it calculated for two compounds (HSP [$\delta D_1$, $\delta P_1$, $\delta H_1$] and [$\delta D_2$, $\delta P_2$, $\delta H_2$]) by applying Equation 2.1 (Abbott et al., 2012:218).

$$\text{Distance} = \sqrt[4]{(\delta D_1 - \delta D_2)^2 + (\delta H_1 - \delta H_2)^2 + (\delta P_1 - \delta P_2)^2}$$  \hspace{1cm} \text{Equation 2.1}

A small distance demonstrates likeness between compounds that would therefore easily dissolve in one another. By calculating the volume-weighted average of individual ingredients, the HSP of a formulation can be determined. This means that a good solvent can be formed by mixing non-solvents, provided that the average of the HSP works out favourably (Abbott et al., 2012:218).

The HSP can therefore be used to manipulate the composition of a formulation in order to enhance the solubility of the active ingredient in the formulation, the solubility of the formulation in the skin, or to obtain a certain degree of both possibilities (Abbott et al., 2012:218).
2.5.2.2 Skin delivery gap principle

When the SDG is known, it mathematically indicates which active ingredient and topical delivery vehicle to use. The calculation (Equation 2.2) incorporates both the intrinsic activity of the active ingredient (demonstrated by the minimum effective concentration), as well as the dermal penetrability thereof (demonstrated by the local tissue concentration reached) (JW Solutions, 2013c).

\[
SDG = \frac{MEC}{LTC}
\]

Equation 2.2

Where:
- \( MEC \) = minimum effective concentration,
- \( LTC \) = local tissue concentration.

The FFE\textsuperscript{TM} software can be applied to calculate SDG once an active ingredient and formulation have been selected. The smaller the SDG (< 1), the more effective the formulation of the active ingredient would be. Formulating an active ingredient with an SDG of 1 might be successful, but utilising FFE\textsuperscript{TM} to optimise the formulation is recommended (using the FFE\textsuperscript{TM} flux method to determine the SDG). An SDG above 1 is undesirable, since the effective formulation and delivery of the active ingredient would be impossible and might only be realised with much effort, research and intricate delivery systems (JW Solutions, 2013c). By utilising the SDG principle, the minimum amount of active ingredient required to obtain an optimal effect in the skin can be determined (JW Solutions, 2013a).

2.6 SUMMARY

Everyday infections and tissue injury activate the inflammatory process. Pain is a key feature of inflammation, brought about by stimuli that would normally cause no discomfort (Kidd & Urban, 2001:3).

NSAIDs are widely used in the treatment of inflammation and pain, diclofenac being the first in its class of phenylacetic acids, commonly administered for this purpose (Wang & Fang, 2008:131). However, due to the variety of undesirable effects associated with the use of oral diclofenac (as for most other NSAIDs), the necessity for effective, topical dosage forms have been identified.

Various transdermal delivery vehicles are available for the delivery of active pharmaceutical ingredients, such as gels, creams and lotions. Employing the FFE\textsuperscript{TM} software and taking the SDG of compounds into consideration, products can be formulated that would optimally deliver active ingredients into the skin at selected concentrations.
During this study, three diclofenac salts were formulated in emulgels. The effects of formulation polarity on skin permeation of diclofenac from these formulations were evaluated and the transdermal delivery of diclofenac from emulgels of different diclofenac salts were compared. These procedures and outcomes are discussed in the following chapters.
REFERENCES


Chapter 3 is written in article format for the purpose of publication in *The International Journal of Pharmaceutics*. The complete Guide for authors by this journal is attached in Appendix D. No formatting was used during the writing of this article, other than advised by the Guide for authors.
Formulation of diclofenac salts by implementing the delivery gap principle

Hanri Smith, Jan L. Du Preez, Minja Gerber, Jeanetta du Plessis*

Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom 2520, South Africa

* Corresponding author. Tel.: +27 18 299 2274, Fax: +27 18 299 2225. E-mail address: Jeanetta.duPlessis@nwu.ac.za
ABSTRACT

This study was conducted to evaluate the effect of formulation polarity on skin permeation of diclofenac from emulgels, and to determine whether different diclofenac salts would influence transdermal delivery in these polarity differenicated emulgels. Diclofenac sodium (DNa), diclofenac diethylamine (DDEA) and diclofenac N-(2-hydroxyethyl) pyrrolidine (DHEP) were each formulated as an emulgel optimised towards the stratum corneum (SC) (oily phase ~30%), a more hydrophilic emulgel (oily phase ~15%) and a more lipophilic emulgel (oily phase ~45%). Components of the oily phase and their respective amounts, as well as the skin delivery gap (SDG) of formulations were determined, utilising the Formulating for Efficacy (FFE™) software of JW Solutions (2013). Transdermal diffusion studies were performed over periods of 12 hours each, followed by tape stripping experiments. Results showed that formulation polarity significantly influenced skin permeation of diclofenac. Transdermal delivery from emulgels, prepared from different salts, was similar. DNa formulations showed only slightly better results. It was concluded that the most efficient skin permeation of diclofenac was obtained from formulations with polarities higher than that of formulations optimised towards the SC.

Keywords: Formulation polarity, skin permeation, diclofenac, FFE™
1. **INTRODUCTION**

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of inflammation and pain (Escribano et al., 2003). Diclofenac, a traditional NSAID, is considerably more effective as an analgesic, antipyretic and anti-inflammatory drug than other traditional NSAIDs, such as indomethacin and naproxen (Grosser et al., 2011). Diclofenac is known for its side effects, of which gastric disorders and fluid and sodium retention are commonly observed. Less common side effects include hypersensitivity reactions, nephrotoxicity, mild central nervous system effects (headache, dizziness, drowsiness and depression), hepatic dysfunction, haematological disturbances (e.g. inhibition of platelet aggregation and occasionally pancytopenia), visual disorders and tinnitus (Rossiter, 2012). In the United States of America a black box warning was issued for its possible cardiovascular side effects (Grosser et al., 2011).

According to Roth and Fuller (2011), topical diclofenac is a valuable substitute for oral NSAID therapy in the treatment of osteoarthritis, due to its more favourable safety profile. Topical application of NSAIDs is significantly restricted, due to the therapeutic effect being limited to the site of application. Prolonged contact of the formulation with the skin may further lead to skin reactions, including irritant dermatitis and erythema (Lionberger & Brennan, 2010).

The benefits of topically applied NSAIDs, compared to oral administration and systemic delivery, include the easy cessation of treatment, should effects become troublesome (Brown et al., 2006), avoidance of extensive first-pass metabolism (Cleary, 1993; Kornick 2003; Lionberger & Brennan, 2010; Prausnitz & Langer, 2008), reduced systemic side effects (Colin Long, 2002), convenience of application and improved patient compliance (Cleary, 1993; Prausnitz & Langer, 2008).

Diclofenac, 2-[(2,6-dichlorophenyl)amino]-benzeneacetic acid (D) is an acidic compound (pKₐ 3.80 at 25 °C) with very low aqueous solubility in the unionised form (Kozakevych et al., 2013). An approach regularly applied in the optimisation of the solubility and dissolution rate of poorly water soluble, weak electrolytes is to prepare a salt of the active pharmaceutical ingredient (API) (Minghetti et al., 2007; O’Connor & Corrigan, 2001). In comparison with the unionised form of an API, the solubility and dissolution rate of the salt form are usually much more
favourable (Fini et al., 1999; Wells & Aulton, 2007). APIs in salt form can continue to partition to a certain extent, for as long as the anion and cation can couple as ion-pairs (Fini et al., 1999). According to Fini et al. (1999), the behaviour of organic cations, such as diethylamine and hydroxyethyl pyrrolidine, is more desirable than that of inorganic ones, like sodium. The hydrophobic natures of organic counter ions also lead to an agreeable increase in the partition coefficient of the ion-pairs. The high lipid solubility of diclofenac N-(2-hydroxyethyl) pyrroldine (DHEP) can be attributed to tight ion-pairs that form, while diclofenac diethylamine (DDEA) displays a higher partition coefficient of the ion-pair.

Due to the water solubility of a salt, an API can be formulated as such in a hydrophilic dosage form, such as a gel, be deposited on the skin and travel across the lipophilic stratum corneum (SC) as ion-pair. Because pH changes are slow and limited, the transdermal delivery route prevents breakdown of a salt form before its dermal absorption, thereby allowing the API to maintain its partitioning abilities (Fini et al., 1999). The dissociation of salts and the subsequent formation of ion-pairs improve transdermal API absorption, as it also offers alternative percutaneous pathways (lipophilic and hydrophilic routes) (Fini et al., 1999).

Diclofenac is frequently administered as a salt, due to the high partition coefficient and very low water solubility of this molecule. Salt formation of diclofenac, however, does not completely eliminate the high lipophilicity of the parent molecule and some salts retain a moderate affinity towards an oily environment (Fini et al., 1999; Maitani et al., 1994). Hydrophilic diclofenac salts can cross the skin via the lipid pathway or hydrophilic pores. It most likely permeates lipophilic membranes as ion-pairs, although diffusion of diclofenac as the free acid is possible (Maitani et al., 1994).

Formulating for Efficacy (FFE™) is a software programme, designed by JW Solutions, to facilitate the formulation of cosmetic ingredients, or solvents into a product that would optimally deliver active ingredients into the skin (JW Solutions Software, 2013a).

The notion is built upon solubility, i.e. solubility of the active ingredient in the formulation and solubility of the formulation in the skin. For optimal results and delivery, good solubility in both instances is imperative, although a certain balance between the two should be established. This balance, as well as approximate solubilities can be calculated by utilising the Hansen
solubility parameters (HSP). A formulation that is soluble in the skin is often said to open up the skin in order for active ingredients to permeate into it more readily (JW Solutions Software, 2013a).

The programme can also be employed to optimise the amounts of predetermined ingredients, to propose formulations that would ensure optimal drug delivery, to calculate the skin delivery gap (SDG) and to demonstrate transdermal permeation of active ingredients and excipients (JW Solutions Software, 2013a).

When optimising the formulation, there are three possibilities to choose from (JW Solutions, 2013b), i.e. (1) optimise towards the active ingredient and obtain the maximum amount of dissolved active in the formulation, (2) optimise towards the SC and enhance drug delivery into this layer and subsequently into the deeper skin tissues (the preferred option), or (3) optimise towards the target concentration to attain the highest possible driving force to push the active ingredient from the formulation into the skin.

When the SDG is known, it mathematically indicates which active ingredient and topical delivery vehicle to use. The calculation (Equation 1.1) incorporates both the intrinsic activity of the active ingredient (demonstrated by the minimum effective concentration), as well as the dermal penetrability thereof (demonstrated by the local tissue concentration reached).

\[
SDG = \frac{MEC}{LTC}
\]

Equation 1.1

Where: \( MEC \) is the minimum effective concentration and \( LTC \) is the local tissue concentration.

The FFE™ software can be applied to calculate SDG, once an active ingredient and formulation have been selected. The smaller the SDG (< 1), the simpler the effective formulation of the active ingredient would be. Formulating an active ingredient with a SDG equal to 1 may prove successful, but utilisation of FFE™ to optimise the formulation is recommended (using the FFE™ flux method to determine the SDG). An SDG above 1 is undesirable, since the effective formulation and delivery of the active ingredient would not be possible and may it only realise with much effort, research and intricate delivery systems (JW Solutions Software, 2013c). By utilising the SDG principle, the minimum amount of active ingredient required to obtain an optimal effect in the skin can be determined (JW Solutions Software, 2013a).
In this study, three diclofenac salts, also used in current commercial, topical application products, including diclofenac sodium (DNa), DDEA and DHEP, were formulated as emulgels at different polarities. Absorption of diclofenac through the SC and epidermis from these topical dosage forms were compared. Diclofenac concentrations were fixed at 1% (w/w). Emulgels, optimised towards the SC (oil phase ~30% of the formulation), more hydrophilic (oil phase ~15%) and more lipophilic emulgels than the first (oil phase ~45%), were prepared. Components of the oil phase and their respective amounts, as well as the SDG of each formulation were determined by utilising the FFE™ software.

2. MATERIALS AND METHODS

2.1 Analytical method

The concentration of diclofenac in the receiver medium was determined by high pressure liquid chromatography (HPLC) (Agilent® 1200 Series). A 25 µl sample was injected onto a C18 Venusil® XBP column (5 µm, 100Å, 4.6 x 150 mm). The mobile phase consisted of acetonitrile/water/acetic acid (70/29/1, v/v). The flow rate was 1.0 ml/min and UV detection was set at 275 nm. Retention time for diclofenac was 4.5 min.

2.2 Materials

DNa, DDEA and DHEP were acquired from Amoli Organics (Mumbai, India). Other excipients used in these emulgel formulations included Arlasove® dimethylisosorbide (Croda, Boksburg, South Africa), Carbopol® Ultrez 10 Polymer (Lubrizol, Brussels, Belgium), ethanol, UniAR® triethanolamine and polyethylene glycol 400 (PEG400) (Merck Millipore, Halfway House, South Africa). Methanol AR grade (Merck Millipore, Halfway House, South Africa) was used in the preparation of samples. UniAR® potassium dihydrogen orthophosphate and UniLAB® sodium hydroxide were used for the preparation of the phosphate buffer solution (PBS) (pH 7.4) and were both obtained from Merck, Wadeville, South Africa. HPLC analytical grade LiChrosolv® acetonitrile and HiPerSolv® 100% acetic acid, supplied by Merck Millipore (Halfway House, South Africa), were used for the HPLC mobile phase preparation. Deionised HPLC grade water was prepared with a Milli-Q® water purification system (Millipore, Milford, USA) and was used throughout this study.
2.3 Formulation of emulgels

Carbomer was dispersed in water to form a hydrogel. The oil phase consisted of dimethylisosorbide (DMI), polyethylene glycol 400 (PEG400) and ethanol. Triethanolamine (TEA) was added to the oil phase. Each salt was dissolved in the oil phase and the TEA mixture and resulting solution were added to the hydrogel. Once all ingredients were added together, an increase in viscosity was induced by the presence of TEA. The pH of all nine formulations was measured between pH 7.20 – 7.60, using a Mettler Toledo pH meter (Switzerland).

