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

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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^{TM}) 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^{TM} 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/cm^2.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^{TM}, 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).

Aangesien topikale diklofenak 'n meer gunstige veiligheidsprofiel bied, is dit 'n waardevolle plaasvervanger vir orale NSAIM-toedienings in die behandeling van osteoartritis (Roth & Fuller, 2011:166). Die voordele van topikale NSAIM-aanwendings, in vergelyking met orale toediening en sistemiese aflewering, sluit die maklike staking van behandeling indien effekte hinderlik raak (Brown *et al*., 2006:177), die ontduiking van ekstensiewe eerste-deurgangmetabolisme (Cleary, 1993:19; Kornick, 2003:953; Prausnitz & Langer, 2008:1261; Lionberger & Brennan, 2010:225), verminderde sistemiese newe-effekte (Colin Long, 2002:41), gerieflike aanwending en verhoogde pasiëntmeewerkendheid (Cleary, 1993:19; Prausnitz & Langer, 2008:1261) in.

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^{TM}) 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, naamlik 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 formulerings met optimale geneesmiddelaflewering 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 vervoersisteem gebruik kan word (JW Solutions, 2013b).

In hierdie studie is diklofenaknatrium (DNa), diklofenakdiëtielamien (DDEA) en diklofenak-N-(2-hidroksi-etiel) pirrolidien (DHEP) elk in die volgende emulgels geformuleer:

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

Die komponente van die oliefase en hul onderskeie hoeveelhede, asook die SDG van die formulerings is met behulp van die FFE^{TM}–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 vervoersisteem, 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 formulerings vrygestel is, te bepaal. Resultate het bevestig dat diklofenak inderdaad uit al nege hierdie geformuleerde emulgels vrygestel is. Die meer hidrofiele DNa-formulering het die hoogste gemiddelde persentasie diklofenakvrystelling (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 persentasie veldiffusie (0.09%) na 12 ure getoon, asook die hoogste gemiddelde vloed (1.42 ± 0.20 µg/cm^2.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-formulering, wat vir die SC geoptimaliseer was, die hoogste konsentrasie in die SCE (0.77 µg/ml) getoon het.

Sleutelwoorde: diklofenak, FFE^{TM}, SDG, formulering, transdermale diffusie

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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 (pK_a 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 ibufropen, 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 $\log_{10}$ 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:

Polarity of formulation > Polarity of penetrant + PPG **Equation 1.1**

Polarity of formulation < Polarity of penetrant - PPG **Equation 1.2**

Equations 1.1 and 1.2 are schematically represented in Figure 1.1.

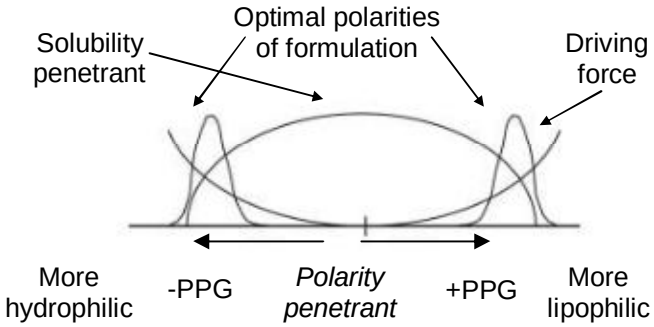

Figure 1.1: Schematic representation of optimal polarity of the formulation relative to the polarity of the penetrant to ensure 50% of the API penetrating the SC (Wiechers *et al*., 2004:177).

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^{TM}) 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^{TM} 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^{TM} 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^{TM} 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.

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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^{TM} 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).

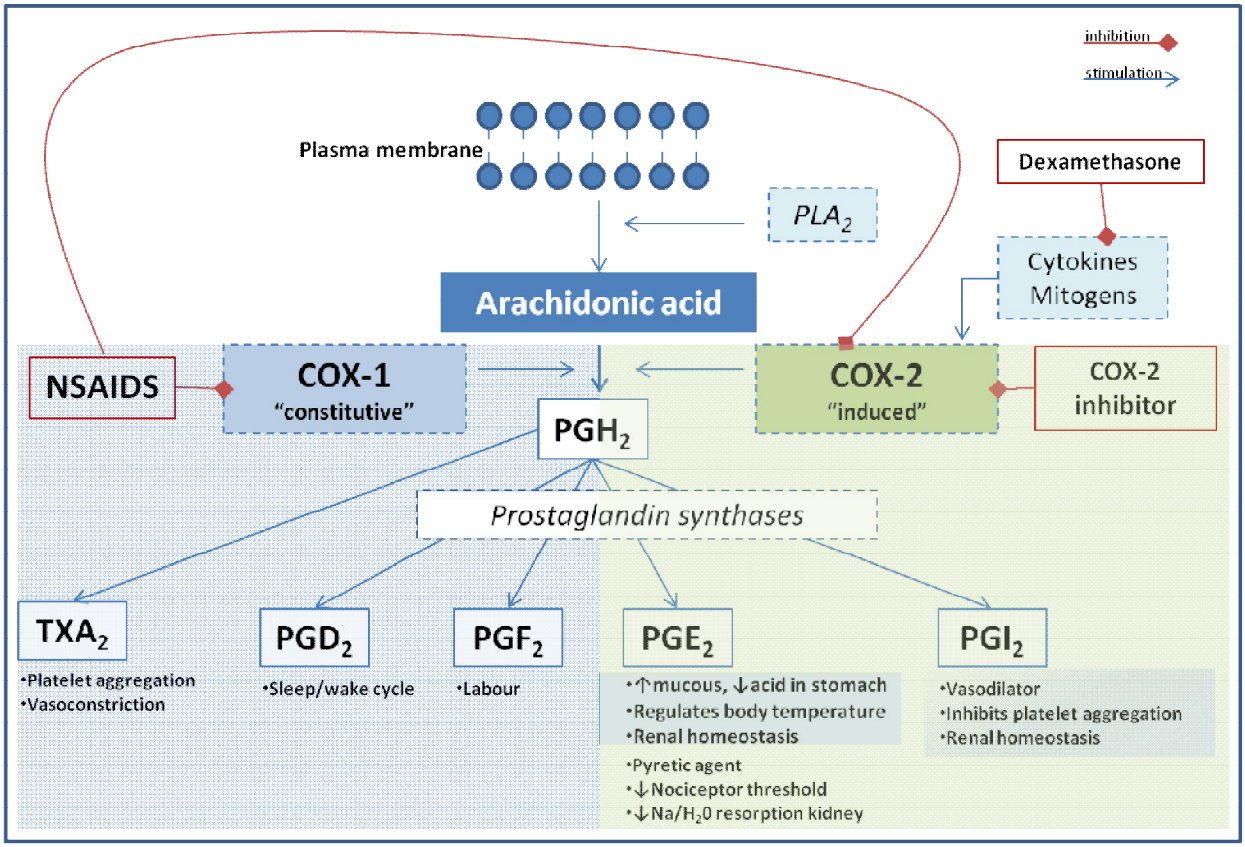


Figure 2.1: Illustration of the prostaglandin synthesis pathway, showing the roles of COX-1 and COX-2 in the production of prostaglandins (PGD_2, PGF_2, PGE_2) from arachidonic acid (Botting, 2006:210; Chell *et al*., 2006:109).

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 pancytopaenia), 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)

Description	Specification
Peak concentration	2 - 3 hours
Protein binding	99%
Metabolites	Glucuronide and sulphide (renal 65%, bile 35%)
$T_{1/2}$ (oral preparations)	1 - 2 hours
Oral bioavailability (subject to first-pass effect)	50%
Urinary excretion of unchanged drug	< 1%

2.3.2 PHYSICOCHEMICAL CHARACTERISTICS OF DICLOFENAC

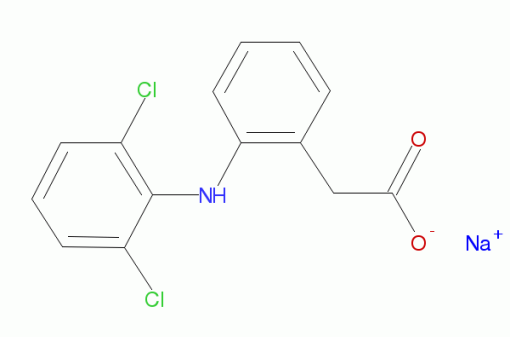
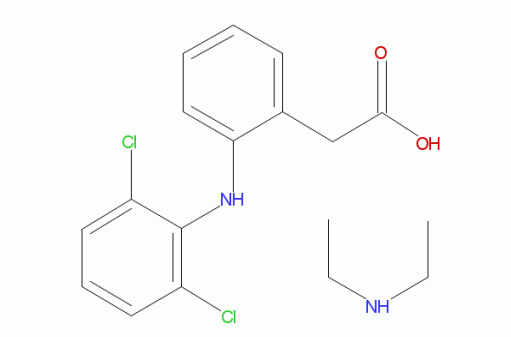
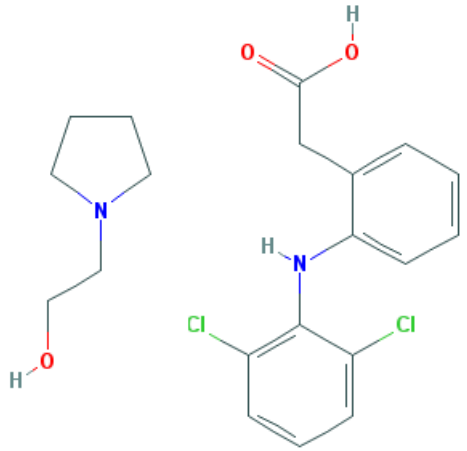
Table 2.2 summarises the physicochemical characteristics of diclofenac.

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

Description	Specification
Molecular formula	$C_{14}H_{11}Cl_2NO_2$
Molecular weight	296.14864 [g/mol]
Dissociation constant (pK_a)	4.15
Partition coefficient (LogP)	4.51
Solubility (mg/l)	17.8

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)

Description	DNa	DDEA	DHEP
Molecular structure			
Molecular formula	$C_{14}H_{10}Cl_2NNaO_2$	$C_{18}H_{22}Cl_2N_2O_2$	$C_{20}H_{24}Cl_2N_2O_3$
Molecular weight	318.130469 [g/mol]	369.28548 [g/mol]	411.32216 [g/mol]
Appearance	White or slightly yellowish, slightly hygroscopic, crystalline powder	White to light beige, crystalline powder	White to cream, crystalline powder
Solubility	Sparingly soluble in water Freely soluble in methanol Soluble in ethanol (96%) Slightly soluble in acetone	Sparingly soluble in water and in acetone Freely soluble in ethanol (96%) and in methanol Practically insoluble in 1M sodium hydroxide	Freely soluble in methanol and in ethanol Sparingly soluble in water and in acetic acid
Melting point	~280 °C, with decomposition	~154 °C, with decomposition.	101 °C - 104 °C

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.

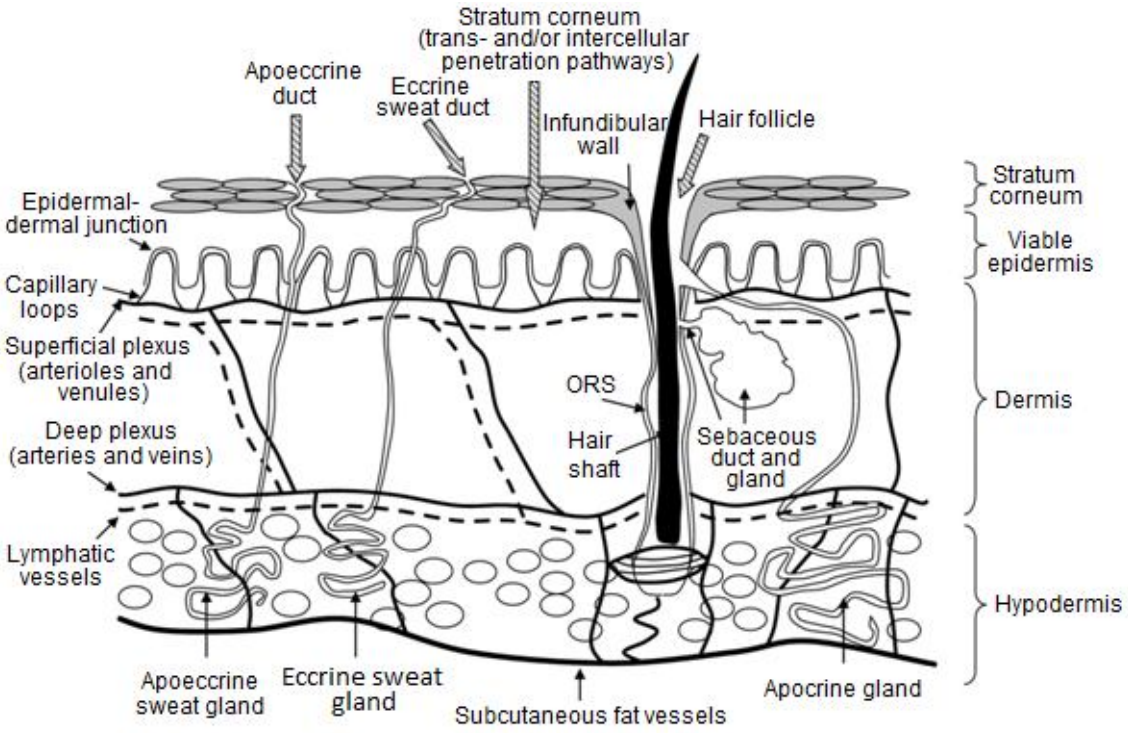


Figure 2.2: Diagrammatic representation of skin layers, appendages, blood and lymphatic vessels (Jepps *et al*., 2013:154).