2.4 Standard preparation

DNA (20 mg) was accurately weighed in a 100 ml volumetric flask and made up to volume with methanol. Six standards solutions, with different concentrations (20 µg/ml, 15 µg/ml, 10 µg/ml, 5 µg/ml, 0.2 µg/ml, 0.1 µg/ml) were prepared by diluting the DNA/methanol solution with PBS (pH 7.4). Each of the six standards was injected in duplicate into the HPLC (injection volume 5 µl).

2.5 Physicochemical properties

2.5.1 Aqueous solubility determinations

PBS (pH 7.4) was prepared and a shaker bath preheated to 32 °C. Saturated solutions of each diclofenac salt in PBS were prepared and placed in the shaker bath and left overnight. Solutions were each centrifuged and 1 ml of the supernatant was diluted to 7 ml with methanol and analysed using HPLC. This experiment was performed in triplicate.

2.5.2 Octanol-buffer distribution coefficient (log D) determinations

Equal volumes of n-octanol and PBS (pH 7.4) were added together and co-saturation of the two phases allowed to equilibrate over a period of at least 24 hours. Solutions of each model compound were prepared using the pre-saturated n-octanol phase as solvent. An equal volume of pre-saturated PBS was added to each of these solutions. The mixtures were then placed in a shaker bath and left overnight and centrifuged thereafter. After centrifugation the concentrations of diclofenac in the two phases were determined by HPLC. The log D values were calculated as the ratio of diclofenac concentration in the octanol phase to that in the buffer phase. This experiment was performed in triplicate.
2.6  Diffusion studies

2.6.1  Preparation of the skin

Full thickness, abdominal skin was collected from plastic surgeons, following abdominoplastic surgery on female, Caucasian patients. Written informed consent was obtained from each patient for use of their skin for research purposes. Patient information is kept confidential. The research was conducted in a laboratory (type 2) that is approved for transdermal studies and equipped for working with human skin. The Ethics Committee of the North-West University approved the research as from 25 August 2011 to 24 August 2016 (reference number: NWU-00114-11-A5).

The skin was kept in a freezer at -20 °C for not more than 6 months. Prior to preparation, the skin was thawed and a thickness of 400 µm removed, using an electric dermatome (Zimmer Inc. Warsaw, Indiana, USA). The dermatomed skin (containing SC, viable epidermis and upper dermis (ED)) was cut into circles and placed on Whatman® filter paper. Samples were wrapped in aluminium foil and frozen at -20 °C. Prior to diffusion studies, these skin samples were thawed, visually examined for defects and mounted on Franz diffusion cells (with filter paper still attached).

2.6.2  Membrane diffusion studies

Franz cells with a diffusion area of 1.075 cm² and a receptor capacity of approximately 2 ml were used in this study. FP Vericel® (PVDF) membrane filters (25 mm diameter, 0.45 µm pore size) (Pall Corporation, Mexico, USA) were mounted on the outer half of the receptor compartment of the Franz cells. The upper and lower parts of the Franz cells were sealed with vacuum grease and joined together with a clamp. 1 ml of the emulgel (~32 °C) was placed in the donor compartment and immediately covered with Parafilm® to minimise evaporation. Each receptor compartment was filled with 2 ml PBS (pH 7.4, 37 °C), equipped with a stirring magnet driven at 750 rpm. A temperature of 32 °C was maintained for the cell system, using a water bath. In order to assure sink conditions throughout the experiment, the entire receptor volume was withdrawn every hour for up to 6 hours and replaced with 2 ml fresh PBS. The extracted samples were assayed by HPLC to determine the concentrations of diclofenac that had
permeated through the membrane filters, as well as the release rates of diclofenac from the nine emulgel formulations.

2.6.3 Transdermal diffusion studies

The same method, as discussed in Section 2.6.2, was employed to perform in vitro skin permeation experiments. Instead, skin samples were mounted on the lower part of the Franz cells with the SC facing the upper donor compartment and the underlying filter paper (covering the dermis) facing downwards. Samples from the receptor phase were withdrawn at predetermined time intervals (every 20 min for up to 2 hours, followed by 2 hourly extractions for up to 12 hours) and replaced with fresh PBS (37 °C, pH 7.4). The withdrawn samples were assayed by HPLC and the concentrations of diclofenac that had permeated the skin samples were determined.

2.6.4 Tape stripping

After completion of the 12 hours transdermal diffusion studies, Franz cells were dismantled and each skin sample pinned onto Whatman® filter paper, attached to a solid surface. These skin samples were gently dabbed dry with paper towel to remove any remaining formulation. Pieces of 3M Scotch® Magic™ tape, large enough to cover the area of diffusion, were consecutively placed onto each skin sample, rubbed and removed. Due to possible contamination from formulation still left on the skin, the first tape strip for each sample was discarded. The skin was stripped until the SC over the diffusion area was completely removed from the underlying epidermis (the stripped area appeared white once the viable epidermis had been exposed). These strips contained amounts of diclofenac (Lademann et al., 2008). The strips, with visible pieces of SC removed through stripping, were placed in a polytop each per skin sample, containing 5 ml of methanol. The remaining skin samples (the viable epidermis and upper dermis of the diffusion area and part of the skin not exposed to the emulgel formulations) were cut into smaller pieces and each sample’s transferred into a vial containing 5 ml of methanol. Polytops (those containing tape strips and SC, as well as those containing epidermal skin pieces) were left overnight at 4 °C in order for the API on the strips and present in the skin cuttings to dissolve in the methanol. Each solution was analysed by means of HPLC to
determine the concentration of diclofenac that penetrated the respective skin layers during the diffusion studies.

2.7 Data analysis

The cumulative concentration of diclofenac that had permeated through the skin was plotted as a function of time. The flux was determined as the slope of the linear portion of the plot. The yield from every Franz cell was calculated as a percentage of the volume and strength of formulation in the donor compartment. Every transdermal diffusion study was conducted over a period of 12 hours and was the yield calculated at the end of this period. The percentage yield was also determined for the membrane diffusion studies after 6 hours to establish possible API release.

2.8 Statistical analysis

Two-way and three-way analysis of variance (ANOVA) tests, as well as Tukey’s Honestly Significant Difference (HSD) tests were performed using Statistical Analysis System (SAS Institute Inc., 2011). Differences among groups were considered statistically significant when the $P$ value was less than 0.05 (Dawson & Trapp, 2001).

3. RESULTS AND DISCUSSION

3.1 Formulation of emulgels with different polarities

Three 1% diclofenac gels (calculated as free acid) were prepared using DNa. The salt was formulated in an emulgel, optimised towards the SC (oily phase ~30%) (DNa-O), a more hydrophilic emulgel (oily phase ~15%) (DNa-H) and a more lipophilic emulgel (oily phase ~45%) (DNa-L). This was repeated using DDEA (DDEA-O, DDEA-H and DDEA-L) and DHEP (DHEP-O, DHEP-H and DHEP-L). In total, nine 1% diclofenac emulgels were thus prepared. The more polar formulations had a cloudy, greyish colour. The remaining six formulations were clear and colourless.

The composition of the oily phase of the formulation optimised towards the SC was determined by utilising the FFE™ software of JW Solutions. This formula was subsequently adapted by decreasing the oily phase and increasing the aqueous phase accordingly, to establish a formula for a gel with an increased polarity. The formula of a product with a polarity lower than that of the gel optimised towards the SC was similarly obtained by increasing the oily phase with a
subsequent decrease in aqueous phase. The SDG of all nine formulations was calculated using the FFE™ software and was established at values below 1.

3.2 Aqueous solubility and partition coefficient

Aqueous solubility of the respective diclofenac salts used in this study was determined at 32 °C, using PBS (pH 7.4) as solvent. The solubility and log D values obtained respectively were 11.4 mg/ml and 1.270 for DNa, 8.0 mg/ml and 1.291 for DDEA and 11.9 mg/ml and 1.285 mg/ml for DHEP. According to Naik et al. (2000), an aqueous solubility above 1 mg/ml is required for molecules to passively cross the skin. The generated values for the aqueous solubility of the diclofenac salts during this study were thus all indicative of effortless percutaneous delivery.

3.3 Diffusion studies

3.3.1 Membrane diffusion studies

Results confirmed that diclofenac was indeed released from all nine emulgel formulations. Evaluation of the separate groups of emulgels, containing different diclofenac salts, indicated that the more hydrophilic emulgels and those gels optimised towards the SC, released the highest percentage of diclofenac (DNa gel: 8.375% and 8.232%, DDEA gel: 7.895% and 8.058% and DHEP gel: 8.232% and 8.006%, respectively) after 6 hours. There was only a slight difference between the percentages of diclofenac released from these two types of formulations. In the DNa and DHEP groups, the more hydrophilic gels released the highest percentage of diclofenac. The gel optimised towards the SC, prepared using DDEA, however, showed the highest percentage of diclofenac released in that group. Yet, in all three groups, those two types of formulations released more than twice the percentage of diclofenac than the more lipophilic formulation (DNa gel: 2.979%, DDEA gel: 3.483% and DHEP gel: 3.763%). This may have been, because both the free acid (lipophilic) and salt forms of diclofenac have an affinity towards the oily phase (Fini et al., 1999; Maitani et al., 1994). Diclofenac, therefore, favours the oily composition of the more lipophilic formulation over the aqueous environment of the receptor solution, whether in salt form or having changed to the free acid (Abbott, 2012). The diclofenac would therefore not leave the formulation readily in order to cross the membrane filter.
A comparison of formulations with similar polarities and different diclofenac salt groups showed no pattern. In the group of gels optimised towards the SC, the highest percentage of diclofenac was released from the formulation prepared from DNa, followed by DDEA and DHEP. When comparing the more hydrophilic gels, the gel formulated with DNa released the highest percentage of diclofenac, followed by DHEP and DDEA. Finally, measurement of the more lipophilic gels showed that the percentage of diclofenac released in order of highest to lowest was from the DHEP, DDEA and DNa formulated gels.

3.3.2 Transdermal diffusion studies

The more hydrophilic formulations in every group of gels, formulated with a certain diclofenac salt, showed the highest average percentage of diclofenac having diffused (DNa gel: 0.089%, DDEA gel: 0.086% and DHEP gel: 0.082%) after 12 hours, followed by either the more lipophilic gel (as was the case for gels made with DDEA with 0.014%), or the preparation optimised towards the SC (DNa gel: 0.029% and DHEP gel: 0.012%). There was a significant difference between the average percentages of diclofenac having diffused from the more hydrophilic gels and the remaining two types of formulations. Between three and seven times more diclofenac had diffused through the dermatomed skin from the more hydrophilic gels, compared to the other two types of formulations. This could have been due to these preparations having had polarities higher than that of the SC (and were possibly even more hydrophilic than the epidermis), and since the diclofenac acid is lipophilic and the salt form retains affinity for an oily environment, it would rather leave the formulation to permeate the fatty SC and underlying epidermis.

No pattern was observed when formulations with similar polarities, but prepared with different diclofenac salts, were compared. In the group of gels optimised towards the SC, the DNa gel showed the highest average percentage of diclofenac diffusion (0.029%), followed equally by the gels prepared with DDEA and DHEP (both 0.012%). The average percentage of diclofenac having diffused from the more hydrophilic gels was highest for the DNa gel (0.089%) and the average percentage having diffused from the more lipophilic gels for the DDEA gel (0.014%). The lowest percentage of diclofenac diffusion was for the DHEP gels (0.082% and 0.011%, respectively) in both instances.
According to Wells and Aulton (2007:341), different salt forms of an API can change the dissolution rate and solubility of the compound in varying degrees. This in turn would affect the bioavailability of the API. The difference in effects of the various salts on the physicochemical properties of diclofenac acid could, however, not be confirmed during this study.