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

Topical delivery vehicle	Favourable characteristics	Undesirable effects
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^{TM} 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 δD (the dispersion force amongst molecules), δP (the dipolar force amongst molecules) and δH (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 δP or δH value, but the behaviour of such compounds would differ. Contrary, non-polar or lipophilic compounds are distinguished from each other through the difference in δD values, i.e. the higher the δD, 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 [δD1, δP1, δH1] and [δD2, δP2, δH2]) by applying Equation 2.1 (Abbott *et al*., 2012:218).

$$\text{Distance} = \sqrt{4(\delta D_1 - \delta D_2)^2 + (\delta H_1 - \delta H_2)^2 + (\delta P_1 - \delta P_2)^2} \qquad \textbf{Equation 2.1}$$

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).

$$SDG = \frac{MEC}{LTC} \qquad \textbf{Equation 2.2}$$

Where:
MEC = minimum effective concentration,
LTC = 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 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™ 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 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™ 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 the transdermal delivery of diclofenac from emulgels of different diclofenac salts were compared. These procedures and outcomes are discussed in the following chapters.

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CHAPTER 3

ARTICLE FOR PUBLICATION IN THE INTERNATIONAL JOURNAL OF PHARMACEUTICS

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

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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 differenciated 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^{TM}) 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^{TM}

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 pancytopaenia), 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 (pK_a 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^{TM}) 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).

$$SDG = \frac{MEC}{LTC} \qquad \textbf{Equation 1.1}$$

Where: *MEC* is the minimum effective concentration and *LTC* is the local tissue concentration. The FFE^{TM} 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^{TM} to optimise the formulation is recommended (using the FFE^{TM} 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 C_{18} 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 poly-ethylene 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 into 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 cm^2 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 Corportion, 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^{TM} 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^{TM} 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 SCE (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).

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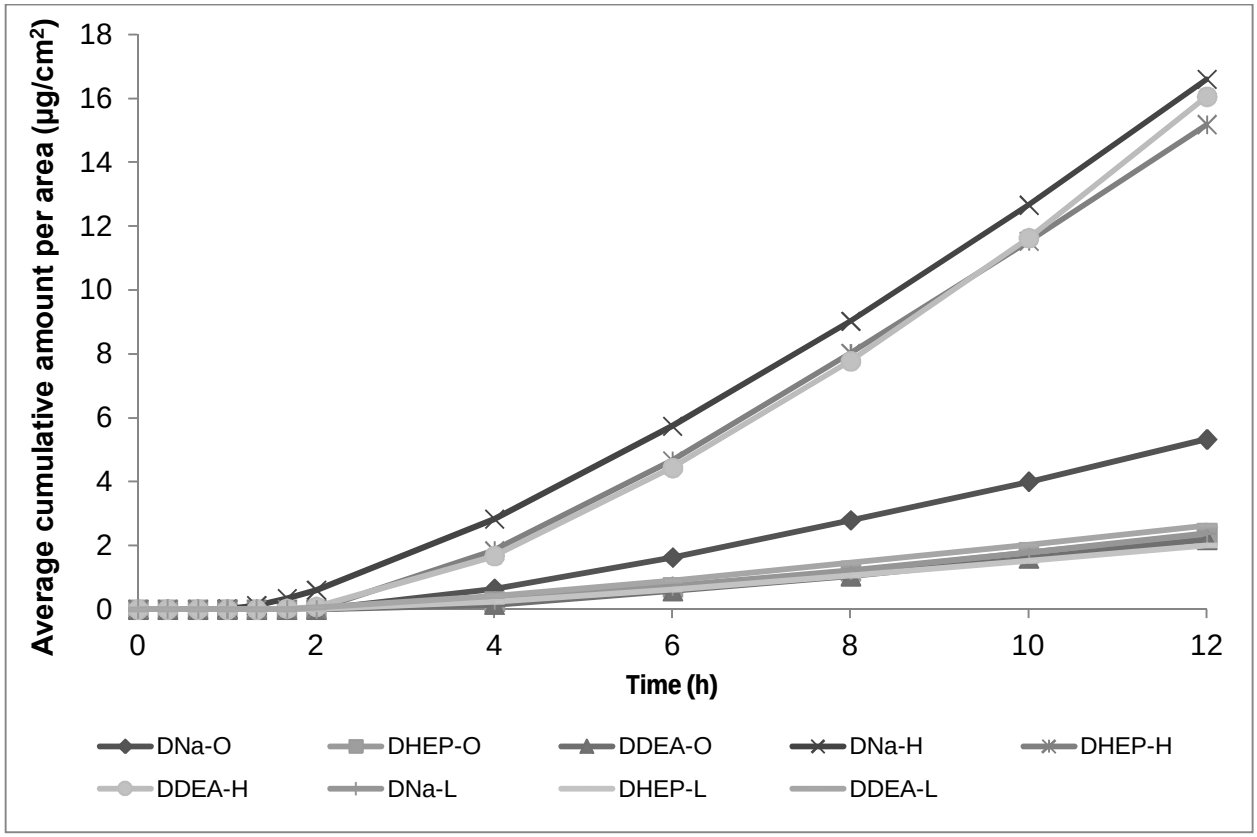
FIGURE LEGENDS

Figure 1: Average cumulative amount of diclofenac per area (µg/cm^2) 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:



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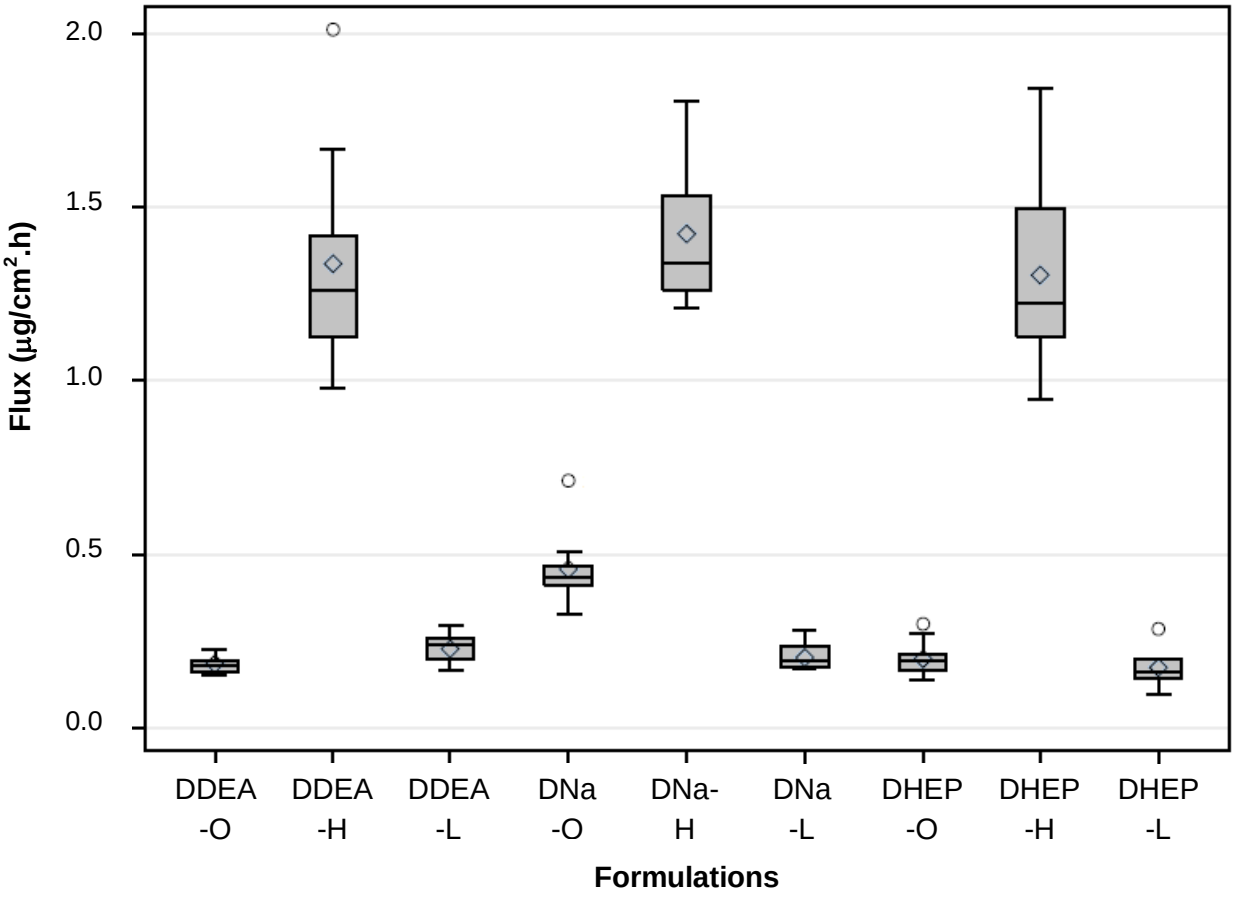

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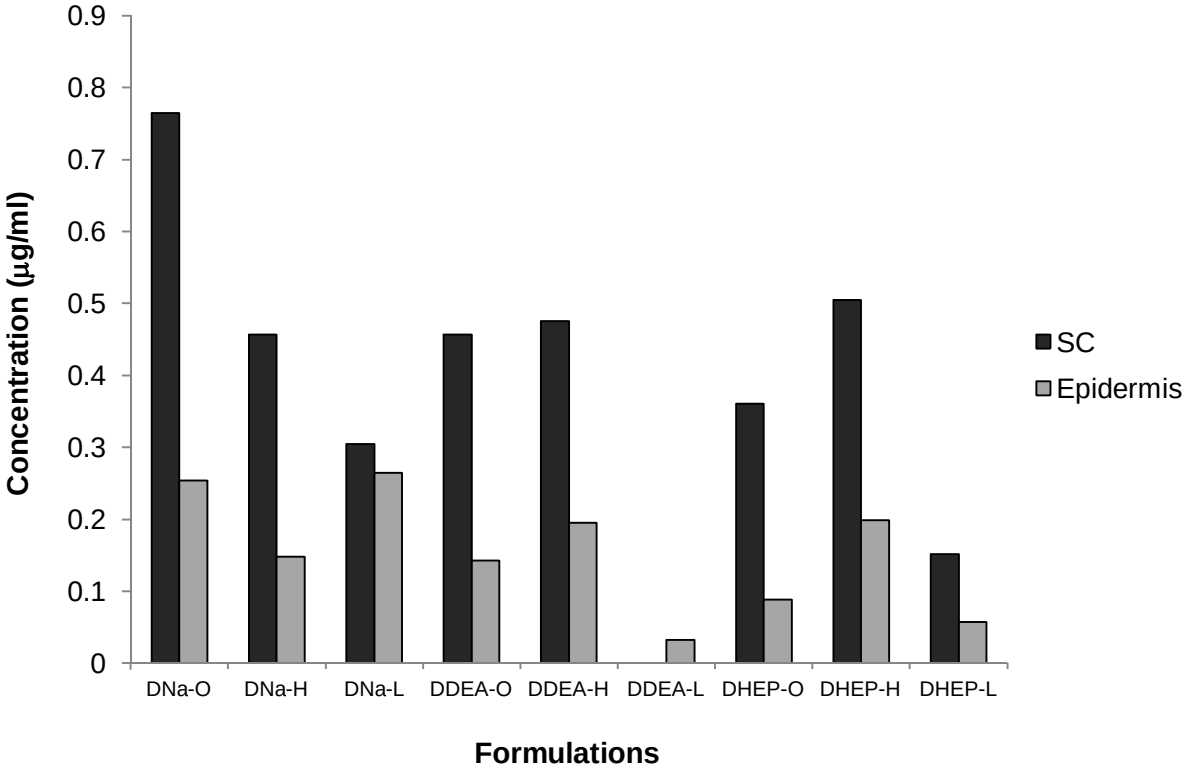

Figure 3: Comparison of the concentration (µg/ml) of diclofenac in the SCE and ED among different formulations.

CHAPTER 4

FINAL CONCLUSIONS AND FUTURE PROSPECTS

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^{TM} 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 DNa, DDEA and DHEP.
- Employment of the JW Solutions software, FFE^{TM}, 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,

Potchefstroom Campus, South Africa (Steyn, 2010:69-111). Revalidation of this existing method was performed and the scope extended to include the assay of formulated products and of test samples generated during diffusion and membrane studies. Analysis of samples collected from *in vitro* and tape stripping experiments were performed by HPLC.

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^{TM} 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 dicofenac (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/cm^2.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 outomes 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 extention 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^{TM}.
- Preparing formulations with higher polarity variances.
- Employing and evaluating a different API in emulgel form.
- Preparing formulations with different SDG values, including ≥ 1.

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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 C_{18} 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.3 PREPARATION OF SAMPLES AND STANDARD SOLUTION

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).

$$y = mx + c \qquad \textbf{Equation A.1}$$

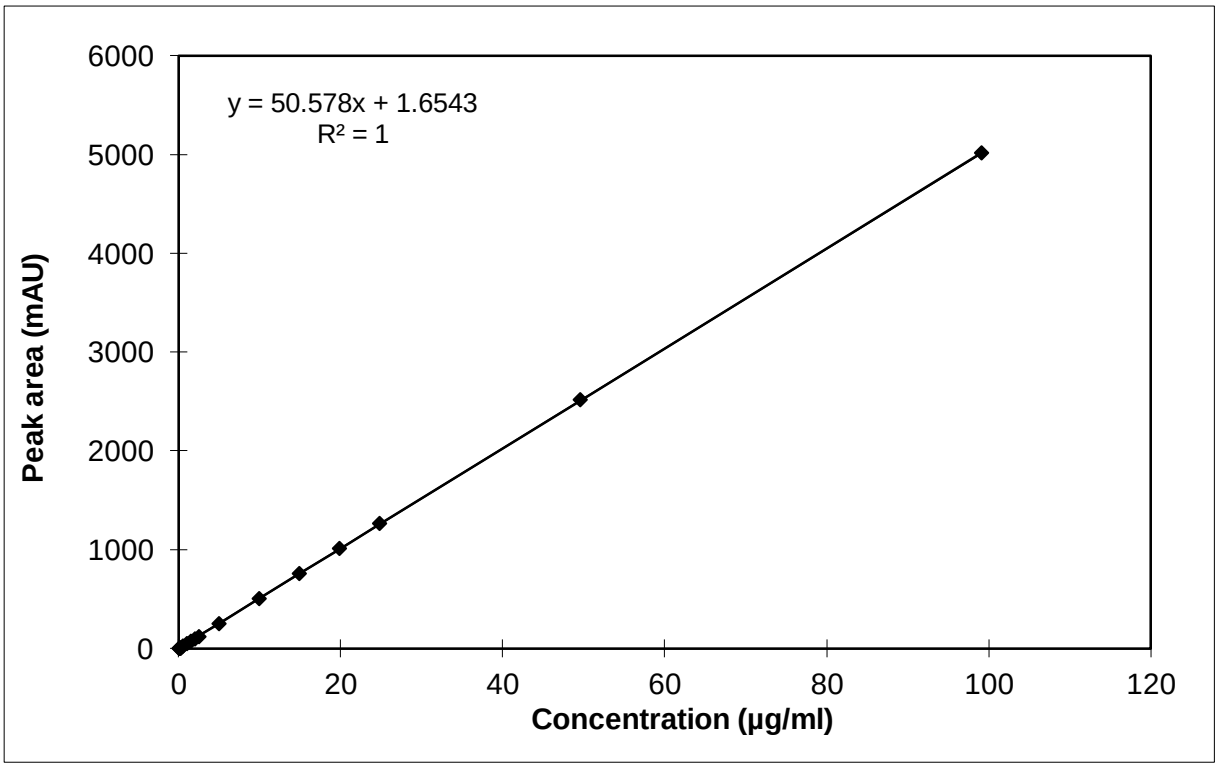

Figure A.1: Linear regression curve of diclofenac standard solutions.

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

Concentration (µg/ml)	Peak area 1	Peak area 2	Mean
0.0248	1.256	1.387	1.3
0.0495	2.322	2.297	2.3
0.0991	4.151	4.307	4.2
0.1486	4.400	5.055	4.7
0.1982	5.100	5.054	5.1
0.2477	4.603	4.939	4.8
0.4954	25.151	24.798	25.0
0.9908	50.106	49.512	49.8
1.4862	73.279	73.826	73.6
1.9816	96.718	97.751	97.2
2.4770	120.095	120.087	120.1
4.9540	252.448	251.000	251.7
9.9080	505.846	504.833	505.3
14.8620	759.493	758.523	759.0
19.8160	1013.437	1013.410	1013.4
24.7700	1266.473	1266.345	1266.4
49.5400	2521.027	2517.503	2519.3
99.0800	5016.940	5020.073	5018.5
148.6200	7517.913	7528.725	7523.3
198.1600	10042.000	10037.200	10039.6
247.7000	12516.900	12499.000	12508.0
R squared	0.999548	Lower 95%	Upper 95%
Intercept	45.15099	-55.51567	145.8177
Slope	55.05241	54.43173	55.67309

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

Concentration spiked (ug/ml)	Peak area 1	Peak area 2	Mean	Recovery (ug/ml)	%
96.3	4950.00	4940.00	4945	97.4	101.2
96.3	4970.90	4973.40	4972	98.0	101.8
96.3	4955.80	4927.80	4942	97.4	101.1
120.3	6181.76	6179.00	6180	121.8	101.2
120.3	6159.00	6154.80	6157	121.3	100.8
120.3	6164.00	6156.70	6160	121.4	100.9
144.4	7393.80	7416.7	7405	145.9	101.0
144.4	7432.40	7427.6	7430	146.4	101.4
144.4	7430.30	7443.7	7437	146.5	101.5
				Mean	101.2
				SD*	0.3
				%RSD**	0.3

*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

Mass (g)	Peak area 1	Peak area 2	Mean	Concentration (µg/ml)	%
0.8025	5045.6	5055.2	5050	85.6	106.6
0.8008	5026.7	5020.9	5024	85.1	106.3
0.8029	5035.2	5018.2	5027	85.2	106.1
1.0015	6330.6	6340.5	6336	107.3	107.2
1.0016	6250.9	6232.5	6242	105.7	105.6
1.0016	6245.3	6243.8	6245	105.8	105.6
1.2033	7535.7	7641.7	7589	128.6	106.8
1.2037	7622.0	7625.1	7624	129.2	107.3
1.2032	7595.8	7610.5	7603	128.8	107.1
				Mean	106.51
				SD*	0.61
				%RSD**	0.58

*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

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

*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

Mass (g)	Day 1	Day 2	Day 3	Between days
	107.2 105.6 105.6	105.7 104.5 105.3	105.6 104.9 105.1	
Mean	106.13	105.15	105.21	105.50
SD*	0.74	0.48	0.28	0.45
%RSD**	0.70	0.46	0.27	0.42

*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

Injection	Peak area	Retention time
1	1171.0	4.427
2	1168.7	4.418
3	1159.8	4.409
4	1164.0	4.413
5	1164.3	4.445
6	1156.8	4.465
Mean	1164	4.430
SD*	4.83	0.020
%RSD**	0.41	0.445

*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.

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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^{TM} 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)

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

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

Diclofenac salt	Quantity in grams (g) equivalent to 1 g of diclofenac free acid
DNa	1.074 g
DDEA	1.274 g
DHEP	1.375 g

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

Ingredient	Quantity
Diclofenac (free acid)	1.0 g
DMI	18.3 ml
Ethanol	0.9 ml
TEA	2.0 ml
PEG400	9.9 ml
Carbopol® Ultrez 20*	1.5 g
Water (ad)	100.0 g

*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

Ingredient	Quantity
Diclofenac (free acid)	1.0 g
DMI	3.5 ml
Ethanol	6.3 ml
TEA	2.0 ml
PEG400	4.2 ml
Carbopol® Ultrez 20*	1.5 g
Water (ad)	100.0 g

*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

Ingredient	Quantity
Diclofenac (free acid)	1.0 g
DMI	11.0 ml
Ethanol	19.8 ml
TEA	2.0 ml
PEG400	13.2 ml
Carbopol® Ultrez 20*	1.5 g
Water (ad)	100.0 g

*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 H_3PO_4, 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.

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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 *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 *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 (KH_2PO_4, 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 KH_2PO_4 solution until a pH of 7.4 was reached. 10% NaOH or 10% KH_2PO_4 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

Diclofenac salt	Average percentage released after 6 h	Average cumulative concentration after 6 h (µg/cm^2)	Median cumulative concentration after 6 h (µg/ml)
DNa-O	8.23 ± 0.94	1530.70 ± 175.34	1554.66
DNa-H	8.38 ± 0.57	1557.39 ± 105.59	1574.86
DNa-L	2.98 ± 0.48	553.89 ± 89.27	560.78
DDEA-O	8.06 ± 0.24	1498.42 ± 230.08	1577.01
DDEA-H	7.90 ± 1.08	1468.05 ± 200.74	1403.07
DDEA-L	3.48 ± 0.60	647.64 ± 110.61	628.15
DHEP-O	8.01 ± 1.05	1488.75 ± 195.68	1489.50
DHEP-H	8.23 ± 0.99	1530.72 ± 183.91	1541.25
DHEP-L	3.76 ± 0.47	699.75 ± 86.79	662.86

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

Diclofenac salt	Average percentage diffused after 12 h	Average flux (µg/cm^2.h)	Average cumulative concentration after 12 h (µg/ml)	Median flux (µg/cm^2.h)	Median cumulative concentration after 12 h (µg/ml)
DNa-O	0.03 ± 0.01	0.46 ± 0.10	5.33 ± 1.20	0.43	5.13
DNa-H	0.09 ± 0.01	1.42 ± 0.20	16.60 ± 2.39	1.34	15.76
DNa-L	0.01 ± 0.00	0.20 ± 0.04	2.38 ± 0.40	0.19	2.24
DDEA-O	0.01 ± 0.00	0.18 ± 0.03	2.18 ± 0.30	0.18	2.13
DDEA-H	0.09 ± 0.02	1.34 ± 0.32	16.05 ± 3.54	1.26	14.95
DDEA-L	0.01 ± 0.00	0.23 ± 0.04	2.63 ± 0.46	0.24	2.76
DHEP-O	0.01 ± 0.00	0.20 ± 0.05	2.32 ± 0.59	0.19	2.19
DHEP-H	0.08 ± 0.02	1.30 ± 0.28	15.18 ± 3.20	1.23	14.25
DHEP-L	0.01 ± 0.00	0.17 ± 0.05	2.01 ± 0.62	0.16	1.95

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.

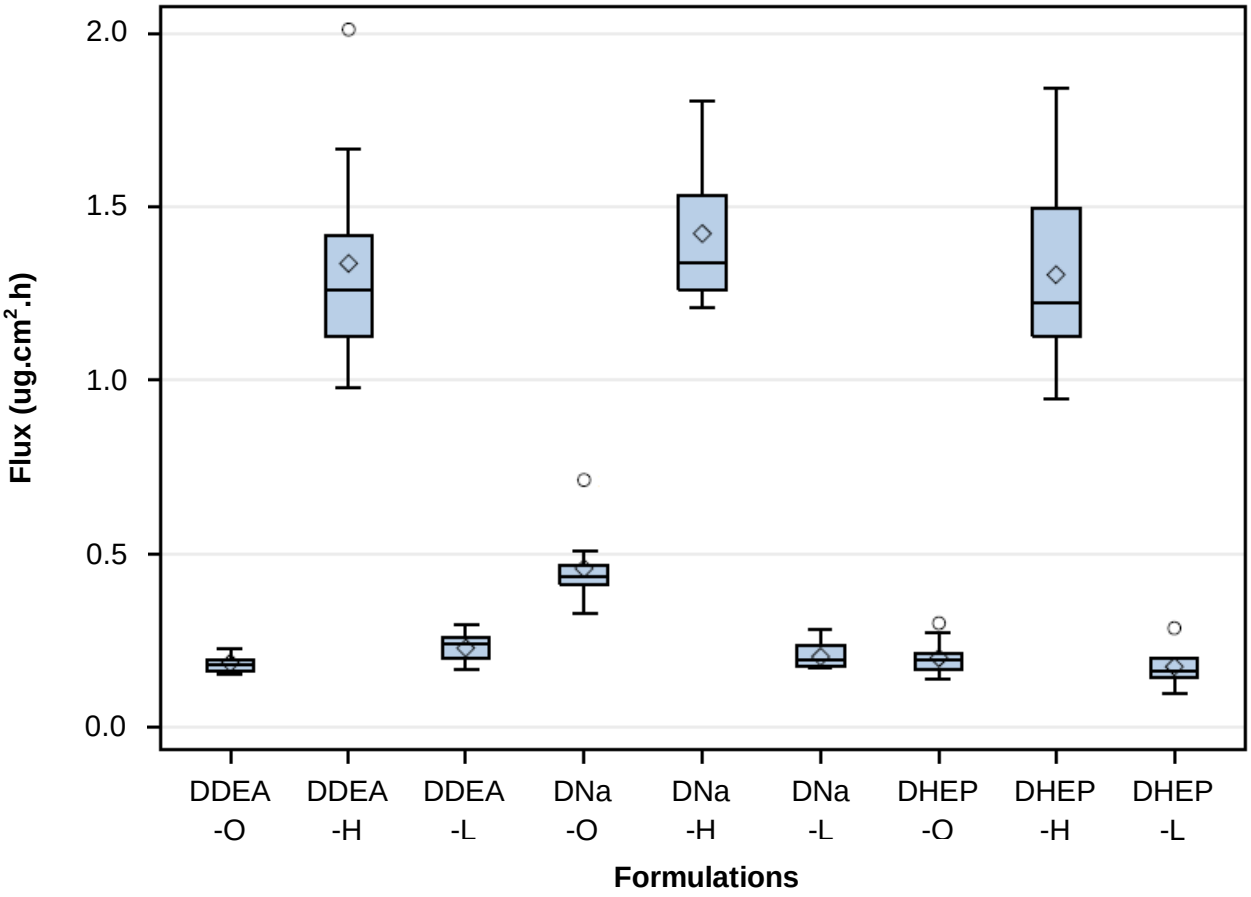

Figure C.1: Box plots of flux values of nine emulgel formulations after topical application, illustrating the median (indicated with a horizontal line in the box) and average flux (indicated with diamond) of diclofenac.