3.3.3 Tape stripping

The average diclofenac concentration in the SC-epidermis (SCE) was higher than in the ED for all the formulations, except for the more lipophilic gel prepared with DDEA (which showed zero diclofenac in the SCE). This could have been attributed to the epidermis being an aqueous environment, compared to the lipophilic SC, causing the lipophilic diclofenac to remain in the SCE rather than to permeate into the viable epidermis (Abbott, 2012; Naik et al., 2000). For the group of formulations prepared using DNa, the average diclofenac concentration in the SCE was highest for gels optimised towards the SC (0.765 µg/ml), followed by the more hydrophilic (0.448 µg/ml) and more lipophilic (0.305 µg/ml) gels. The average diclofenac concentration in the ED was highest for the more lipophilic formulation (0.265 µg/ml) and lowest for the more hydrophilic (0.148 µg/ml) gel. Emulgels made with DDEA showed the highest concentration of diclofenac for the more polar gel (0.475 µg/ml), followed by the formulation optimised towards the SC (0.457 µg/ml), possibly due to the high polarity of the more hydrophilic DDEA formulation, which created a driving force to push the lipophilic diclofenac out of the formulation into the SCE (Abbott, 2012:218). Epidermal diclofenac penetration of this group was best for the more hydrophilic type (0.195 µg/ml) and lowest for the more lipophilic type (0.032 µg/ml), in agreement with the findings of Wenkers and Lippold (1999). The more polar DHEP gel showed the highest diclofenac concentration in both the SCE (0.505 µg/ml) and ED (0.199 µg/ml), compared to the other types being prepared using this salt, whilst the more lipophilic gel had the lowest concentration in both layers (SCE: 0.152 and ED: 0.057 µg/ml). Again, the high polarity of the more polar DHEP emulgel could have driven the diclofenac to leave the formulation (Abbott, 2012:218). The more diclofenac that permeates into the SCE, the higher the amount available to enter the ED. High concentrations of diclofenac in the SCE were expected for formulations optimised towards the SC, as these would enhance API delivery into this layer.
When the outcomes of similar gel types, prepared with different diclofenac salts, were evaluated, the highest average SCE (0.765 µg/ml) and ED (0.254 µg/ml) diclofenac concentrations for the formulation optimised towards the SC were found in the DNa emulgel, followed by the DDEA (0.457 µg/ml and 0.143 µg/ml, respectively) and DHEP (0.361 µg/ml and 0.088 µg/ml, respectively) gels. As DDEA and DHEP formulations are specifically promoted for their ability to deliver transdermally (Fini et al., 1998; Fini et al., 1999), these results were unexpected. In order of highest to lowest, the average SCE and ED diclofenac concentrations for the more hydrophilic formulations were established for the DHEP (SC: 0.505 µg/ml and ED: 0.199 µg/ml), DDEA (SC: 0.475 µg/ml and ED: 0.195 µg/ml) and DNa (SC: 0.448 µg/ml and ED: 0.148 µg/ml) gels, in accordance with popular belief (Fini et al., 1998; Fini et al., 1999). The DNa gel had both the highest average diclofenac concentration in the SC (0.305 µg/ml) and ED (0.265 µg/ml) in the least polar group of gels, followed by the DHEP (0.152 µg/ml and 0.057 µg/ml, respectively) and DDEA (0.000 µg/ml and 0.032 µg/ml, respectively) gels. As for the formulations optimised towards the SC, these results were unexpected.

3.4 Statistical analysis

The data from the membrane release studies was statistically analysed and compared using a two-way ANOVA and Tukey test. A significant difference between the formulations with different polarities was indicated, having a $P$ value < 0.0001 and different Tukey groupings. Furthermore, no significant differences among those formulations prepared using different diclofenac salts ($P$ value = 0.3956), or among the gels with different combinations of polarities and salts utilised during formulation ($P$ value = 0.3009), were observed.

Statistical analysis of the data generated during transdermal diffusion studies, using a two-way ANOVA, indicated a statistically significant difference among formulations prepared with different salts ($P$ value = 0.0040), as well as a highly significant difference between formulations with different polarities ($P$ value < 0.0001). No significant difference among gels with different combinations of salts being used and with different polarities were indicated ($P$ value = 0.0592). Tukey tests indicated a significant difference between the DNa and DDEA gels, and between the DNa and DHEP emulgels. No significant difference was observed among the DDEA and DHEP formulations. This test also indicated a significant difference between more hydrophilic
gels and formulations optimised towards the SC, and between more hydrophilic gels and more lipophilic gels, with no significant difference between formulations optimised towards the SC and the more lipophilic gels.

Statistical analysis and comparison of tape stripping data, using a three-way ANOVA, revealed a statistically significant difference among average diclofenac concentrations of gels with different polarities ($P$ value < 0.0001), gels prepared using different diclofenac salts ($P$ value < 0.0001) and gels with different combinations of polarities and the salts used during formulation ($P$ value < 0.0001). A significant difference between the average diclofenac concentrations in the SCE and ED was also observed ($P$ value < 0.0001). A statistically significant interaction between the skin location (SCE or ED) and the polarity of gels was indicated ($P$ value < 0.0001).

The salt employed showed no interaction with regards to skin location of any of the formulations ($P$ value = 0.2969). No statistically significant interaction was indicated between the different diclofenac salts, the polarities of the formulations and skin location of diclofenac ($P$ value = 0.1642).

The Tukey test indicated a statistically significant difference between the DNa and DDEA gels, and between the DNa and DHEP gels, with no significant difference between the DHEP and DDEA emulgels. Gels optimised towards the SC and more polar gels displayed a statistically significant difference from the least polar type gels. No significant difference was indicated between gels optimised towards the SC and the more hydrophilic type. This test also indicated a statistically significant difference in diclofenac concentrations between the skin locations, namely the SCE and ED.

The average concentration of diclofenac was higher in the SCE than in the ED for all the formulations, except for the more lipophilic DDEA gel, which had no diclofenac in the SCE. These outcomes were indicative that diclofenac penetrated more effectively into the lower layers of the epidermis beyond the SC.

The average cumulative concentration of diclofenac having diffused the skin after 12 hours was considerably higher than the average concentration of diclofenac in the ED and the SCE. This indicated that diclofenac crossed both layers effectively to enter the receptor phase that resembles the systemic circulation.
4. CONCLUSION

During membrane release studies, the more hydrophilic DNa emulgel formulation showed the highest average percentage of diclofenac being released after 6 hours, followed equally by the DNa gel optimised towards the SC and the more hydrophilic DHEP gel. The average percentage of diclofenac being released after 6 hours from the more lipophilic formulations was much lower than for formulations optimised towards the SC and the more polar emulgels (for each group formulated using a specific salt), possibly due to the diclofenac acid being more lipophilic and the salt form retaining moderate affinity towards an oily phase, making it reside in the oily, less polar formulations, instead of leaving it to enter the aqueous receptor phase (Abbott, 2012).

Results from diffusion studies showed that the more polar DNa emulgel resulted in the highest average cumulative concentration after 12 hours, the highest percentage of diffused diclofenac after 12 hours and the highest average flux, followed by the more polar DDEA and DHEP emulgels. The average cumulative concentration, percentage of diffused diclofenac and average flux were much lower for gels optimised towards the SC, and for the more lipophilic gels of all three groups of gels prepared with a specific salt. These results could have been due to the higher polarity of the more polar formulations having created a higher driving force to push the lipophilic diclofenac out of the formulation and into the lipophilic SC (Abbott, 2012; Naik et al., 2000). Higher amounts of diclofenac were released and therefore available for diffusion across the SCE and ED.

The highest average concentration of diclofenac in the ED was for the more lipophilic DNa formulation, while DNa gel optimised towards the SC displayed the highest concentration in the SCE. The more lipophilic formulations of DNa, DDEA and DHEP indicated the lowest concentrations of diclofenac in the SCE. Since the less polar formulations also released the lowest amounts of diclofenac, according to the membrane release studies, less diclofenac was available for penetration of the SCE and ED than from formulations optimised towards the SC and the more hydrophilic formulations.

The average concentration of diclofenac was higher in the SCE than in the ED for all the emulgel formulations, except for the more lipophilic DDEA gel that resulted in no diclofenac in
the SCE. This was indicative that diclofenac penetrated more effectively into the lower layers of the epidermis beyond the SC.

The average cumulative concentration of diclofenac having diffused after 12 hours was considerably higher than the average concentration of diclofenac present in the ED and in the SCE. This was indicative of diclofenac having crossed both layers effectively to enter the receptor phase that resembles the systemic circulation.

Results from this study supported the delivery gap principle, which states that a small SDG (< 1) facilitates effective formulation and transdermal delivery of an API (JW Solutions, 2013c). It was concluded that the most efficient transdermal delivery of diclofenac, in terms of time and quantity, was obtained from those formulations with polarities higher than that of the formulations optimised towards the SC. Although the performances of the various gels, prepared from different salts, were very similar, the DNA (containing an inorganic counter-ion) formulations showed slightly better results, unlike one would expect (Fini et al., 1999), particularly since DDEA and DHEP formulations are particularly promoted as transdermal dosage forms (Fini et al., 1998; Fini et al., 1999; Rossiter, 2012).
5. ACKNOWLEDGEMENTS

We would like to thank the National Research Foundation (NRF) of South Africa and the Centre of Excellence for Pharmaceutical Sciences (Pharmacen), Potchefstroom Campus, North-West University, South Africa, for funding this project. Thank you to Prof Faans Steyn for statistical analysis and Ms Julia Handford for language editing.

Any opinions, findings and conclusions, or recommendations expressed in this material are those of the author(s) and therefore the NRF does not accept any liability in regard thereto.
REFERENCES


FIGURE LEGENDS

**Figure 1:** Average cumulative amount of diclofenac per area (µg/cm²) of all nine formulations that permeated the skin between 4 - 12 hours as a function of time (n=10 for all formulations, except DDEA-H en DHEP-L where n=9).

**Figure 2:** Box plots and diamonds of the flux values of all nine formulations after topical application illustrating median (indicated by a horizontal line in the box) and average flux (indicated by diamond).

**Figure 3:** Comparison of the concentration (µg/ml) of diclofenac in the stratum corneum-epidermis and epidermis-dermis among different formulations.
FIGURES:

**Figure 1:** Average cumulative amount of diclofenac per area (µg/cm²) of all nine formulations that permeated the skin between 4 - 12 hours as a function of time (n=10 for all formulations, except DDEA-H en DHEP-L where n=9).
Figure 2: Box plots and diamonds of the flux values of all nine formulations after topical application illustrating median (indicated by a horizontal line in the box) and average flux (indicated by diamond).
Figure 3: Comparison of the concentration (µg/ml) of diclofenac in the SCE and ED among different formulations.
NSAIDs are widely used in the treatment of inflammation and pain (Escribano et al., 2003:203). Diclofenac, a traditional NSAID, is considerably more effective as an analgesic, antipyretic and anti-inflammatory drug than other traditional NSAIDs, such as indomethacin and naproxen (Grosser et al., 2011:986). Oral NSAIDs are, however, known for their side effects (Grosser et al., 2011:987; Rossiter, 2012:391), which makes topical administration a valuable alternative to oral diclofenac therapy in the treatment of osteoarthritis, for example, as it offers a more favourable safety profile (Roth & Fuller, 2011:166). Diclofenac is frequently administered as a salt, due to the high partition coefficient and very low water solubility of this molecule. FFE™ is a software programme designed by JW Solutions to facilitate the formulation of cosmetic ingredients, or solvents into a product that would optimally deliver active ingredients into the skin (JW Solutions Software, 2013).

The aim of this study was to evaluate the effect of the polarity of formulations on the permeation of diclofenac from emulgels through the skin, and to determine whether there would be a difference in transdermal delivery of diclofenac among emulgels, consisting of different diclofenac salts. The objectives of this study thus included the following:

- Revalidation of an existing HPLC method to determine concentrations of the different ingredients, including the API, in nine newly formulated diclofenac emulgels.
- Determination of the aqueous solubility and distribution coefficients of DNα, DDEA and DHEP.
- Employment of the JW Solutions software, FFE™, to determine optimal formulae for topical preparations containing diclofenac, according to particular polarity indexes and to calculate the SDG of these formulations.
- Development of emulgel formulations, using three diclofenac salts.
- Determination of the rate of API release by conducting membrane diffusion studies.
- Determination of the transdermal and topical delivery of diclofenac by conducting diffusion studies, by utilising excised human skin and subsequently employing the tape stripping method to determine the amount of API present in the different skin layers.

An HPLC method for analysis of diclofenac salts had already been developed and validated by Prof. J.L. du Preez at the Analytical Technology Laboratory, North-West University,
Aqueous solubility was determined for DNa, DDEA and DHEP and the corresponding values obtained were 11.4 mg/ml, 8.0 mg/ml and 11.9 mg/ml, all indicative of effortless percutaneous delivery (Naik et al., 2000:319). Log D (pH 7.4) determinations for DNa, DDEA and DHEP were performed and values established at 1.270 (DNa), 1.291 (DDEA) and 1.285 (DHEP). According to these outcomes, diclofenac (topically applied as a salt in a suitable vehicle) should therefore permeate transdermally without the aid of radical intervention (Naik et al., 2000:319; Walters, 2007:1312).

DNa, DDEA and DHEP were each formulated as an emulgel optimised towards the SC (oily phase ~30%), a more hydrophilic emulgel (oily phase ~15%) and a more lipophilic emulgel (oily phase ~45%). Components of the oily phase and their respective amounts, as well as the SDG of formulations were determined, utilising the FFE™ software of JW Solutions (2013).

Membrane release studies were conducted to determine the rate of API release from the new emulgel formulations. Results confirmed that diclofenac was indeed released from all nine emulgels. The more hydrophilic DNa formulation (DNa-H) released the highest average percentage of diclofenac (8.375%) after 6 hours. Subsequent transdermal diffusion studies were performed to determine the concentration of diclofenac that had permeated the skin. The DNa-H emulgel showed the highest average percentage skin diffusion (0.089%) after 12 hours, as well as the highest average flux (1.422 ± 0.198 µg/cm².h).

The concentrations of diclofenac in the SCE and ED were determined by tape stripping experiments. DNa-L (the more lipophilic emulgel) achieved the highest average concentration in the ED (0.265 µg/ml), while the DNa-O emulgel (optimised towards the SC) showed the highest concentration in the SCE.

Results showed that the polarity of the formulation significantly influenced skin permeation of diclofenac. Transdermal delivery from emulgels, prepared from different salts, was similar. DNa formulations, however, unexpectedly displayed slightly better results. It was concluded that the most efficient skin permeation of diclofenac was achieved from formulations with polarities higher than that of the formulations optimised towards the SC, possibly due to the higher polarity of the hydrophilic formulations that created a stronger driving force to push the lipophilic diclofenac (Fini et al., 1999:164) out of the formulation and into the skin (Abbott, 2012:218; Naik et al., 222:319). The outcomes of this study supported the delivery gap principle,
which states that a small SDG (< 1) facilitates effective formulation and transdermal delivery of an API (JW Solutions, 2013).