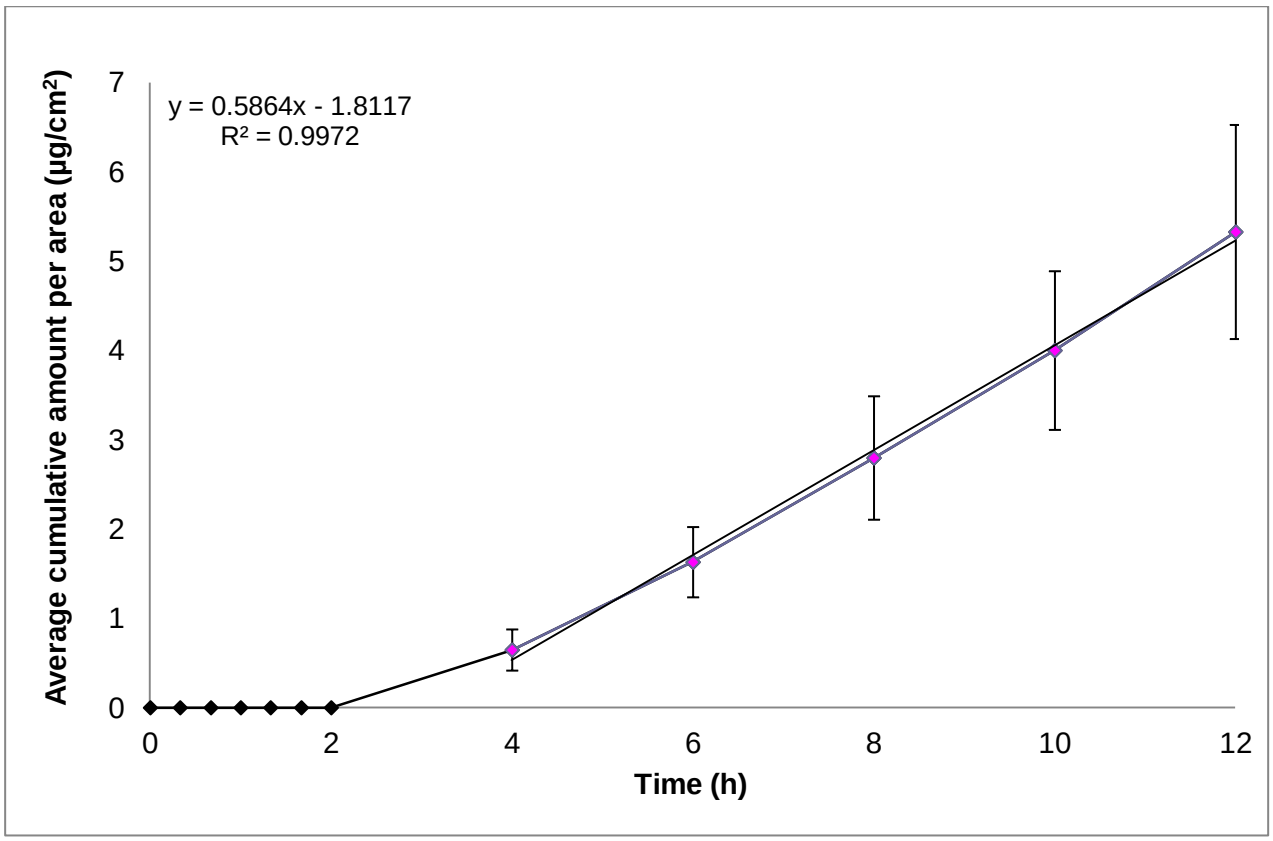

Figure C.2: Average cumulative concentrations of diclofenac per area (µg/cm^2) for the DNa-O emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

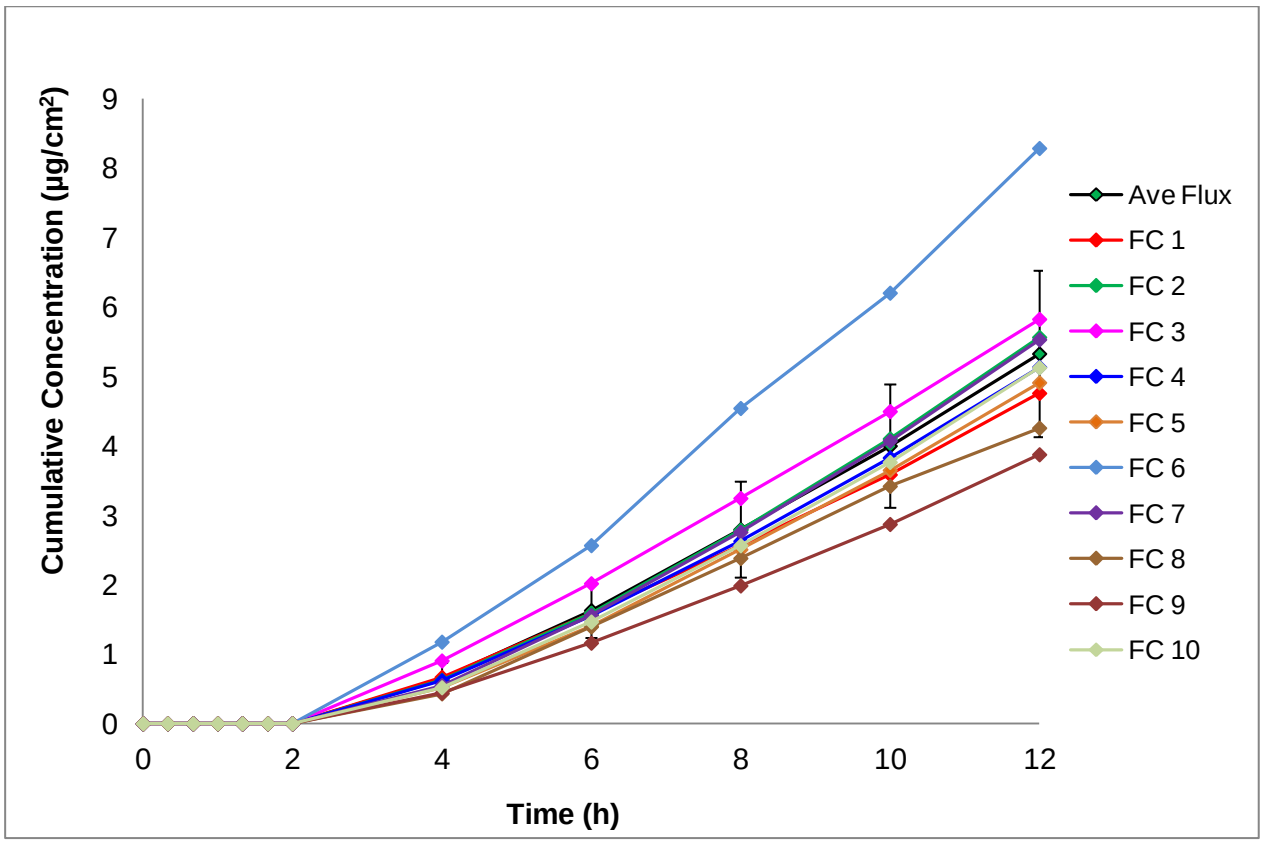

Figure C.3: Cumulative concentrations of diclofenac per area (µg/cm^2) for each individual DNa-O emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

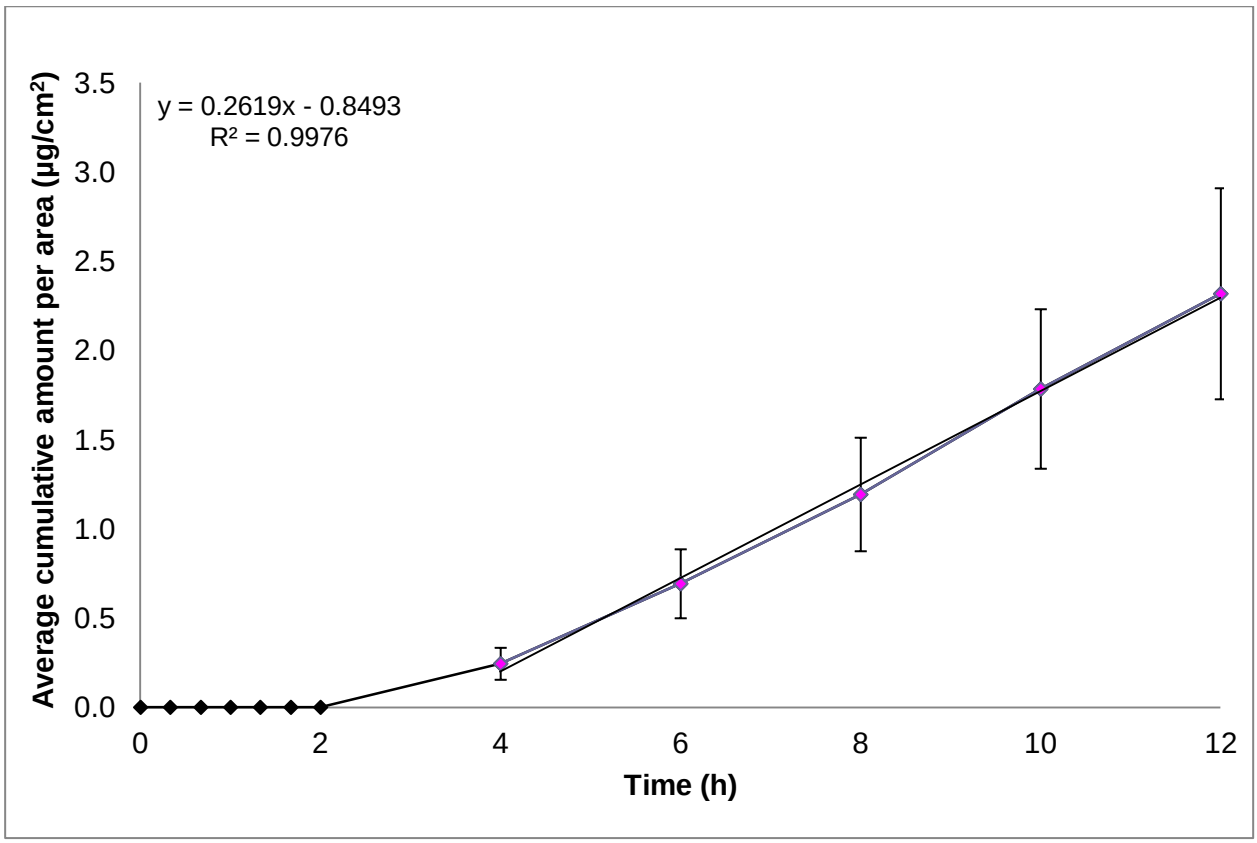

Figure C.4: Average cumulative concentrations of diclofenac per area (µg/cm^2) for the DHEP-O emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