Future prospects for further investigation with extension of this study would include:

- Formulating diclofenac into another dosage form, such as an ointment or cream.
- Optimising all formulations at different polarity indexes towards the SC, using FFE™.
- Preparing formulations with higher polarity variances.
- Employing and evaluating a different API in emulgel form.
- Preparing formulations with different SDG values, including ≥ 1.
REFERENCES


APPENDIX A

VALIDATION OF AN HPLC ANALYTICAL METHOD FOR THE
DETERMINATION OF DICLOFENAC CONCENTRATION

A.1 PURPOSE OF THE VALIDATION

An HPLC method for analysis of diclofenac salts had been developed and validated previously by Prof. Jan L. du Preez at the Analytical Technology Laboratory, North-West University, Potchefstroom Campus, South Africa, to ensure analytical method sensitivity and reliability when the amount of the API in study samples is quantified (Steyn, 2010:69-111). Revalidation of this existing method was performed and the scope extended to include the assay of formulated products and test samples generated during diffusion and membrane studies.

A.2 CHROMATOGRAPHIC CONDITIONS

Analytical instrument: An Agilent® 1200 Series HPLC system (Chemetrix, Midrand, South Africa) was used for analysis. The instrument was equipped with an Agilent® 1200 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, reverse phase C₁₈ Venusil® XBP column (5 μm, 100Å, 4.6 x 150 mm) (Stargate Scientific, Roodepoort, South Africa) was used.

Mobile phase: The mobile phase consisted of a filtered mixture of acetonitrile/water/acetic acid (70/29/1 v/v).

Solvent: Methanol or PBS (pH 7.4)

Flow rate: 1.0 ml/min

Injection volume: 25 μl

Retention volume: Approximately 4.5 min

Run time: 6.5 min with a post run time of 1 min

Detection wavelength: UV at 275 nm
A diclofenac-containing emulgel and placebo formulation were prepared (see Section B.3). The placebo gel was added to nine 100 ml volumetric flasks (0.8 g, 1.0 g and 1.2 g gel in three flasks each). DNa (240 mg) was accurately weighed into a 100 ml volumetric flask and filled up to volume with methanol (producing a 2.4 mg/ml solution). For the preparation of samples, 4 ml of this solution was added to each of the volumetric flasks containing 0.8 g of placebo gel. Similarly, 5 ml of the solution was added to the flasks containing 1.0 g of placebo gel and 6 ml to each containing 1.2 g of placebo gel. All nine flasks were filled up to volume with methanol. This procedure was repeated for the diclofenac-containing formulation. A standard solution was prepared by transferring 5 ml of the 2.4 mg/ml solution into a 100 ml volumetric flask and filling up to volume with methanol.
A.4 VALIDATION PARAMETERS

A.4.1 LINEARITY

The linearity of an analytical method is a measure of how well (within a specified range) a calibration plot of response against concentration approximates a straight line (ICH, 2005:5). The data is best described by the linear equation (Equation A.1), where $y$ is the peak area (response), $m$ is the slope, $x$ is the concentration and $c$ is the $y$-intercept (ICH, 2005:5; Snyder et al., 1997:644).

$$y = mx + c$$  

Equation A.1

![Linear regression curve of diclofenac standard solutions.](image)

Figure A.1: Linear regression curve of diclofenac standard solutions.

The linearity of diclofenac was determined by performing linear regression analysis on the plot of the peak areas against concentration (µg/ml). A minimum of five different concentrations is recommended to confirm linearity (ICH, 2005:8; Snyder et al., 1997:694).

Figure A.1 and Table A.1 illustrate the linearity data of diclofenac. A generally acceptable linear correlation coefficient ($R^2$) is above 0.999 (Snyder et al., 1997:691). Diclofenac demonstrated an $R^2$ value of 1, indicative of a perfectly positive linear relationship (Dawson & Trapp, 2001:49).
Table A.1: Peak area values of diclofenac

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Peak area 1</th>
<th>Peak area 2</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0248</td>
<td>1.256</td>
<td>1.387</td>
<td>1.3</td>
</tr>
<tr>
<td>0.0495</td>
<td>2.322</td>
<td>2.297</td>
<td>2.3</td>
</tr>
<tr>
<td>0.0991</td>
<td>4.151</td>
<td>4.307</td>
<td>4.2</td>
</tr>
<tr>
<td>0.1486</td>
<td>4.400</td>
<td>5.055</td>
<td>4.7</td>
</tr>
<tr>
<td>0.1982</td>
<td>5.100</td>
<td>5.054</td>
<td>5.1</td>
</tr>
<tr>
<td>0.2477</td>
<td>4.603</td>
<td>4.939</td>
<td>4.8</td>
</tr>
<tr>
<td>0.4954</td>
<td>25.151</td>
<td>24.798</td>
<td>25.0</td>
</tr>
<tr>
<td>0.9908</td>
<td>50.106</td>
<td>49.512</td>
<td>49.8</td>
</tr>
<tr>
<td>1.4862</td>
<td>73.279</td>
<td>73.826</td>
<td>73.6</td>
</tr>
<tr>
<td>1.9816</td>
<td>96.718</td>
<td>97.751</td>
<td>97.2</td>
</tr>
<tr>
<td>2.4770</td>
<td>120.095</td>
<td>120.087</td>
<td>120.1</td>
</tr>
<tr>
<td>4.9540</td>
<td>252.448</td>
<td>251.000</td>
<td>251.7</td>
</tr>
<tr>
<td>9.9080</td>
<td>505.846</td>
<td>504.833</td>
<td>505.3</td>
</tr>
<tr>
<td>14.8620</td>
<td>759.493</td>
<td>758.523</td>
<td>759.0</td>
</tr>
<tr>
<td>19.8160</td>
<td>1013.437</td>
<td>1013.410</td>
<td>1013.4</td>
</tr>
<tr>
<td>24.7700</td>
<td>1266.473</td>
<td>1266.345</td>
<td>1266.4</td>
</tr>
<tr>
<td>49.5400</td>
<td>2521.027</td>
<td>2517.503</td>
<td>2519.3</td>
</tr>
<tr>
<td>99.0800</td>
<td>5016.940</td>
<td>5020.073</td>
<td>5018.5</td>
</tr>
<tr>
<td>148.6200</td>
<td>7517.913</td>
<td>7528.725</td>
<td>7523.3</td>
</tr>
<tr>
<td>198.1600</td>
<td>10042.000</td>
<td>10037.200</td>
<td>10039.6</td>
</tr>
<tr>
<td>247.7000</td>
<td>12516.900</td>
<td>12499.000</td>
<td>12508.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R squared</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.999548</td>
<td>-55.51567</td>
<td>145.8177</td>
</tr>
</tbody>
</table>

A.4.2 ACCURACY

The accuracy of an analytical procedure is defined as the closeness of agreement between the measured value and the true value (ICH, 2005:4; Snyder et al., 1997:687). According to the ICH (1997:10) and APVMA (2004:5), at least nine determinations, with a minimum of three concentrations (80%, 100% and 120%) should be used to ascertain accuracy.

Table A.2 summarises the accuracy results of diclofenac. The mean percentage recovery should be within the range of 98 – 102% (APVMA, 2004:5). Diclofenac recovery ranged between 100.8 - 101.8%, with a mean percentage recovery of 101.2% and did it thus comply with the said requirements.
Table A.2: Accuracy parameters of diclofenac

<table>
<thead>
<tr>
<th>Concentration spiked (ug/ml)</th>
<th>Peak area 1</th>
<th>Peak area 2</th>
<th>Mean</th>
<th>Recovery (ug/ml)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>96.3</td>
<td>4950.00</td>
<td>4940.00</td>
<td>4945</td>
<td>97.4</td>
<td>101.2</td>
</tr>
<tr>
<td>96.3</td>
<td>4970.90</td>
<td>4973.40</td>
<td>4972</td>
<td>98.0</td>
<td>101.8</td>
</tr>
<tr>
<td>96.3</td>
<td>4955.80</td>
<td>4927.80</td>
<td>4942</td>
<td>97.4</td>
<td>101.1</td>
</tr>
<tr>
<td>120.3</td>
<td>6181.76</td>
<td>6179.00</td>
<td>6180</td>
<td>121.8</td>
<td>101.2</td>
</tr>
<tr>
<td>120.3</td>
<td>6159.00</td>
<td>6154.80</td>
<td>6157</td>
<td>121.3</td>
<td>100.8</td>
</tr>
<tr>
<td>120.3</td>
<td>6164.00</td>
<td>6156.70</td>
<td>6160</td>
<td>121.4</td>
<td>100.9</td>
</tr>
<tr>
<td>144.4</td>
<td>7393.80</td>
<td>7416.7</td>
<td>7405</td>
<td>145.9</td>
<td>101.0</td>
</tr>
<tr>
<td>144.4</td>
<td>7432.40</td>
<td>7427.6</td>
<td>7430</td>
<td>146.4</td>
<td>101.4</td>
</tr>
<tr>
<td>144.4</td>
<td>7430.30</td>
<td>7443.7</td>
<td>7437</td>
<td>146.5</td>
<td>101.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD*</th>
<th>%RSD**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>101.2</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*SD refers to standard deviation
**%RSD refers to relative standard deviation

A.4.3 PRECISION

The precision of an analytical method is defined as the closeness of agreement between individual test results, following multiple sampling of the same homogeneous sample. It is often considered at three levels, i.e. repeatability, intermediate precision and reproducibility (ICH, 2005:4).

Tables A.3, A.4 and A.5 summarise the repeatability, intermediate precision and reproducibility of diclofenac, respectively. A percentage relative standard deviation (%RSD) of 2% or less is recommended to confirm precision (APVMA, 2004:5).

A.4.3.1 Repeatability (intra-day precision)

Repeatability expresses the precision of a method under the same operating conditions over a short period of time (ICH, 2005:5; Snyder et al., 1997:690). The %RSD obtained during this study for this HPLC method was 0.58, which confirmed its repeatability.
Table A.3: Repeatability parameters of diclofenac

<table>
<thead>
<tr>
<th>Mass (g)</th>
<th>Peak area 1</th>
<th>Peak area 2</th>
<th>Mean</th>
<th>Concentration (µg/ml)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8025</td>
<td>5045.6</td>
<td>5055.2</td>
<td>5050</td>
<td>85.6</td>
<td>106.6</td>
</tr>
<tr>
<td>0.8008</td>
<td>5026.7</td>
<td>5020.9</td>
<td>5024</td>
<td>85.1</td>
<td>106.3</td>
</tr>
<tr>
<td>0.8029</td>
<td>5035.2</td>
<td>5018.2</td>
<td>5027</td>
<td>85.2</td>
<td>106.1</td>
</tr>
<tr>
<td>1.0015</td>
<td>6330.6</td>
<td>6340.5</td>
<td>6336</td>
<td>107.3</td>
<td>107.2</td>
</tr>
<tr>
<td>1.0016</td>
<td>6250.9</td>
<td>6232.5</td>
<td>6242</td>
<td>105.7</td>
<td>105.6</td>
</tr>
<tr>
<td>1.0016</td>
<td>6245.3</td>
<td>6243.8</td>
<td>6245</td>
<td>105.8</td>
<td>105.6</td>
</tr>
<tr>
<td>1.2033</td>
<td>7535.7</td>
<td>7641.7</td>
<td>7589</td>
<td>128.6</td>
<td>106.8</td>
</tr>
<tr>
<td>1.2037</td>
<td>7622.0</td>
<td>7625.1</td>
<td>7624</td>
<td>129.2</td>
<td>107.3</td>
</tr>
<tr>
<td>1.2032</td>
<td>7595.8</td>
<td>7610.5</td>
<td>7603</td>
<td>128.8</td>
<td>107.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>106.51</td>
<td></td>
</tr>
<tr>
<td>SD*</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>%RSD**</td>
<td>0.58</td>
<td></td>
</tr>
</tbody>
</table>

*A SD refers to standard deviation
**%RSD refers to relative standard deviation

A.4.3.2 Intermediate precision

Intermediate precision is the agreement of results, when the same method is applied repeatedly in the same laboratory, while other factors, such as days, analysts and equipment vary (ICH, 2005:5; Snyder et al., 1997:690). A %RSD of 0.33 confirmed intermediate precision.
Table A.4: Sample stability parameters of diclofenac

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Peak Area</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2441</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>2435</td>
<td>99.8</td>
</tr>
<tr>
<td>2</td>
<td>2445</td>
<td>100.2</td>
</tr>
<tr>
<td>3</td>
<td>2444</td>
<td>100.1</td>
</tr>
<tr>
<td>4</td>
<td>2448</td>
<td>100.3</td>
</tr>
<tr>
<td>5</td>
<td>2453</td>
<td>100.5</td>
</tr>
<tr>
<td>6</td>
<td>2459</td>
<td>100.7</td>
</tr>
<tr>
<td>7</td>
<td>2462</td>
<td>100.9</td>
</tr>
<tr>
<td>8</td>
<td>2464</td>
<td>100.9</td>
</tr>
<tr>
<td>9</td>
<td>2447</td>
<td>100.2</td>
</tr>
<tr>
<td>10</td>
<td>2443</td>
<td>100.1</td>
</tr>
<tr>
<td>11</td>
<td>2445</td>
<td>100.2</td>
</tr>
<tr>
<td>12</td>
<td>2433</td>
<td>99.7</td>
</tr>
<tr>
<td>13</td>
<td>2443</td>
<td>100.1</td>
</tr>
<tr>
<td>14</td>
<td>2449</td>
<td>100.3</td>
</tr>
<tr>
<td>15</td>
<td>2448</td>
<td>100.3</td>
</tr>
<tr>
<td>16</td>
<td>2436</td>
<td>99.8</td>
</tr>
<tr>
<td>17</td>
<td>2447</td>
<td>100.2</td>
</tr>
<tr>
<td>18</td>
<td>2451</td>
<td>100.4</td>
</tr>
<tr>
<td>19</td>
<td>2456</td>
<td>100.6</td>
</tr>
<tr>
<td>Mean</td>
<td>2447.7</td>
<td>100.3</td>
</tr>
<tr>
<td>SD*</td>
<td>8.04</td>
<td>0.33</td>
</tr>
<tr>
<td>%RSD**</td>
<td>0.33</td>
<td>0.33</td>
</tr>
</tbody>
</table>

*SD refers to standard deviation
**%RSD refers to relative standard deviation

A.4.3.3 Reproducibility (inter-day precision)

Reproducibility expresses the precision of a method between different laboratories. It is commonly determined through collaborative studies, or method transfer experiments (ICH, 2005:5; Snyder et al., 1997:690). Reproducibility was confirmed by a %RSD value of 0.42.
Table A.5: Reproducibility parameters of diclofenac

<table>
<thead>
<tr>
<th>Mass (g)</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Between days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>107.2</td>
<td>105.7</td>
<td>105.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>105.6</td>
<td>104.5</td>
<td>104.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>105.6</td>
<td>105.3</td>
<td>105.1</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>106.13</td>
<td>105.15</td>
<td>105.21</td>
<td>105.50</td>
</tr>
<tr>
<td>SD*</td>
<td>0.74</td>
<td>0.48</td>
<td>0.28</td>
<td>0.45</td>
</tr>
<tr>
<td>%RSD**</td>
<td>0.70</td>
<td>0.46</td>
<td>0.27</td>
<td>0.42</td>
</tr>
</tbody>
</table>

*SD refers to standard deviation  
**%RSD refers to relative standard deviation

A.4.4 SYSTEM REPEATABILITY (SUITABILITY)

System suitability tests are conducted prior to sample analyses to determine whether the HPLC system and method are able to provide accurate and precise results (Snyder et al., 1997:705). A %RSD of 2% or less is required for peak area and retention time (Du Preez, 2010:8). Values obtained were 0.41% and 0.445% for peak area and retention time, respectively, which were indicative of the standards being met.