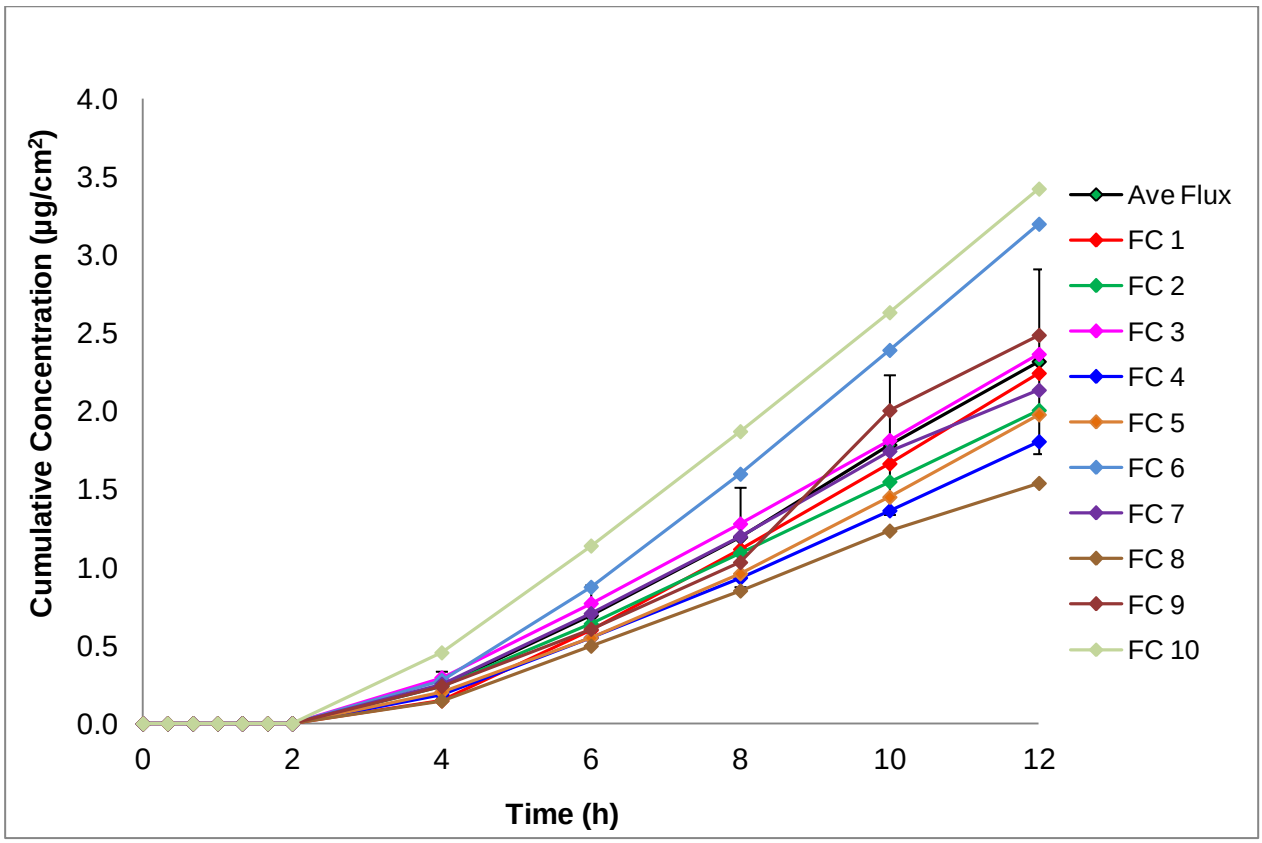

Figure C.5: Cumulative concentrations of diclofenac per area (µg/cm^2) for each individual DHEP-O emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

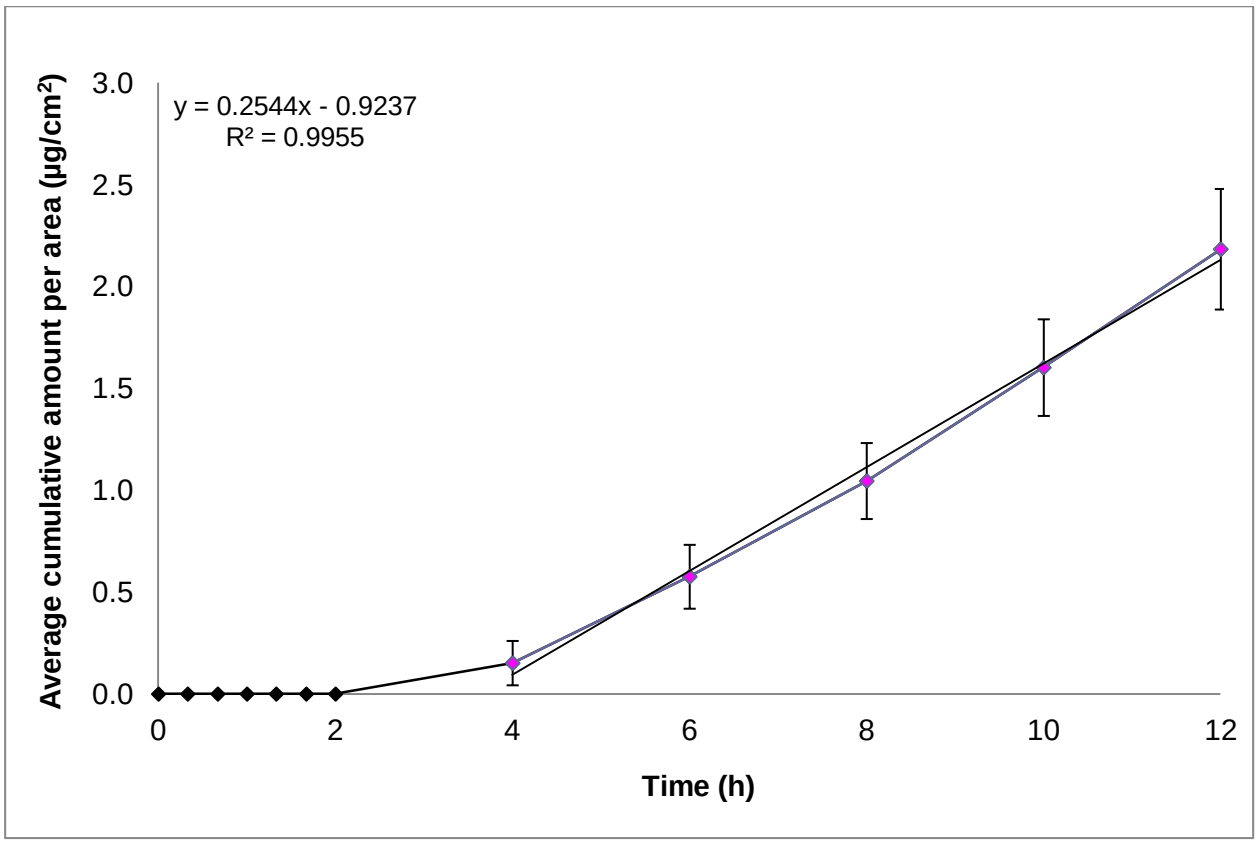

Figure C.6: Average cumulative concentrations of diclofenac per area (µg/cm^2) for the DDEA-O emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

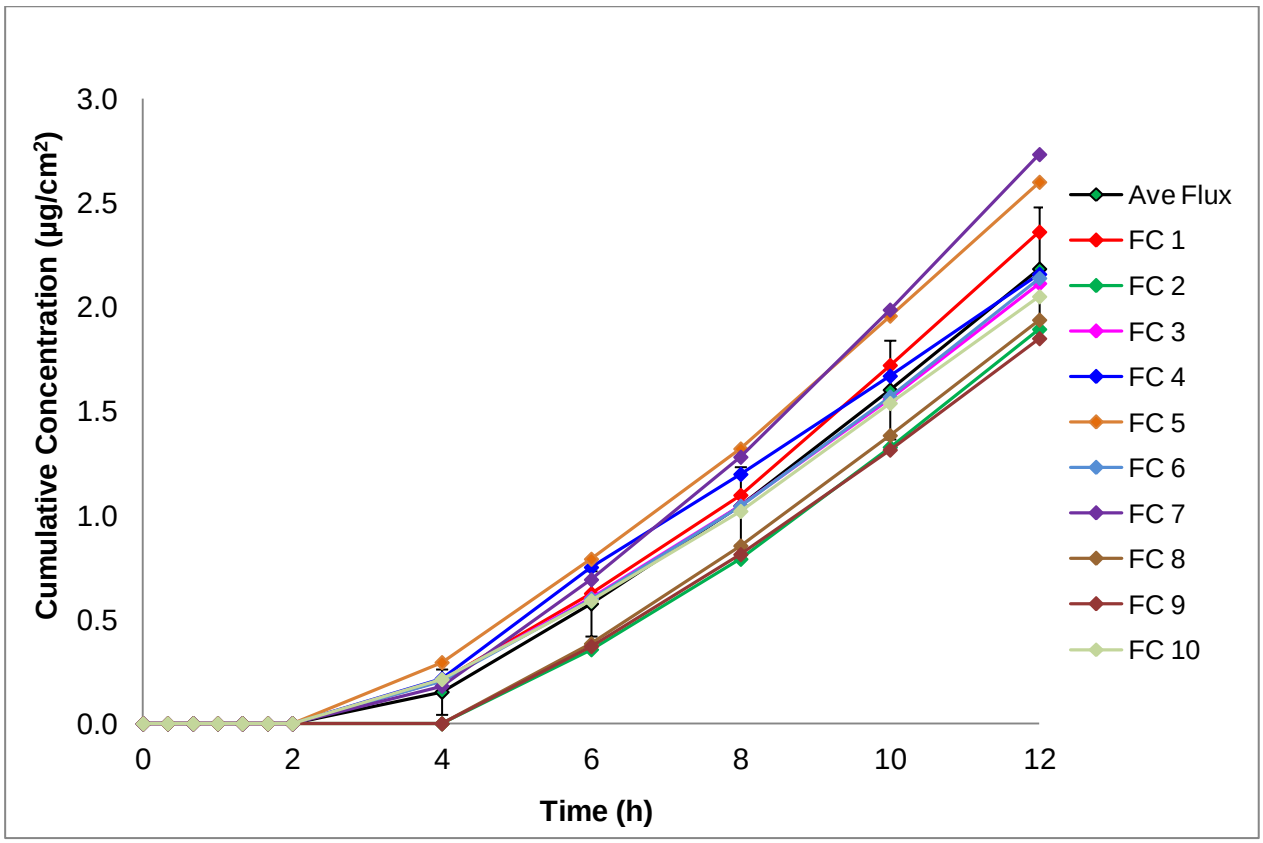

Figure C.7: Cumulative concentrations of diclofenac per area (µg/cm^2) for each individual DDEA-O emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

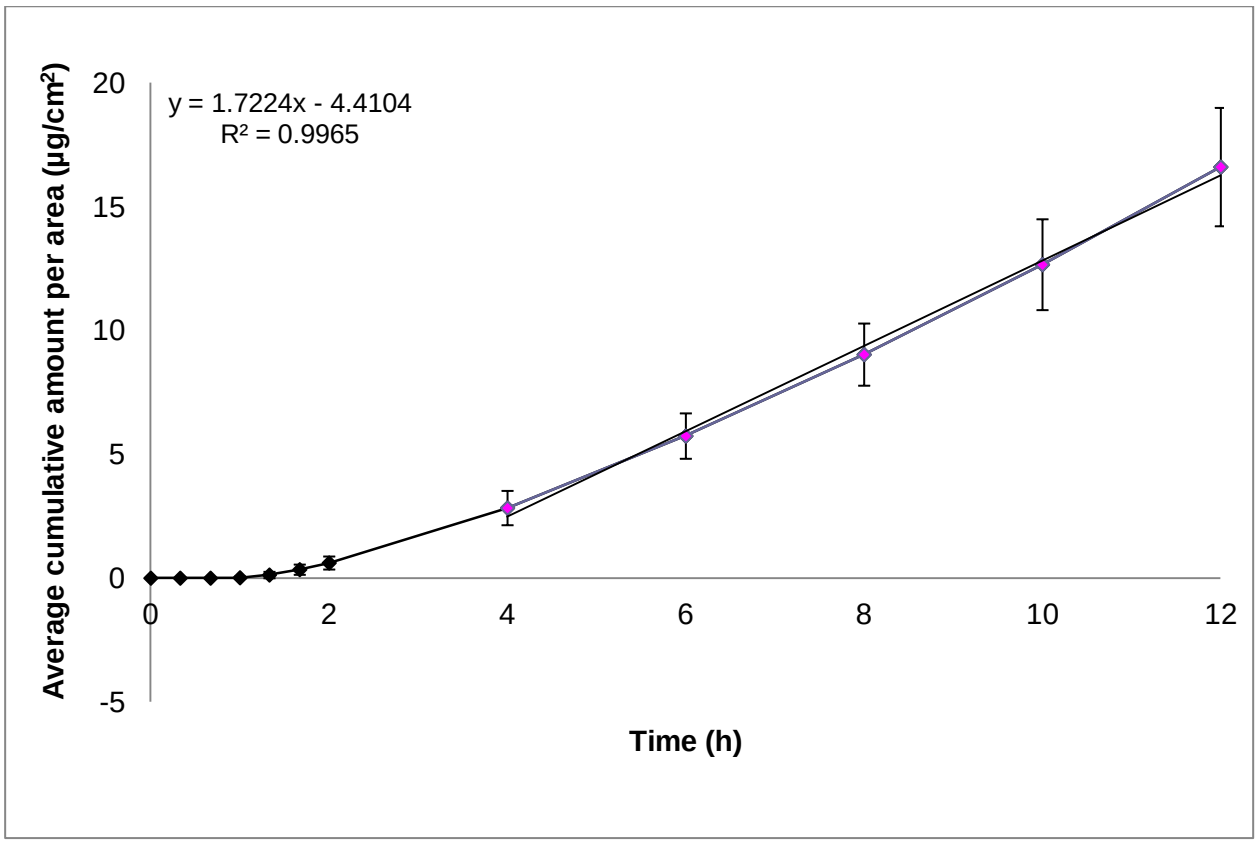

Figure C.8: Average cumulative concentrations of diclofenac per area (µg/cm^2) for the DNa-H emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