Table A.6: Variations in response (%RSD) of the detection system with regard to peak area and retention time of diclofenac

<table>
<thead>
<tr>
<th>Injection</th>
<th>Peak area</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1171.0</td>
<td>4.427</td>
</tr>
<tr>
<td>2</td>
<td>1168.7</td>
<td>4.418</td>
</tr>
<tr>
<td>3</td>
<td>1159.8</td>
<td>4.409</td>
</tr>
<tr>
<td>4</td>
<td>1164.0</td>
<td>4.413</td>
</tr>
<tr>
<td>5</td>
<td>1164.3</td>
<td>4.445</td>
</tr>
<tr>
<td>6</td>
<td>1156.8</td>
<td>4.465</td>
</tr>
<tr>
<td>Mean</td>
<td>1164</td>
<td>4.430</td>
</tr>
<tr>
<td>SD*</td>
<td>4.83</td>
<td>0.020</td>
</tr>
<tr>
<td>%RSD**</td>
<td>0.41</td>
<td>0.445</td>
</tr>
</tbody>
</table>

*SD refers to standard deviation  
**%RSD refers to relative standard deviation

A.5 CONCLUSION

The HPLC method was validated and found to be adequately sensitive and reliable for the determination of the API, diclofenac, in emulgel formulations, as well as in membrane and diffusion study samples.
REFERENCES


APPENDIX B

FORMULATION OF AN EMULGEL
WITH DICLOFENAC AS ACTIVE INGREDIENT

B.1 INTRODUCTION

The objective of this study was to formulate preparations with different polarities, each containing DNa, DDEA or DHEP. Each of these diclofenac salts was formulated into three emulgels, i.e. preparations optimised towards the SC (enhancing drug delivery into this layer and deeper tissues), formulations with increased polarities, and formulations with decreased polarities, compared to the optimised preparation. The SDG values of all nine formulations as calculated were below 1, hence predicting effective formulation and transdermal delivery (JW Solutions, 2013). When using the FFE™ software to formulate an API into a product, optimising towards the SC is one of the three options that JW Solutions offer (Section 2.5.2). These new emulgel formulations were then prepared and exposed to tests to determine the release and transdermal delivery of diclofenac.

B.2 FORMULATION OF AN EMULGEL

B.2.1 MAIN INGREDIENTS OF AN EMULGEL

Table B.1: Typical formula of an emulsion type gel, i.e. emulgel (Mitsui, 1997:353)

<table>
<thead>
<tr>
<th>Component</th>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil component</td>
<td>Liquid paraffin</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>Glycerol tri-2-ethylhexanoate</td>
<td>50.0</td>
</tr>
<tr>
<td>Humectant</td>
<td>Sorbitol</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Poly-ethylene glycol (PEG) 400</td>
<td>5.0</td>
</tr>
<tr>
<td>Surfactant</td>
<td>Acylmethyl taurate</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>POE octyl dodecyl alcohol ether</td>
<td>10.0</td>
</tr>
<tr>
<td>Purified water</td>
<td>Purified water</td>
<td>8.0</td>
</tr>
</tbody>
</table>

B.2.2 GENERAL METHOD FOR MANUFACTURING A GEL

Components of the formulation were mixed uniformly and air bubbles removed. The gel end product was clear. Special attention was given to the dissolution and homogeneity of the ground substances (Mitsui, 1997:353).
B.3 METHODS AND MATERIALS

B.3.1 DETERMINATION OF THE AMOUNTS OF DICLOFENAC SALTS EQUAL TO 1 g OF DICLOFENAC FREE ACID

A salt is formed when two ionisable components (acidic and basic in relation to each other) are chemically bound to improve solubility of the parent molecule. In solution, dissociation of these moieties from each other occurs (Wells & Aulton, 2007:340). In order to compare results with regards to the release and transdermal delivery of diclofenac from formulations (prepared by using different diclofenac salts as API), the amount of diclofenac free acid following dissolution has to be equal in all preparations.

The molecular weight of the respective diclofenac salts and the free acid was thus used to calculate the amount of every salt that is equivalent to 1 g diclofenac of free acid.

Table B.2: Quantity of diclofenac salts equivalent to 1 g of diclofenac free acid

<table>
<thead>
<tr>
<th>Diclofenac salt</th>
<th>Quantity in grams (g) equivalent to 1 g of diclofenac free acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNa</td>
<td>1.074 g</td>
</tr>
<tr>
<td>DDEA</td>
<td>1.274 g</td>
</tr>
<tr>
<td>DHEP</td>
<td>1.375 g</td>
</tr>
</tbody>
</table>

B.3.2 INGREDIENTS FOR THE PREPARATION OF A FORMULATION OPTIMISED TOWARDS THE STRATUM CORNEUM CONTAINING DICLOFENAC

Table B.3: Quantity of ingredients in emulgel formulations optimised towards the SC containing diclofenac

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac (free acid)</td>
<td>1.0 g</td>
</tr>
<tr>
<td>DMI</td>
<td>18.3 ml</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.9 ml</td>
</tr>
<tr>
<td>TEA</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>PEG400</td>
<td>9.9 ml</td>
</tr>
<tr>
<td>Carbopol® Ultrez 20*</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Water (ad)</td>
<td>100.0 g</td>
</tr>
</tbody>
</table>

*Acrylate crosspolymer (Lubrizol, Brussels, Belgium)

The oily phase (DMI, PEG400 and ethanol) comprised ~30% of the total formulation.
B.3.3 INGREDIENTS FOR THE PREPARATION OF A MORE HYDROPHILIC FORMULATION CONTAINING DICLOFENAC

Table B.4: Quantity of ingredients in more hydrophilic emulgel formulations containing diclofenac

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac (free acid)</td>
<td>1.0 g</td>
</tr>
<tr>
<td>DMI</td>
<td>3.5 ml</td>
</tr>
<tr>
<td>Ethanol</td>
<td>6.3 ml</td>
</tr>
<tr>
<td>TEA</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>PEG400</td>
<td>4.2 ml</td>
</tr>
<tr>
<td>Carbopol® Ultrez 20*</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Water (ad)</td>
<td>100.0 g</td>
</tr>
</tbody>
</table>

*Acrylate crosspolymer (Lubrizol, Brussels, Belgium)

The oily phase (DMI, PEG400 and ethanol) comprised ~15% of the total formulation.

B.3.4 INGREDIENTS FOR THE PREPARATION OF A MORE LIPOPHILIC FORMULATION CONTAINING DICLOFENAC

Table B.5: Quantity of ingredients in more lipophilic emulgel formulations containing diclofenac

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac (free acid)</td>
<td>1.0 g</td>
</tr>
<tr>
<td>DMI</td>
<td>11.0 ml</td>
</tr>
<tr>
<td>Ethanol</td>
<td>19.8 ml</td>
</tr>
<tr>
<td>TEA</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>PEG400</td>
<td>13.2 ml</td>
</tr>
<tr>
<td>Carbopol® Ultrez 20*</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Water (ad)</td>
<td>100.0 g</td>
</tr>
</tbody>
</table>

*Acrylate crosspolymer (Lubrizol, Brussels, Belgium)

The oily phase (DMI, PEG400 and ethanol) comprised ~45% of the total formulation.

B.3.5 METHOD FOR THE PREPARATION OF A FORMULATION CONTAINING DICLOFENAC

Ingredients, of which amounts were indicated in volume, were measured using a calibrated pipette. These volumes were subsequently weighed, using a Sartorius or Mettler Toledo balance. Either of these two balances was employed for the measurement of the remaining ingredients. Carbopol® Ultrez 20 was measured and added to HPLC water and left in order for proper wetting of the Carbopol® to occur. During the preparation of formulations optimised
towards the SC, and formulations with higher and lower polarities than the optimised formulation, this amount of water was ± 50 ml, ± 60 ml and ± 40 ml, respectively. The DMI, ethanol, TEA and PEG400 were added together to prepare the oily phase. The diclofenac salt was then added to the oily phase and the mixture placed on an ultrasonic bath for a few minutes to enhance dissolution of the salt. As soon as the salt dissolution was complete, the oily phase was removed from the ultrasonic bath and the Carbopol® in water was added to the solution. The final weight of each formulation had to be 100 g and hence the required remaining weight was calculated (by subtracting the weights of all measured ingredients from the total 100 g) and the appropriate amount of HPLC water subsequently added. Every formulation was then mixed using a homogeniser, until a smooth gel was formed. The pH of each preparation was determined using a pH meter and adjusted to a pH between 7.20 – 7.60 by adding 2 M of H₃PO₄, or 2 M of NaOH, as required. The removal of air bubbles was attempted, but unsuccessful. Gels were stored in a refrigerator for two days to allow proper dissolution of all the ingredients and to attain clear emulgel formulations.

B.4 SUMMARY

A gel optimised towards the SC (O), a gel with a higher polarity (H) and a gel with a lower polarity than the optimised gel (L) were prepared, using DNA, DDEA and DHEP, hence nine formulations in total. The three formulations with an increased polarity were opaque. The remaining six formulations (optimised towards the SC and decreased polarity) were clear and colourless.
REFERENCES


APPENDIX C
FRANZ CELL DIFFUSION STUDIES

C.1 INTRODUCTION
Franz cells (vertical diffusion cells) have been employed over the past number of decades for the \textit{in vitro} testing of drug release from semi-solid dosage forms. Even today, it is preferred over any other method designed for this purpose (Copley Scientific, 2013:60).

The receptor compartment is filled with a solution that is maintained at 37 °C (in order to mimic body temperature) (Silverthorn, 2004:715) and separated from the upper donor compartment by a membrane, or skin as passage for diffusion. The donor compartment is filled with the relevant semi-solid formulation. The receptor solution is extracted at predetermined intervals and analysed by means of HPLC, or similar analytical technique (Copley Scientific, 2013:60).

During this study, membrane diffusion studies were conducted to determine the release of diclofenac from the emulgel dosage form. Transdermal diffusion studies followed to determine whether topical and transdermal delivery had been achieved.

C.2 METHODS

C.2.1 HPLC ANALYSIS OF DICLOFENAC
An HPLC method for analysis of diclofenac salts had previously been developed by Prof. Jan L. du Preez at the Analytical Technology Laboratory, North-West University, Potchefstroom Campus, South Africa. Revalidation during this study was performed to prove its suitability and reproducibility for emulgel formulations and test samples collected during diffusion and membrane studies. Analysis of samples collected from \textit{in vitro} and tape stripping experiments were performed using HPLC.

An Agilent® 1200 Series HPLC system (Chemetrix, Midrand, South Africa) was used for analysis. The instrument was equipped with an Agilent® 1200 pump, diode array detector, autosampler injection mechanism and Chemstation Rev. A.06.02 software for data acquisition and analysis. A high performance, silica based, reverse phase C$_{18}$ Venusil® XBP column (5 μm, 100 Å, 4.6 x 150 mm) (Stargate Scientific, Roodepoort, South Africa) was used. Mobile phase was prepared using HPLC grade acetonitrile, HPLC grade water and glacial acetic acid in a 70:29:1 ratio. The operating flow rate was 1.0 ml/min and the injection volume 25 μl. Retention time of diclofenac was approximately 4.5 min and the run time was set at 6.5 min with a post
run time of 1 min. Methanol was used as solvent. Analyses were performed in a controlled laboratory environment at 25 °C.

C.2.2 AQUEOUS SOLUBILITY

Equilibrium between excesses of each diclofenac salt and PBS was established (pH adjusted to 7.4 as per the pH of blood) in order to obtain saturated solutions of the model compounds. Test tubes, containing these solutions, were placed in a shaker water bath with temperature maintained at 32 °C. After a period of roughly 24 hours, the solutions were centrifuged. Supernatant (1 ml) was diluted to 7 ml with methanol and analysed using HPLC. The experiment was performed in triplicate.

C.2.3 OCTANOL BUFFER DISTRIBUTION COEFFICIENT (LOG D)

Log P is a routine measure of lipophilicity and describes the partition coefficient of unionised molecules between aqueous and lipophilic phases, usually water and octanol. Ionisable groups, however, are likely to be charged at physiological pH of 7.4. Log D (distribution constant), therefore, describes the lipophilicity of a molecule more accurately. Instead of using water, the aqueous phase is buffered to a particular pH. Because log D is pH dependent, the pH at which log D is measured must be specified. Log D, at pH 7.4, the physiological pH of blood serum, is of particular interest (CMC, 2012).

A mixture of equal volumes of n-octanol and PBS (pH 7.4) was left to equilibrate over a period of at least 24 hours, for co-saturation of the two phases to be reached. Solutions of each model compound were prepared using the pre-saturated n-octanol phase as solvent. An equal volume of pre-saturated PBS was added to each of these solutions. The mixtures were placed into and left in a shaker water bath overnight and centrifuged thereafter. After centrifugation, the concentrations of the API in the separated phases were determined on HPLC. The partition coefficients were calculated as the ratio of API concentration in the octanol phase to that in the buffer phase. These experiments were performed in triplicate.

C.2.4 SKIN PREPARATION

Full-thickness, abdominal skin was collected from plastic surgeons, following abdominoplastic surgery on female, Caucasian patients. Ethical approval was obtained from the Research Ethics Committee of the North-West University (reference number: NWU-00114-11-A5). The research was conducted in a laboratory (type 2) that is approved for transdermal studies and is equipped for working with human skin. Written informed consent was obtained from every patient for use of their skin for research purposes. Patient information remains confidential. After completion of the experiments, all biological waste was legally disposed of, according to
prescribed methods. The skin was kept in a freezer at -20 °C for not more than 6 months. Dermatomed skin with a thickness of 400 µm (containing SC, viable epidermis and upper dermis (ED)) was cut into circles and placed on Whatman® filter paper to dry, after which it was wrapped in aluminium foil and stored in a freezer at -20 °C. Before commencing the diffusion studies, the frozen skin samples were thawed, examined visually for defects and mounted onto the diffusion apparatus.

C.2.5 PREPARATION OF PHOSPHATE BUFFER SOLUTION (pH 7.4)
Potassium dihydrogen orthophosphate (KH₂PO₄, 6.805 g) and sodium hydroxide (NaOH, 1.537 g) were measured and dissolved into 250 ml and 393.4 ml HPLC water, respectively. The NaOH solution was slowly added to the KH₂PO₄ solution until a pH of 7.4 was reached. 10% NaOH or 10% KH₂PO₄ was utilised to further adjust the pH, as required (BP, 2013).

C.2.6 MEMBRANE DIFFUSION STUDIES
During execution of the membrane diffusion studies, vertical Franz cells were used. FP Vericel® PVDF membrane filters (25 mm diameter, 0.45 µm pore size) (Pall Corportion, Mexico, USA) were mounted onto the outer half of the diffusion cells. While PBS (pH 7.4) was used to fill the receptor compartment, the formulation containing the diclofenac was added to the donor compartment. A temperature of 32 °C was maintained for the cell system. In order to ensure sink conditions throughout the experiment, the entire receptor volume was extracted every hour for up to 6 hours and the receptor compartment refilled with PBS (pH 7.4) at a temperature of ~37 °C. Samples were analysed using HPLC (see Section C.2.1). The concentration of API that had permeated the membrane filter into the receiver fluid, and the release rate of API from the formulation were subsequently determined.

C.2.7 TRANSDERMAL DIFFUSION STUDIES
The method discussed in Section C.2.6 was utilised to perform the in vitro skin permeation experiments. The epidermal layer of the skin was mounted onto the outer half of the diffusion cells with the SC facing the upper donor compartment. Samples from the receptor phase were withdrawn at predetermined time intervals (every 20 min for up to 2 hours, followed by 2 hourly extractions for up to 12 hours) and replaced with fresh PBS (pH 7.4, 37 °C). Again, samples were analysed, using HPLC, while the API concentration that had permeated the skin into the receiver fluid was determined.
C.2.8 TAPE STRIPPING

This technique was employed for analysing the amounts of API present in the SCE and ED after completion of the 12 hour diffusion studies. Franz cells were carefully dismantled and each piece of skin pinned onto Parafilm®, attached to a solid surface. The skin was then dabbed dry with paper towel to remove any remaining formulation. 3M Scotch® Magic™ tape was cut into pieces the size of the diffusion area and placed onto these areas. Due to possible contamination with formulation still left on the skin, the first tape strip per skin sample was disposed of. Each skin sample was stripped until the SCE over the diffusion area was completely removed from the underlying epidermis (the white epidermis of the diffusion area visibly exposed). In addition to the obvious pieces of SC, these strips contained traces of the API, diclofenac (Lademann et al., 2008:318). These strips were placed in a polytop filled with 5 ml of methanol, per skin sample. The remaining skin (the ED of the diffusion area and the rest of the skin sample not exposed to formulation) were cut into smaller pieces and each sample’s cuttings placed into a vial containing 5 ml of methanol. Polytops (those containing tape strips and SCE, as well as those containing skin pieces) were left overnight at 4 °C in order for the API to dissolve in the methanol. The amount of diclofenac that was extracted from the SCE and ED were then analysed by means of HPLC.

C.2.9 DATA ANALYSIS

The cumulative concentration of API that permeated the dermatomed skin (referring to transdermal diffusion studies) or was released from a gel (for membrane diffusion studies) was plotted against time. The slope of this curve between 4 - 12 hours (for transdermal diffusion studies) and between 3 - 6 hours (for membrane release studies) represented the flux of diclofenac acid. For transdermal diffusion studies, the yield of every Franz cell sample was calculated as a percentage of the volume and strength of formulation in the donor compartment. Transdermal diffusion studies were conducted over a period of 12 hours and the yield was also calculated at the end of this period. This was repeated for membrane diffusion studies after 6 hours to determine possible API release.

C.3 RESULTS AND DISCUSSION

C.3.1 AQUEOUS SOLUBILITY

According to Naik et al. (2000:319), an aqueous solubility above 1 mg/ml is required for molecules to passively cross the skin. Aqueous solubility was determined for DNa, DDEA and DHEP by utilising PBS (pH 7.4) at 37 °C. The corresponding values obtained were 11.4 mg/ml, 8.0 mg/ml and 11.9 mg/ml, all indicative of effortless percutaneous delivery.
C.3.2 DISTRIBUTION COEFFICIENT (LOG D)

Compounds with a log octanol/water partition coefficient of 1 – 3 are expected to readily travel across the skin (Naik et al., 2000:319; Walters, 2007:1312). Log D (pH 7.4) determinations for DNa, DDEA and DHEP were performed and values established at 1.270 (DNa), 1.291 (DDEA) and 1.285 (DHEP). According to these outcomes, diclofenac (topically applied as a salt in a suitable vehicle) should therefore permeate transdermally without the aid of radical interventions.

C.3.3 MEMBRANE DIFFUSION STUDIES

Table C.1: Data generated during membrane diffusion studies

<table>
<thead>
<tr>
<th>Diclofenac salt</th>
<th>Average percentage released after 6 h</th>
<th>Average cumulative concentration after 6 h (µg/cm²)</th>
<th>Median cumulative concentration after 6 h (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNa-O</td>
<td>8.23 ± 0.94</td>
<td>1530.70 ± 175.34</td>
<td>1554.66</td>
</tr>
<tr>
<td>DNa-H</td>
<td>8.38 ± 0.57</td>
<td>1557.39 ± 105.59</td>
<td>1574.86</td>
</tr>
<tr>
<td>DNa-L</td>
<td>2.98 ± 0.48</td>
<td>553.89 ± 89.27</td>
<td>560.78</td>
</tr>
<tr>
<td>DDEA-O</td>
<td>8.06 ± 0.24</td>
<td>1498.42 ± 230.08</td>
<td>1577.01</td>
</tr>
<tr>
<td>DDEA-H</td>
<td>7.90 ± 1.08</td>
<td>1468.05 ± 200.74</td>
<td>1403.07</td>
</tr>
<tr>
<td>DDEA-L</td>
<td>3.48 ± 0.60</td>
<td>647.64 ± 110.61</td>
<td>628.15</td>
</tr>
<tr>
<td>DHEP-O</td>
<td>8.01 ± 1.05</td>
<td>1488.75 ± 195.68</td>
<td>1489.50</td>
</tr>
<tr>
<td>DHEP-H</td>
<td>8.23 ± 0.99</td>
<td>1530.72 ± 183.91</td>
<td>1541.25</td>
</tr>
<tr>
<td>DHEP-L</td>
<td>3.76 ± 0.47</td>
<td>699.75 ± 86.79</td>
<td>662.86</td>
</tr>
</tbody>
</table>

Results confirmed that diclofenac was indeed released from all nine emulgels. Considering the separate groups of gels being formulated, using the three different diclofenac salts (DNa, DDEA and DHEP), the more hydrophilic emulgels (H gels) and the emulgels optimised towards the SC (O gels) resulted in the highest percentage of diclofenac released after 6 hours. There was only a slight difference between the percentages released from these two types of formulations. In the DNa and DHEP groups, the more hydrophilic gels released the highest percentage of diclofenac. The DDEA-O gel, however, achieved the highest percentage of diclofenac release in that group. Yet, in all three groups, O and H gels released more than twice the percentage of diclofenac than the more lipophilic formulation (L gels). This may have been, because both the free acid (lipophilic) and salt forms of diclofenac have an affinity towards the oily phase (Fini et al., 1999; Maitani et al., 1994). Diclofenac, therefore, favours the oily composition of the more lipophilic formulation over the more aqueous environment of the receptor solution, whether in salt form or having changed to the free acid (Abbott, 2012:218). The diclofenac would hence not leave the formulation readily in order to cross the membrane filter.
A comparison of formulations with similar polarities and different diclofenac salt groups showed no pattern. In the O group, the highest percentage of diclofenac release was from the DNa-O, followed by DDEA-O and DHEP-O. A comparison of the more hydrophilic gels showed that DNa-H released the highest percentage of diclofenac, followed by DHEP-H and DDEA-H. Finally, measurement of the more lipophilic gels indicated that the percentage of diclofenac released was in the order of highest to lowest, DHEP-L, DDEA-L and DNa-L.

The median flux value is the middle value, the point at which half the flux values are smaller and half are larger than the mean. The average (mean) is calculated by adding the values to obtain their total and subsequently dividing it by the number of values (Dawson & Trapp, 2001:28-29). There was no significant difference between average cumulative concentrations after 6 hours and median cumulative concentrations after 6 hours, since very few outliers appeared in the data. Since the median is not as sensitive to extreme values as the average, median values, instead of average values are often utilised to achieve a more accurate representation of data (Dawson & Trapp, 2001:30).

During statistical analysis and comparison of the formulations in terms of polarities and diclofenac salts utilised, two-way ANOVA and Tukey tests were conducted. A significant difference between the formulations with different polarities was indicated with a \( P \) value of less than 0.0001 and different Tukey groupings. However, no statistically significant difference between those formulations prepared using different diclofenac salts (\( P \) value = 0.3956), nor the gels with different combinations of polarities and salts utilised during formulation (\( P \) value = 0.3009), was observed.

C.3.4 TRANSDERMAL DIFFUSION STUDIES

Table C.2: Data generated during transdermal diffusion studies

<table>
<thead>
<tr>
<th>Diclofenac salt</th>
<th>Average percentage diffused after 12 h</th>
<th>Average flux (µg/cm(^2).h)</th>
<th>Average cumulative concentration after 12 h (µg/ml)</th>
<th>Median flux (µg/cm(^2).h)</th>
<th>Median cumulative concentration after 12 h (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNa-O</td>
<td>0.03 ± 0.01</td>
<td>0.46 ± 0.10</td>
<td>5.33 ± 1.20</td>
<td>0.43</td>
<td>5.13</td>
</tr>
<tr>
<td>DNa-H</td>
<td>0.09 ± 0.01</td>
<td>1.42 ± 0.20</td>
<td>16.60 ± 2.39</td>
<td>1.34</td>
<td>15.76</td>
</tr>
<tr>
<td>DNa-L</td>
<td>0.01 ± 0.00</td>
<td>0.20 ± 0.04</td>
<td>2.38 ± 0.40</td>
<td>0.19</td>
<td>2.24</td>
</tr>
<tr>
<td>DDEA-O</td>
<td>0.01 ± 0.00</td>
<td>0.18 ± 0.03</td>
<td>2.18 ± 0.30</td>
<td>0.18</td>
<td>2.13</td>
</tr>
<tr>
<td>DDEA-H</td>
<td>0.09 ± 0.02</td>
<td>1.34 ± 0.32</td>
<td>16.05 ± 3.54</td>
<td>1.26</td>
<td>14.95</td>
</tr>
<tr>
<td>DDEA-L</td>
<td>0.01 ± 0.00</td>
<td>0.23 ± 0.04</td>
<td>2.63 ± 0.46</td>
<td>0.24</td>
<td>2.76</td>
</tr>
<tr>
<td>DHEP-O</td>
<td>0.01 ± 0.00</td>
<td>0.20 ± 0.05</td>
<td>2.32 ± 0.59</td>
<td>0.19</td>
<td>2.19</td>
</tr>
<tr>
<td>DHEP-H</td>
<td>0.08 ± 0.02</td>
<td>1.30 ± 0.28</td>
<td>15.18 ± 3.20</td>
<td>1.23</td>
<td>14.25</td>
</tr>
<tr>
<td>DHEP-L</td>
<td>0.01 ± 0.00</td>
<td>0.17 ± 0.05</td>
<td>2.01 ± 0.62</td>
<td>0.16</td>
<td>1.95</td>
</tr>
</tbody>
</table>
The more hydrophilic formulations in every group of gels formulated from a certain diclofenac salt showed that the highest average percentage of diclofenac having diffused after 12 hours, followed by either the more lipophilic gel (as was the case for gels made with DDEA), or those preparations optimised towards the SC. A Tukey test indicated a statistically significant difference between the average percentages of diclofenac having diffused from the H gels and the remaining two types of formulations. Between three and seven times more diclofenac had diffused the dermatomed skin from the more hydrophilic gels, compared to the other two types of formulations. This could have been because these preparations have polarities higher than that of the SC (and are possibly even more hydrophilic than the epidermis), and since the diclofenac acid is lipophilic and the salt form retains affinity for a lipophilic environment, it would prefer leaving the formulation to permeate the fatty SC and underlying epidermis.