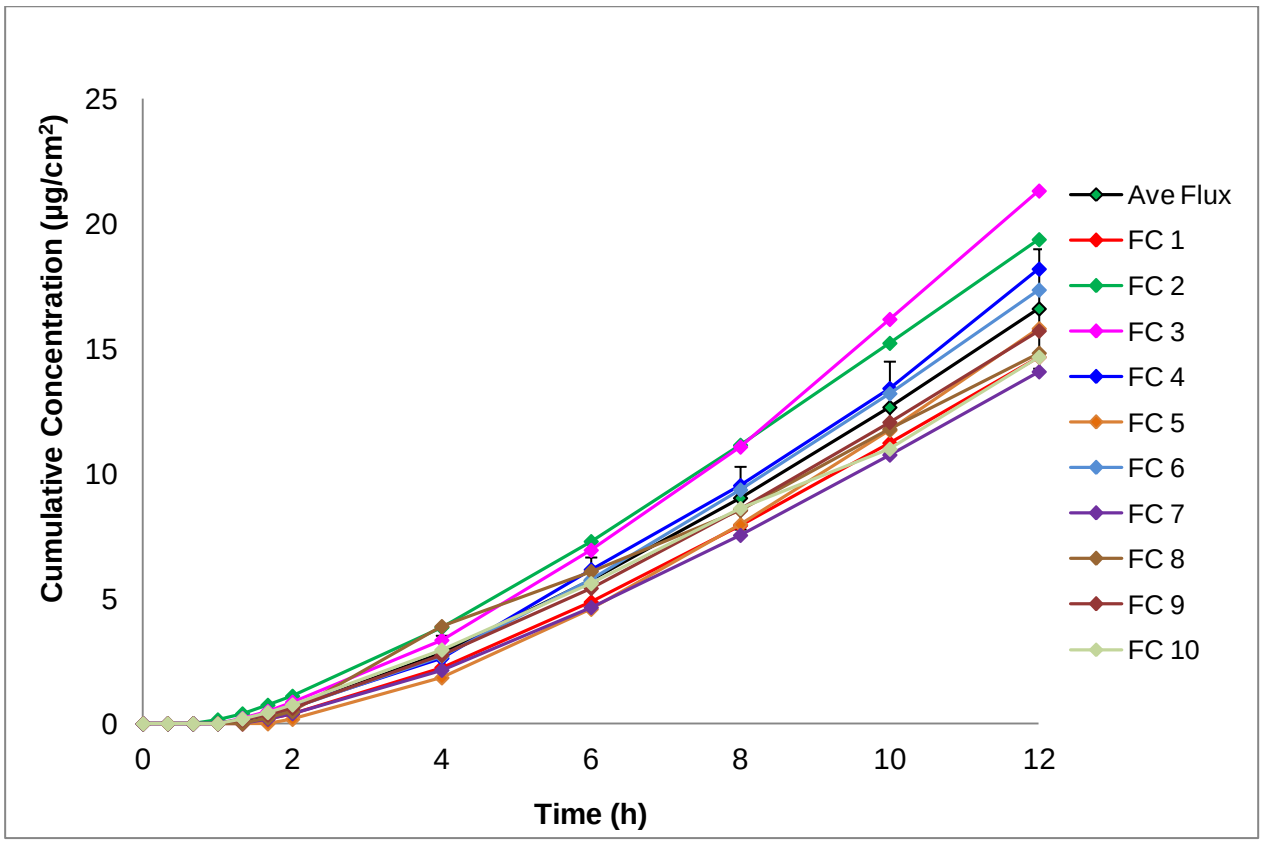

Figure C.9: Cumulative concentrations of diclofenac per area (µg/cm^2) for each individual DNa-H emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

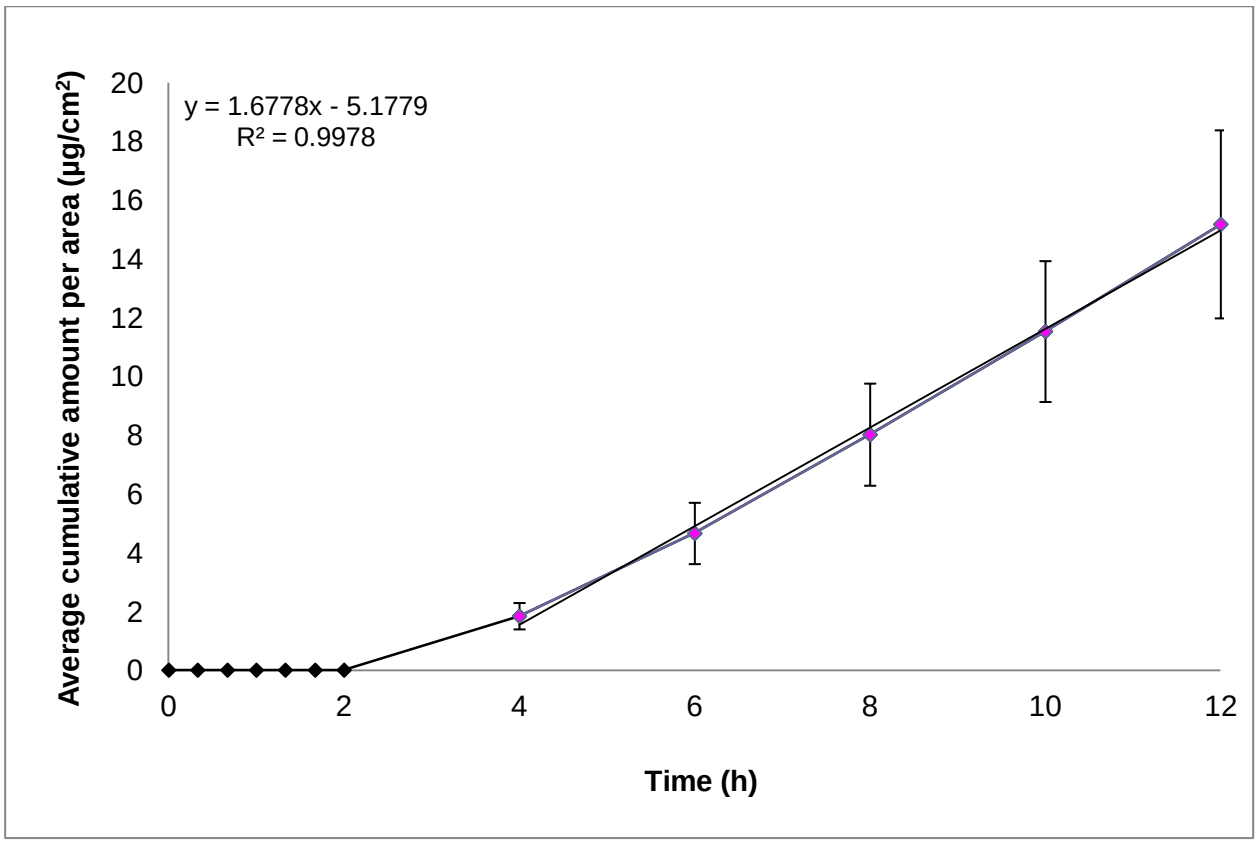

Figure C.10: Average cumulative concentrations of diclofenac per area (µg/cm^2) for the DHEP-H emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

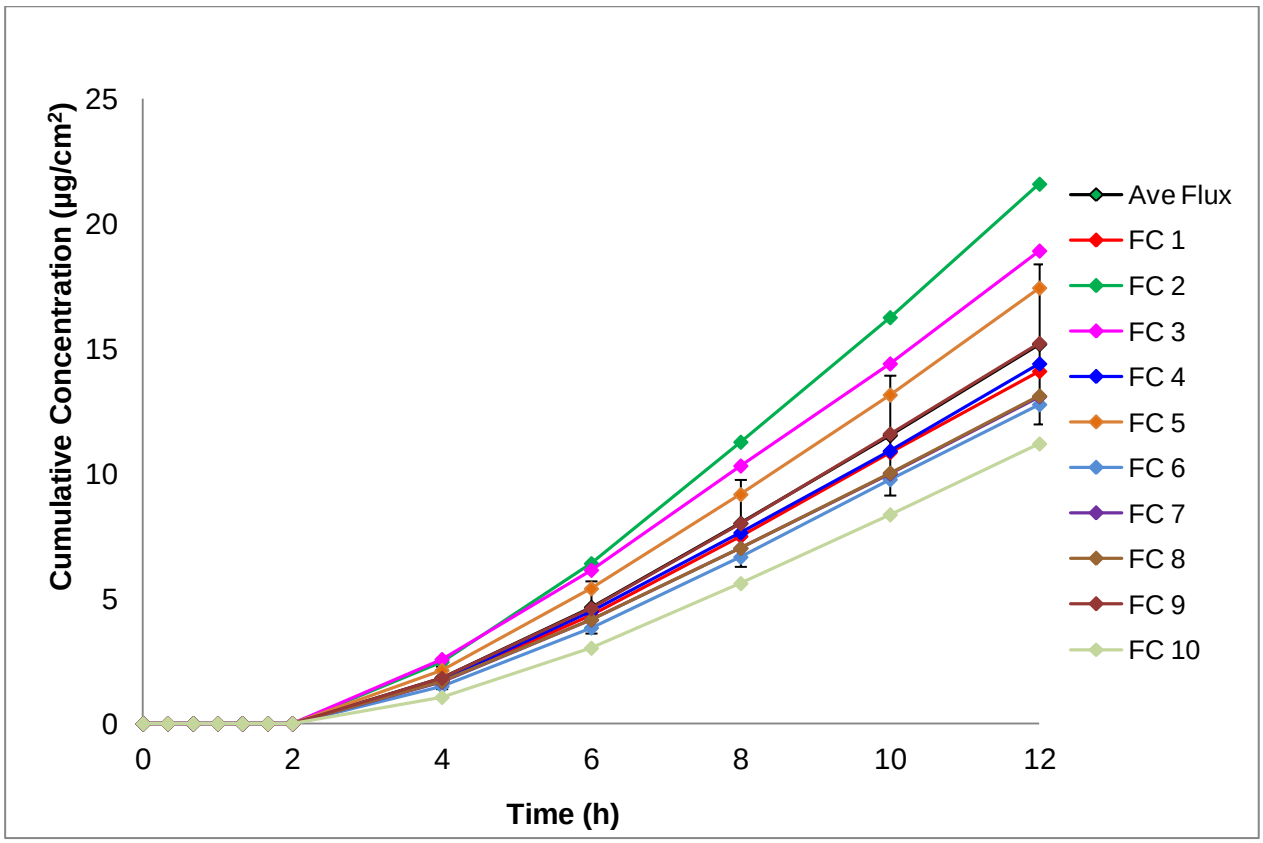

Figure C.11: Cumulative concentrations of diclofenac per area (µg/cm^2) for each individual DHEP-H emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

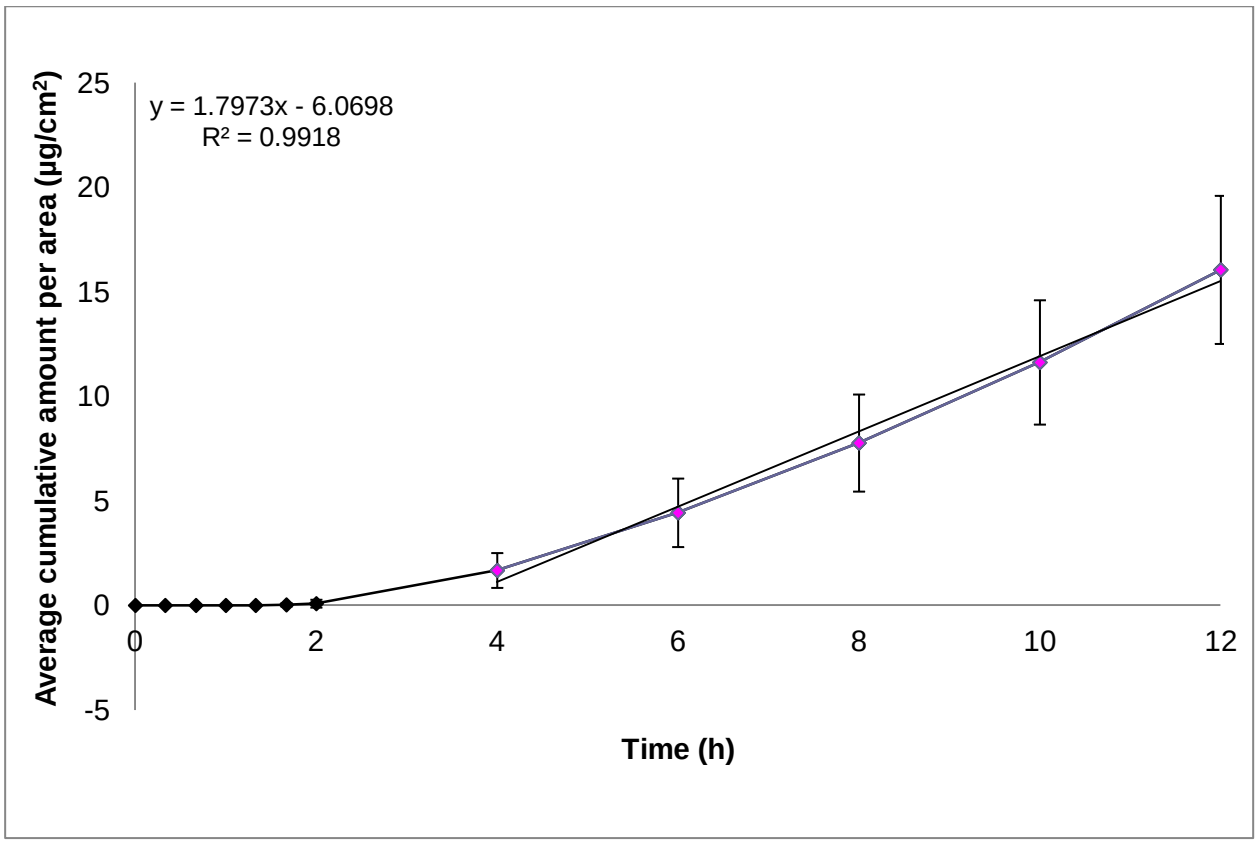

Figure C.12: Average cumulative concentrations of diclofenac per area (µg/cm^2) for the DDEA-H emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 9).