No pattern was observed when comparing emulgels with similar polarities and formulated from different diclofenac salts. In the O group gels, the DNa gel showed the highest average percentage of diffused diclofenac, equally followed by the gels prepared with DDEA and DHEP. The average percentage of diffused diclofenac from H and L gels was highest in the DNa and DDEA gels, respectively and lowest for the DHEP gels, in both instances.

According to Wells and Aulton (2007:341), different salt forms of an API can change the dissolution rate and solubility of the compound to varying degrees. This in turn would affect the bioavailability of the API. The different effects that the three different diclofenac salts had on the physicochemical properties of diclofenac acid could, however, not be confirmed during this study.

With only a few outliers recorded in the study outcomes, variation was small. Average flux values therefore did not differ significantly from median flux values. The average cumulative concentration of diclofenac having diffused after 12 hours also did not differ much from the median cumulative diffusion concentration reached after 12 hours. The highest variation between average and median values was observed for group H. Flux values of all nine formulations are presented in Figures C.1 – C.19.
Figure C.1: Box plots of flux values of nine emulgel formulations after topical application, illustrating the median (indicated with a horizontal line in the box) and average flux (indicated with diamond) of diclofenac.
Figure C.2: Average cumulative concentrations of diclofenac per area (µg/cm\(^2\)) for the DNa-O emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).
**Figure C.3:** Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DNa-O emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).
Figure C.4: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DHEP-O emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).
Figure C.5: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DHEP-O emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).
Figure C.6: Average cumulative concentrations of diclofenac per area (µg/cm$^2$) for the DDEA-O emulgel that had permeated the skin between 4 - 12 hours as a function of time ($n = 10$).
Figure C.7: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DDEA-O emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).
Figure C.8: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DNa-H emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).
Figure C.9: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DNa-H emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).
**Figure C.10:** Average cumulative concentrations of diclofenac per area (µg/cm²) for the DHEP-H emulgel that had permeated the skin between 4 - 12 hours as a function of time \((n = 10)\).
Figure C.11: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DHEP-H emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).
Figure C.12: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DDEA-H emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 9).

\[ y = 1.7973x - 6.0698 \]
\[ R^2 = 0.9918 \]
Figure C.13: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DDEA-H emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).
Figure C.14: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DNa-L emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).
Figure C.15: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DNa-L emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).
Figure C.16: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DHEP-L emulgel that had permeated the skin between 4 - 12 hours as a function of time ($n = 9$).
Figure C.17: Cumulative concentrations of diclofenac per area (µg/cm$^2$) for each individual DHEP-L emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).
Figure C.18: Average cumulative concentrations of diclofenac per area (µg/cm²) for the DDEA-L emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

\[ y = 0.2765x - 0.7227 \]
\[ R^2 = 0.9986 \]
Figure C.19: Cumulative concentrations of diclofenac per area (µg/cm²) for each individual DDEA-L emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

A two-way ANOVA test indicated a statistically significant difference among formulations prepared with the three different diclofenac salts ($P$ value = 0.0040), as well as a highly significant difference between formulations with different polarities ($P$ value < 0.0001). No significant difference between gels with different combinations of salts used and different polarities were indicated ($P$ value = 0.0592).

A Tukey test indicated a significant difference between the DNA and DDEA gels, and between the DNA and DHEP gels. No significant difference was observed between the DDEA and DHEP formulations. This test also indicated a significant difference between H and O gels, and between H and L gels, with no significant difference between O and L.
C.3.5 TAPE STRIPPING

Table C.3: Data generated during tape stripping studies

<table>
<thead>
<tr>
<th>Diclofenac salt</th>
<th>Average concentration in SCE (µg/ml)</th>
<th>Average concentration in ED (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNa-O</td>
<td>0.77 ± 0.31</td>
<td>0.25 ± 0.14</td>
</tr>
<tr>
<td>DNa-H</td>
<td>0.45 ± 0.09</td>
<td>0.15 ± 0.08</td>
</tr>
<tr>
<td>DNa-L</td>
<td>0.31 ± 0.17</td>
<td>0.27 ± 0.29</td>
</tr>
<tr>
<td>DDEA-O</td>
<td>0.46 ± 0.12</td>
<td>0.14 ± 0.11</td>
</tr>
<tr>
<td>DDEA-H</td>
<td>0.48 ± 0.05</td>
<td>0.20 ± 0.17</td>
</tr>
<tr>
<td>DDEA-L</td>
<td>0.00 ± 0.00</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>DHEP-O</td>
<td>0.36 ± 0.20</td>
<td>0.09 ± 0.08</td>
</tr>
<tr>
<td>DHEP-H</td>
<td>0.51 ± 0.16</td>
<td>0.20 ± 0.14</td>
</tr>
<tr>
<td>DHEP-L</td>
<td>0.15 ± 0.07</td>
<td>0.06 ± 0.06</td>
</tr>
</tbody>
</table>

The average diclofenac concentration in the SCE was higher than in the ED for all of the diclofenac formulations, except for DDEA-L, where no diclofenac had reached the SCE. This may have been caused by the ED representing an aqueous environment, compared to the SC, and would the lipophilic diclofenac therefore prefer to remain in the SCE, rather than permeate to the ED (Abbott, 2012:218; Naik, et al., 2000:319).

In the group of formulations prepared from DNa, the average diclofenac concentration in the SCE was highest for DNa-O, followed by DNa-H and DNa-L. The average concentration of diclofenac in the ED was highest for DNa-L and lowest for DNa-H, unlike one would expect, since the viable epidermis is likely to be the primary penetration barrier for NSAIDs from a nonpolar carrier (Wenkers & Lippold, 1999:1330). The diclofenac concentration in the ED was only slightly lower for DNa-O than for DNa-L. Gels made with DDEA had the highest concentration of diclofenac in the SCE for DDEA-H, followed by DDEA-O, possibly due to the high polarity of the DDEA-H formulation that created a driving force to push the lipophilic diclofenac out of the emulgel and into the SCE (Abbott, 2012:218). Epidermal penetration of this group was best for DDEA-H and poorest for DDEA-L, in agreement with the findings by Wenkers and Lippold (1999:1330). DHEP-H had achieved the highest diclofenac concentration in both the SCE and ED, compared to the other types prepared using this salt, i.e. DHEP-L reached the lowest concentration in both layers. This could again have been attributed to the high polarity of the DHEP-H emulgel that drove the diclofenac to leave the formulation (Abbott, 2012:218). The more diclofenac that moves into the SC, the higher the amount available to enter the viable epidermis. High concentrations of diclofenac in the SCE were expected for O formulations, since they were optimised towards the SC (enhancing API delivery into this layer).
Evaluation of similar gel types, prepared from different diclofenac salts, showed that the highest average SCE and ED diclofenac concentrations for type O was reached by the DNa gel, followed by the DDEA and DHEP gels. Since DDEA and DHEP formulations are particularly promoted for their abilities to deliver transdermally (Fini et al., 1998:11; Fini et al., 1999:166), these results were unexpected. In order of highest to lowest, the average SCE and ED diclofenac concentrations for the more hydrophilic formulations were achieved by the DHEP, DDEA and DNa gels, in accordance with popular belief (Fini et al., 1998:11; Fini et al., 1999:166). The DNa gel had both the highest average concentration of diclofenac in the SCE and ED for the more lipophilic group of gels, followed by the DHEP gel and DDEA gels. As for the O formulations, these results were unexpected.

A three-way ANOVA test was performed and statistically significant differences between the average diclofenac concentrations of gels with different polarities ($P$ value $<$ 0.0001), gels prepared using different diclofenac salts ($P$ value $<$ 0.0001) and gels with different combinations of polarities and the salts used during formulation ($P$ value $<$ 0.0001). A significant difference between the average diclofenac concentrations in the SCE and ED was also observed ($P$ value $<$ 0.0001). A significant relationship between the skin location (SCE or ED) and the polarity of gels was indicated ($P$ value $<$ 0.0001). The salt being employed showed no relationship with regards to skin location by any of the formulations ($P$ value = 0.2969). No statistically significant interaction was indicated between the different diclofenac salts, the polarities of the formulations and skin location of diclofenac ($P$ value = 0.1642).

The Tukey test indicated a significant difference between the DNa and DDEA gels, and between the DNa and DHEP gels, with no significant difference among the DHEP and DDEA gels. O and H emulgels showed statistically significant differences from L. No significant difference was indicated between O and H. This test also indicated a significant difference in concentrations between the skin locations, namely the SCE and ED.

### C.4 CONCLUSION

During membrane release studies, DNa-H had achieved the highest average percentage of diclofenac being released after 6 hours (8.38%), equally followed by the DNa-O and DHEP-H emulgels. The average percentage of diclofenac released after 6 hours from L was much lower than for O and H in every group of emulgels formulated from a specific salt, possibly due to the diclofenac acid being more lipophilic, causing it to reside in the oily L formulations, instead of leaving it to enter the aqueous receptor phase (Abbott, 2012:218).

Results from diffusion studies showed that DNa-H had the highest average cumulative diclofenac concentration after 12 hours (16.60 µg/ml), the highest percentage of diclofenac that
had diffused after 12 hours (0.89%) and the highest average flux (1.42 µg/cm².h), followed by DDEA-H and DHEP-H. The average cumulative concentration, percentage of diffused diclofenac and average flux was much lower for O and L in all three groups of emulgels prepared with a specific salt. This could have been as a result of the higher polarity of the H formulations that had created a stronger driving force to push the lipophilic diclofenac acid out of the formulation and into the skin (Abbott, 2012:218; Naik et al., 222:319). Higher amounts of diclofenac were released and therefore available for diffusion across the SC and underlying epidermis.

The highest average concentration of diclofenac in the ED was unexpectedly achieved by the DNA-L emulgel, while DNA-O showed the highest concentration in the SCE. DNA-L, DDEA-L and DHEP-L gels had reached the lowest concentrations of diclofenac in the SCE. Since the L formulations had also released the lowest amounts of diclofenac, according to the membrane release studies, less diclofenac was available for penetration of the SCE and ED, than from the O and H formulations.

The average concentration of diclofenac was higher in the SCE than in the ED for all of the formulations, except for the DDEA-L that had not released any diclofenac in the SCE. This was indicative of diclofenac penetrating more effectively into the lower layers of the epidermis beyond the SC.

The average cumulative concentration of diffused diclofenac after 12 hours was considerably higher than the average concentration of diclofenac in the SCE and the ED. This was indicative that diclofenac crossed both layers effectively to enter the receptor phase that resembled the systemic circulation.

The outcomes of these studies supported the delivery gap principle, which states that a small SDG (< 1) facilitates effective formulation and transdermal delivery of an API (JW Solutions, 2013). It was concluded that the most efficient transdermal delivery of diclofenac, in terms of time and quantity, was obtained from the formulations with polarities higher than those optimised towards the SC. Although the performances of the various gels, prepared from different salts, were very similar, the DNA gels showed slightly better results, despite DDEA and DHEP formulations being specifically promoted as transdermal dosage forms (Fini et al., 1998:11; Fini et al., 1999:166; Rossiter, 2012:391-392,403).
REFERENCES


http://www.pharmacopoeia.co.uk.nwulib.nwu.ac.za/bp2013/ixbin/bp.cgi?a=query&title=%22Phosphate%20Buffer%20Solution%20pH%207.4%22&tab=a-z%20index&l=P&xh=1 Date of access: 29 Oct. 2013.


JW Solutions. 2013. Explaining the importance of the skin delivery gap.


APPENDIX D

AUTHOR’S GUIDE TO THE
INTERNATIONAL JOURNAL OF PHARMACEUTICS

D.1 DESCRIPTION

The *International Journal of Pharmaceutics* is the journal for pharmaceutical scientists concerned with the physical, chemical and biological properties of devices and delivery systems for drugs, vaccines and biologicals, including their design, manufacture and evaluation. This includes evaluation of the properties of drugs, excipients such as surfactants and polymers and novel materials. The journal has special sections on pharmaceutical nanotechnology and personalized medicines, and publishes research papers, reviews, commentaries and letters to the editor as well as special issues.

D.1.1 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.
D.2 GUIDE FOR AUTHORS

D.2.1 INTRODUCTION

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: For details on pharmaceutical nanotechnology, see Editorials in 279/1-2 281/1, and 288/1.

D.2.1.1 Types of paper

1. **Full Length Manuscripts**

2. **Rapid Communications**
   
   a. These articles should not exceed 1500 words or equivalent space.

   b. Figures should not be included otherwise delay in publication will be incurred.

   c. Do not subdivide the text into sections. An Abstract should be included as well as a full reference list.

3. **Notes**

   Should be prepared as described for full length manuscripts, except for the following:

   a. The maximum length should be 1500 words, including figures and tables.

   b. Do not subdivide the text into sections. An Abstract and reference list should be included.
4. **Reviews and Mini-Reviews**

Suggestions for review articles will be considered by the Review-Editor. "Mini-reviews" of a topic are especially welcome.