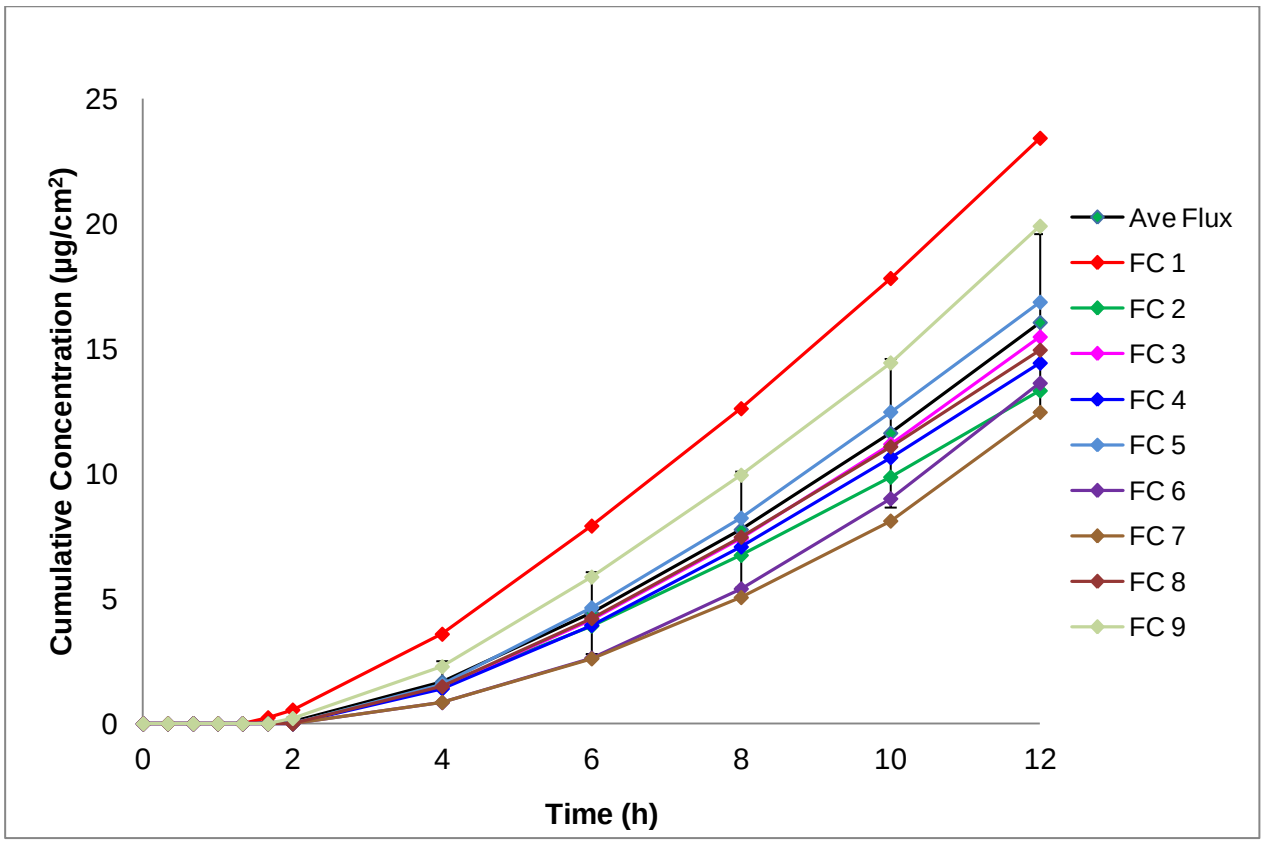

Figure C.13: Cumulative concentrations of diclofenac per area (µg/cm^2) for each individual DDEA-H emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

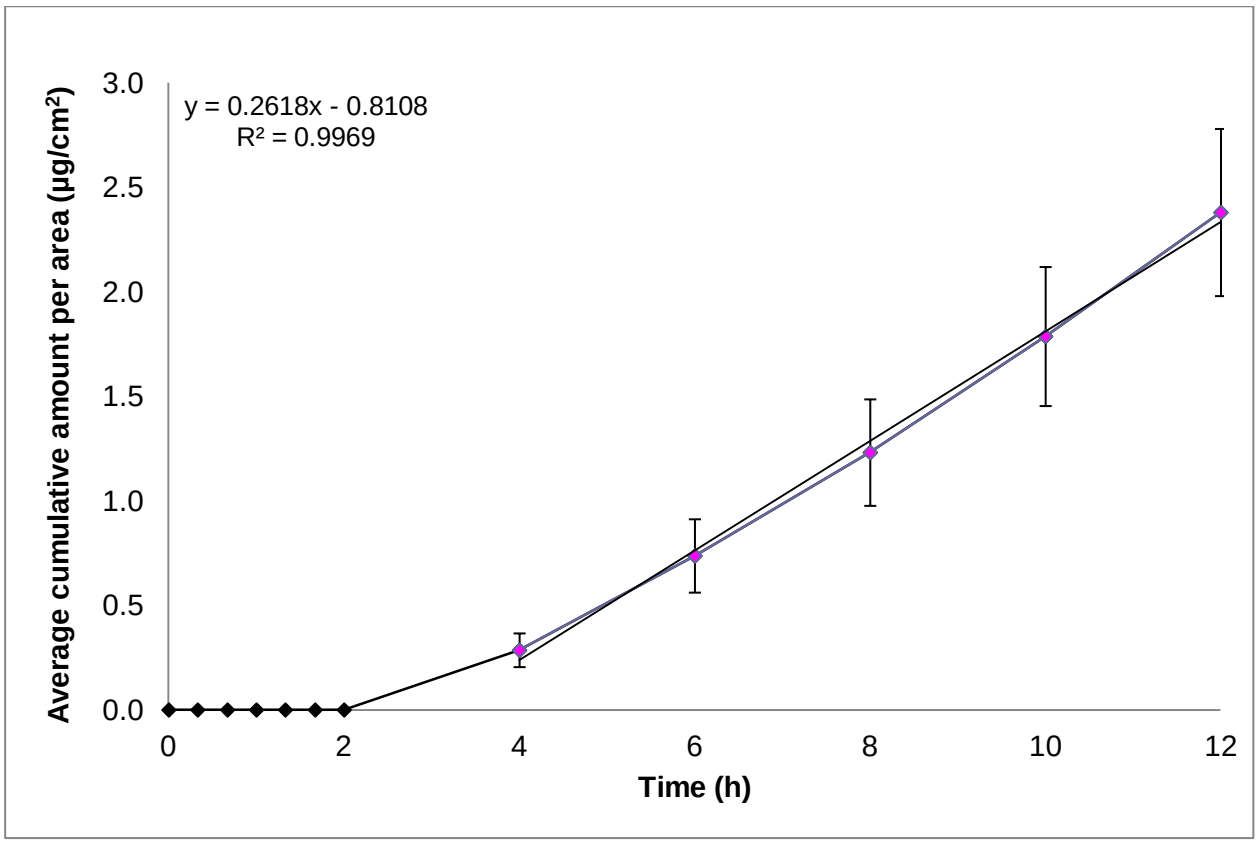

Figure C.14: Average cumulative concentrations of diclofenac per area (µg/cm^2) for the DNa-L emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

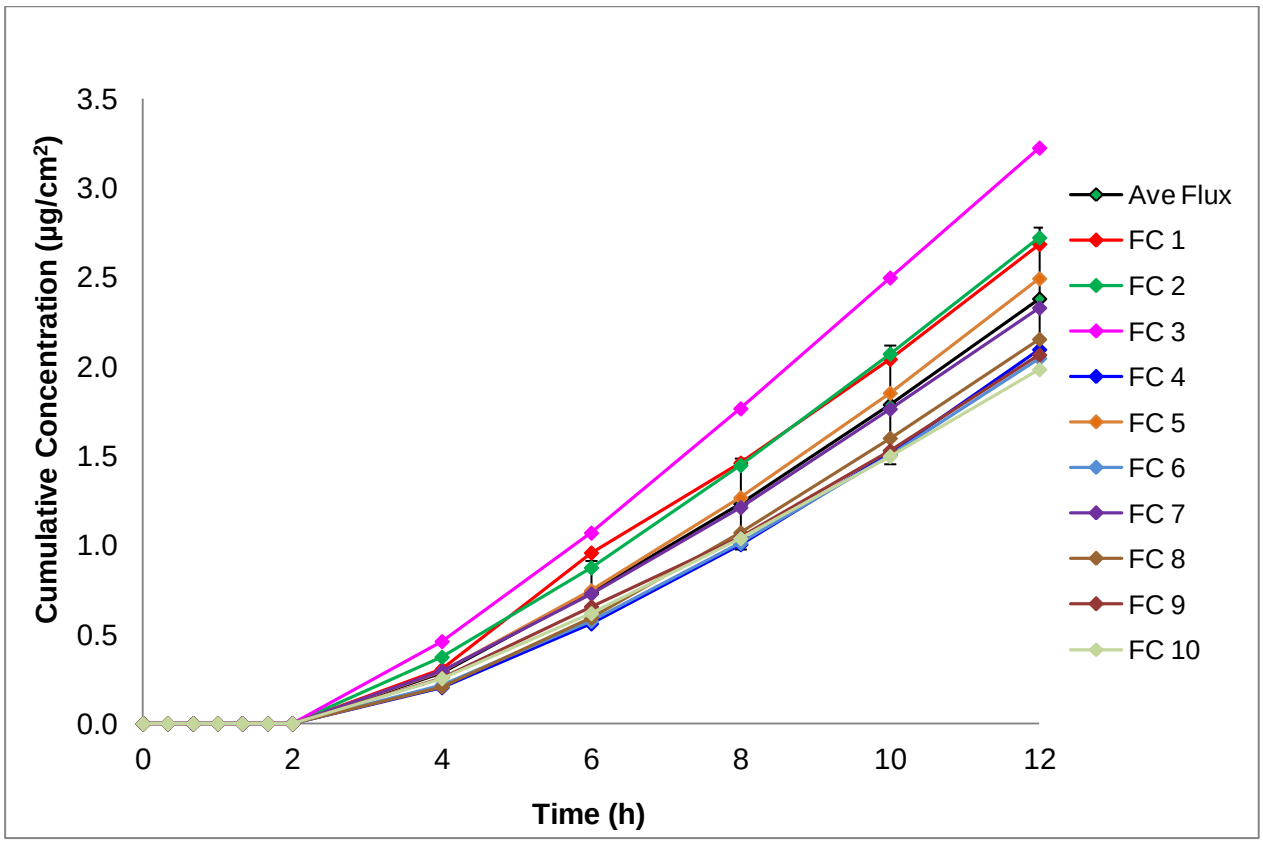

Figure C.15: Cumulative concentrations of diclofenac per area (µg/cm^2) for each individual DNa-L emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

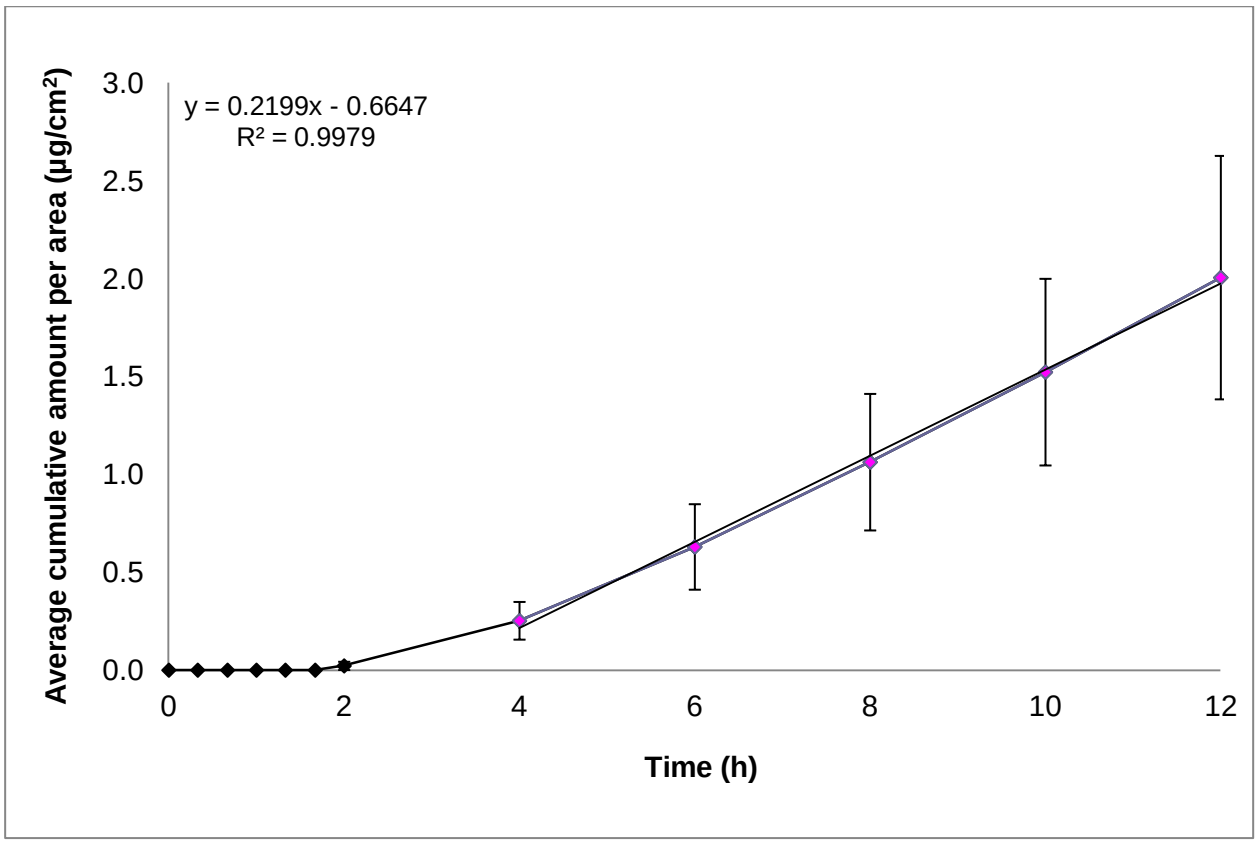

Figure C.16: Average cumulative concentrations of diclofenac per area (µg/cm^2) for the DHEP-L emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 9).

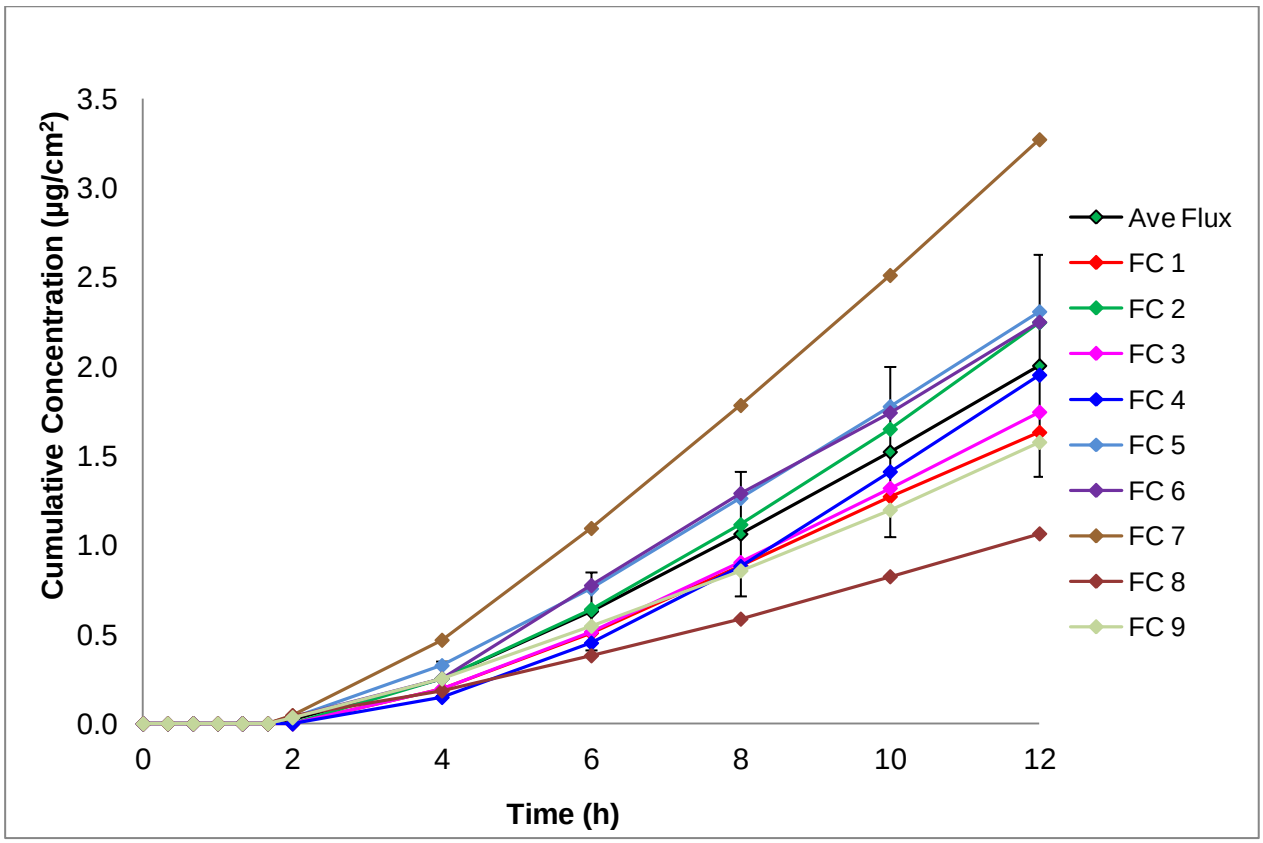

Figure C.17: Cumulative concentrations of diclofenac per area (µg/cm^2) for each individual DHEP-L emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

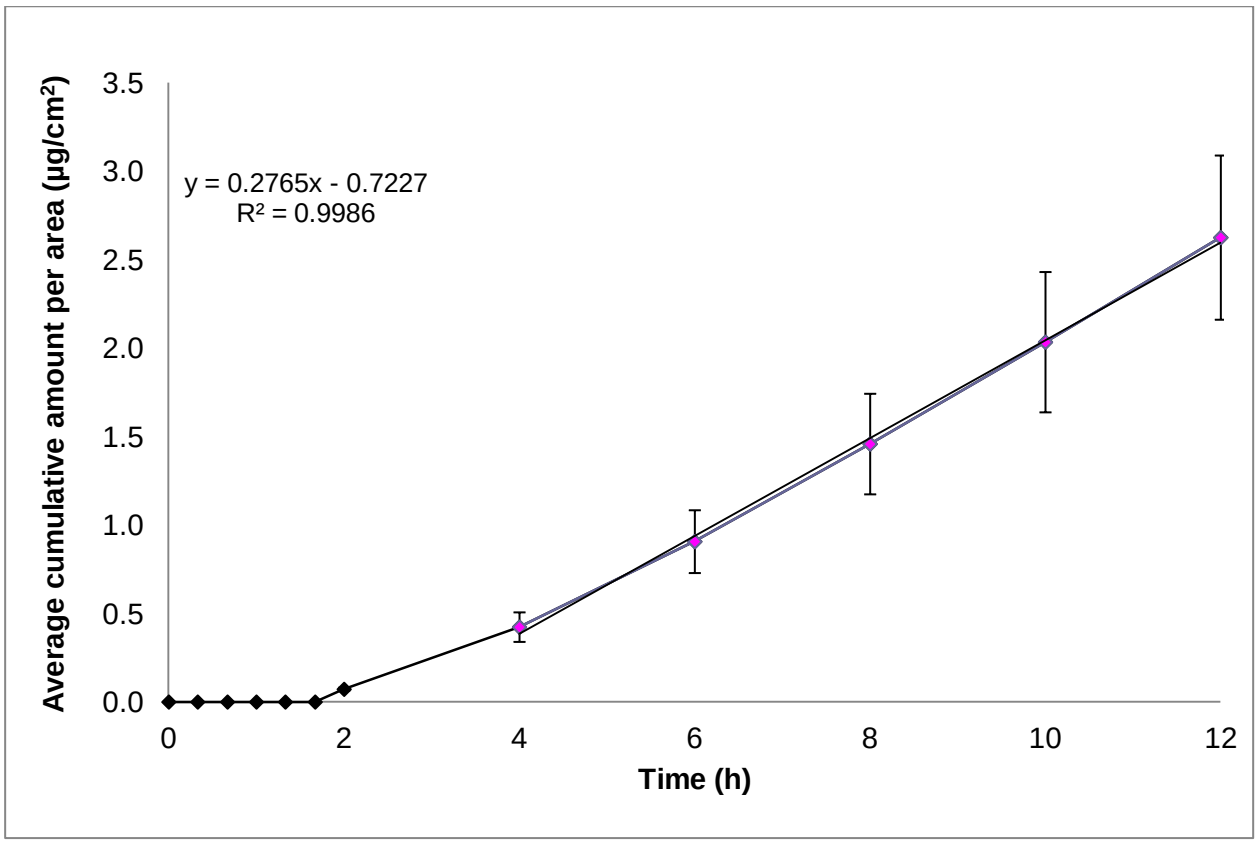

Figure C.18: Average cumulative concentrations of diclofenac per area (µg/cm^2) for the DDEA-L emulgel that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

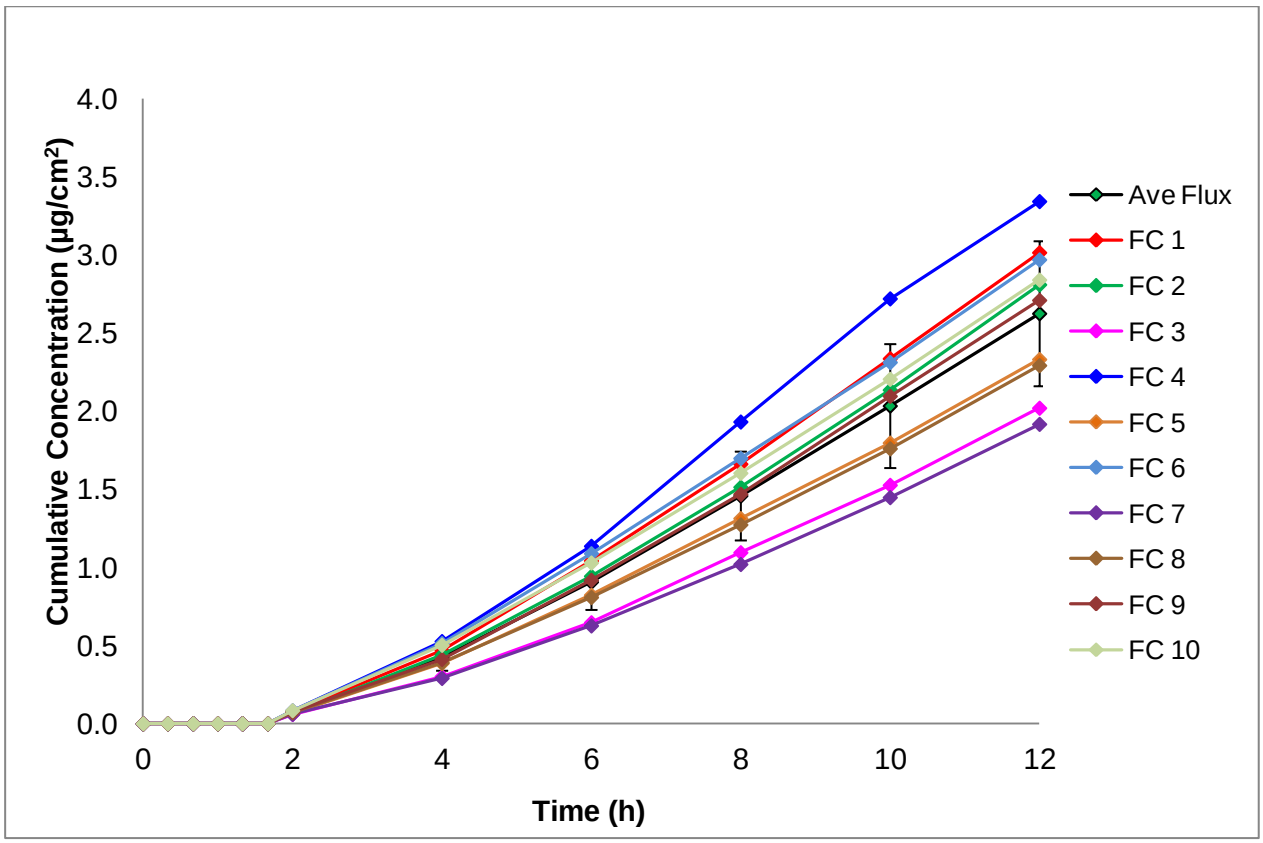

Figure C.19: Cumulative concentrations of diclofenac per area (µg/cm^2) for each individual DDEA-L emulgel sample that had permeated the skin between 4 - 12 hours as a function of time (n = 10).

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 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

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

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/cm^2.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 trandermal dosage forms (Fini *et al*., 1998:11; Fini *et al*., 1999:166; Rossiter, 2012:391-392,403).

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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

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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

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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.

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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.

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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.

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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

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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.

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