D.2.2 **BEFORE YOU BEGIN**

D.2.2.1 Ethics in publishing

For information on Ethics in publishing and Ethical guidelines for journal publication see http://www.elsevier.com/publishingethics and http://www.elsevier.com/journal-authors/ethics.

D.2.2.2 Human and animal rights

If the work involves the use of animal or human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans http://www.wma.net/en/30publications/10policies/b3/index.html; EU Directive 2010/63/EU for animal experiments http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm; Uniform Requirements for manuscripts submitted to Biomedical journals http://www.icmje.org.

Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

D.2.2.3 Conflict of interest

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. See also http://www.elsevier.com/conflictsinterest. Further information and an example of a Conflict of Interest form can be found at: http://help.elsevier.com/app/answers/detail/a_id/286/p/7923. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

D.2.2.4 Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see http://www.elsevier.com/postingpolicy), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly
by the responsible authorities where the work was carried out, and that, if accepted, it will not be
published elsewhere in the same form, in English or in any other language, including
electronically without the written consent of the copyright-holder. To verify originality, your
article may be checked by the originality detection service CrossCheck

D.2.2.5 Contributors

Each author is required to declare his or her individual contribution to the article: all authors
must have materially participated in the research and/or article preparation, so roles for all
authors should be described. The statement that all authors have approved the final article
should be true and included in the disclosure.

D.2.2.6 Authorship

All authors should have made substantial contributions to all of the following: (1) the conception
and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting
the article or revising it critically for important intellectual content, (3) final approval of the
version to be submitted.

D.2.2.7 Changes to authorship

This policy concerns the addition, deletion, or rearrangement of author names in the authorship
of accepted manuscripts: Before the accepted manuscript is published in an online issue:
Requests to add or remove an author, or to rearrange the author names, must be sent to the
Journal Manager from the corresponding author of the accepted manuscript and must include:
(a) the reason the name should be added or removed, or the author names rearranged and (b)
written confirmation (e-mail, fax, letter) from all authors that they agree with the addition,
removal or rearrangement. In the case of addition or removal of authors, this includes
confirmation from the author being added or removed. Requests that are not sent by the
corresponding author will be forwarded by the Journal Manager to the corresponding author,
who must follow the procedure as described above. Note that: (1) Journal Managers will inform
the Journal Editors of any such requests and (2) publication of the accepted manuscript in an
online issue is suspended until authorship has been agreed.

After the accepted manuscript is published in an online issue: Any requests to add, delete, or
rearrange author names in an article published in an online issue will follow the same policies
as noted above and result in a corrigendum.
D.2.2.8 Article transfer service

This journal is part of our Article Transfer Service. This means that if the Editor feels your article is more suitable in one of our other participating journals, then you may be asked to consider transferring the article to one of those. If you agree, your article will be transferred automatically on your behalf with no need to reformat. More information about this can be found here: http://www.elsevier.com/authors/article-transfer-service.

D.2.2.9 Copyright

This journal offers authors a choice in publishing their research: Open Access and Subscription.

For Subscription articles

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright, see http://www.elsevier.com/copyright). An e-mail 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 preprinted forms for use by authors in these cases: please consult http://www.elsevier.com/permissions.

For Open Access articles

Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (for more information see http://www.elsevier.com/OAauthoragreement). Permitted reuse of open access articles is determined by the author's choice of user license (see http://www.elsevier.com/openaccesslicenses).

Retained author rights

As an author you (or your employer or institution) retain certain rights. For more information on author rights for:

Subscription articles please see http://www.elsevier.com/journal-authors/author-rights-and-responsibilities.

Open access articles please see http://www.elsevier.com/OAauthoragreement.
D.2.2.10 Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated. Please see http://www.elsevier.com/funding.

D.2.2.11 Funding body agreements and policies

Elsevier has established agreements and developed policies to allow authors whose articles appear in journals published by Elsevier, 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.

D.2.2.12 Open access

This journal offers authors a choice in publishing their research:

**Open Access**

- Articles are freely available to both subscribers and the wider public with permitted reuse
- An Open Access publication fee is payable by authors or their research funder

**Subscription**

- Articles are made available to subscribers as well as developing countries and patient groups through our access programs (http://www.elsevier.com/access)
- No Open Access publication fee

All articles published Open Access will be immediately and permanently free for everyone to read and download. Permitted reuse is defined by your choice of one of the following Creative Commons user licenses:

**Creative Commons Attribution (CC BY):** lets others distribute and copy the article, to create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), to include in a collective work (such as an anthology), to text or data mine the article, even for commercial purposes, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, and do not modify the article in such a way as to damage the author's honor or reputation.
Creative Commons Attribution-NonCommercial-ShareAlike (CC BY-NC-SA): for noncommercial purposes, lets others distribute and copy the article, to create extracts, abstracts and other revised versions, adaptations or derivative works of or from an article (such as a translation), to include in a collective work (such as an anthology), to text and data mine the article, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, do not modify the article in such a way as to damage the author's honor or reputation, and license their new adaptations or creations under identical terms (CC BY-NC-SA).

Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND): for noncommercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

To provide Open Access, this journal has a publication fee which needs to be met by the authors or their research funders for each article published Open Access.

Your publication choice will have no effect on the peer review process or acceptance of submitted articles.

The publication fee for this journal is $3000, excluding taxes. Learn more about Elsevier's pricing policy: http://www.elsevier.com/openaccesspricing.

D.2.2.13 Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop (http://webshop.elsevier.com/languagediting/) or visit our customer support site (http://support.elsevier.com) for more information.

D.2.2.14 Submission

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF files at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail removing the need for a paper trail.
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.

Note:

The choice of general classifications such as "drug delivery" or "formulation" are rarely helpful when not used together with a more specific classification.

D.2.2.15 Referees

Please submit, with the manuscript, the names, addresses and e-mail addresses of at least three potential reviewers. Reviewers who do not have an institutional e-mail address will only be considered if their affiliations are given and can be verified. Preferably international reviewers should be nominated, and their areas of expertise must be stated clearly. Note that the editor retains the sole right to decide whether or not the suggested reviewers are used.

http://www.elsevier.com/journals/international-journal-of-pharmaceutics/0378-5173/guide-forauthors

D.2.3 PREPARATION

D.2.3.1 Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork. To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.
D.2.3.2 Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.
D.2.3.3  Essential title page information

• **Title.** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

• **Author names and affiliations.** Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors’ affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author’s name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

• **Corresponding author.** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that phone numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.**

• **Present/permanent address.** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

D.2.3.4  Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

The abstract must not exceed 200 words.

D.2.3.5  Graphical abstract

A Graphical abstract is mandatory for this journal. It should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more,
but should be readable on screen at a size of 200 × 500 pixels (at 96 dpi this corresponds to 5 × 13 cm). Bear in mind readability after reduction, especially if using one of the figures from the article itself. Preferred file types: TIFF, EPS, PDF or MS Office files. See http://www.elsevier.com/graphicalabstracts for examples.

D.2.3.6 Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

D.2.3.7 Chemical compounds

You can enrich your article by providing a list of chemical compounds studied in the article. The list of compounds will be used to extract relevant information from the NCBI PubChem Compound database and display it next to the online version of the article on ScienceDirect. You can include up to 10 names of chemical compounds in the article. For each compound, please provide the PubChem CID of the most relevant record as in the following example: Glutamic acid (PubChem CID: 611). The PubChem CIDs can be found via http://www.ncbi.nlm.nih.gov/pccompound. Please position the list of compounds immediately below the 'Keywords' section. It is strongly recommended to follow the exact text formatting as in the example below:

Chemical compounds studied in this article

Ethylene glycol (PubChem CID: 174); Plitidepsin (PubChem CID: 44152164); Benzalkonium chloride (PubChem CID: 15865)


D.2.3.8 Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

D.2.3.9 Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List
here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

D.2.3.10 Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

D.2.3.11 Database linking

Elsevier encourages authors to connect articles with external databases, giving their readers one-click access to relevant databases that help to build a better understanding of the described research. Please refer to relevant database identifiers using the following format in your article: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN). See http://www.elsevier.com/databaselinking for more information and a full list of supported databases.

D.2.3.12 Math formulae

Present simple formulae in the line of normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

D.2.3.13 Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article, using superscript Arabic numbers. Many wordprocessors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Table footnotes

Indicate each footnote in a table with a superscript lowercase letter.

Image manipulation

Whilst it is accepted that authors sometimes need to manipulate images for clarity, manipulation for purposes of deception or fraud will be seen as scientific ethical abuse and will be dealt with accordingly. For graphical images, this journal is applying the following policy: no specific
feature within an image may be enhanced, obscured, moved, removed, or introduced. Adjustments of brightness, contrast, or color balance are acceptable if and as long as they do not obscure or eliminate any information present in the original. Nonlinear adjustments (e.g. changes to gamma settings) must be disclosed in the figure legend.

**Electronic artwork**

**General points**

- Make sure you use uniform lettering and sizing of your original artwork.
- Embed the used fonts if the application provides that option.
- Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
- Number the illustrations according to their sequence in the text.
- Use a logical naming convention for your artwork files.
- Provide captions to illustrations separately.
- Size the illustrations close to the desired dimensions of the printed version.
- Submit each illustration as a separate file.

A detailed guide on electronic artwork is available on our website:

http://www.elsevier.com/artworkinstructions

**You are urged to visit this site; some excerpts from the detailed information are given here.**

**Formats**

If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format. Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

- EPS (or PDF): Vector drawings, embed all used fonts.
- TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
- TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.

Please do not:

- Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
- Supply files that are too low in resolution;
- Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color on the Web (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article. Please indicate your preference for color: in print or on the Web only. 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 color figures to ‘gray scale’ (for the printed version should you not opt for color in print) please submit in addition usable black and white versions of all the color illustrations.

Figure captions

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

D.2.3.14 Tables

Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.
D.2.3.15 References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication and a copy of the title page of the relevant article must be submitted.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is encouraged.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software

This journal has standard templates available in key reference management packages EndNote (http://www.endnote.com/support/enstyles.asp) and Reference Manager (http://refman.com/support/rmstyles.asp). Using plug-ins to wordprocessing packages, authors only need to select the appropriate journal template when preparing their article and the list of references and citations to these will be formatted according to the journal style which is described below.
Reference formatting

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style

Text: All citations in the text should refer to:

1. Single author: the author's name (without initials, unless there is ambiguity) and the year of publication;
2. Two authors: both authors' names and the year of publication;
3. Three or more authors: first author's name followed by 'et al.' and the year of publication.

Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically.

Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999). Kramer et al. (2010) have recently shown ....'

List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference 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.

Examples:

Reference to a journal publication:


Reference to a book:

Reference to a chapter in an edited book:


*Journal abbreviations source*

Journal names should be abbreviated according to the


D.2.3.16  Video data

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 50 MB. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages at http://www.elsevier.com/artworkinstructions. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

D.2.3.17  AudioSlides

The journal encourages authors to create an AudioSlides presentation with their published article. AudioSlides are brief, webinar-style presentations that are shown next to the online article on ScienceDirect. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. More information and examples are available at http://www.elsevier.com/audioslides. Authors of this journal will automatically receive an invitation e-mail to create an AudioSlides presentation after acceptance of their paper.
D.2.3.18 Supplementary data

Elsevier accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. In order to ensure that your submitted material is directly usable, please provide the data in one of our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each file. For more detailed instructions please visit our artwork instruction pages at http://www.elsevier.com/artworkinstructions.

Submission checklist

It is hoped that this list will be useful during the final checking of an article prior to sending it to the journal's Editor for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One Author designated as corresponding Author:

- E-mail address
- Full postal address
- Telephone and fax numbers

All necessary files have been uploaded

- Keywords
- All figure captions
- All tables (including title, description, footnotes)

Further considerations:

- Use continuous line numbering (every 5 lines) to facilitate reviewing of the manuscript.
- Manuscript has been "spellchecked" and "grammar-checked"
- References are in the correct format for this journal
- All references mentioned in the Reference list are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Web)

• Color figures are clearly marked as being intended for color reproduction on the Web (free of charge and in print or to be reproduced in color on the Web (free of charge) and in black-and-white in print

• If only color on the Web is required, black and white versions of the figures are also supplied for printing purposes

For any further information please visit our customer support site at http://support.elsevier.com.

D.2.4 AFTER ACCEPTANCE

D.2.4.1 Use of the Digital Object Identifier

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. Example of a correctly given DOI (in URL format; here an article in the journal Physics Letters B):

http://dx.doi.org/10.1016/j.physletb.2010.09.059

When you use a DOI to create links to documents on the web, the DOIs are guaranteed never to change.

D.2.4.2 Online proof correction

Corresponding authors will receive an e-mail with a link to our ProofCentral system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.

If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately - please upload all of your corrections within 48 hours. It is important to ensure that all 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.

D.2.4.3 Offprints

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. For an extra charge, more paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's WebShop (http://webshop.elsevier.com/myarticleservices/offprints). Authors requiring printed copies of multiple articles may use Elsevier WebShop's 'Create Your Own Book' service to collate multiple articles within a single cover (http://webshop.elsevier.com/myarticleservices/offprints/myarticleservices/booklets).

D.2.5 AUTHOR INQUIRIES

For inquiries relating to the submission of articles (including electronic submission) please visit this journal's homepage. For detailed instructions on the preparation of electronic artwork, please visit http://www.elsevier.com/artworkinstructions. Contact details for questions arising after acceptance of an article, especially those relating to proofs, will be provided by the publisher. You can track accepted articles at http://www.elsevier.com/trackarticle. You can also check our Author FAQs at http://www.elsevier.com/authorFAQ and/or contact Customer Support via http://support.elsevier.com.