The effect of finite dose of ibuprofen on transdermal delivery

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ABSTRACT

Transdermal drug delivery has the advantage over other routes of administration of avoiding the hepatic, first-pass metabolism that would result in better therapeutic efficacy, better patient medication compliance and reduced systemic side-effects (Kydoneieus & Berner, 1987:69). One disadvantage of this mode of drug delivery is the generally poor delivery of drugs through the skin. The intercellular lipid structure of the stratum corneum causes this membrane to be an excellent penetration barrier, which must be breached to enhance drug penetration through the skin.

Factors influencing the drug-skin distribution include the physicochemical properties of the drug, the choice of the delivery vehicle and the drug application mode (finite and infinite dose) being used (Chen *et al*., 2011:224). The permeability of the skin is thus influenced by the physicochemical properties of both the permeant and the penetration enhancer (Dias *et al*., 2007:65).

One way of overcoming this barrier function of the skin is to include penetration enhancer chemicals in the topical application. Such penetration enhancers partition into the stratum corneum and interact with the intercellular lipids, causing a temporary and reversible decrease in this barrier function. With the skin barrier function being reduced, drug transport through the skin increases (Magnusson *et al*., 2001:206). When a drug or the penetration enhancer vehicle does not have the ideal physicochemical properties, penetration through the skin is difficult and manipulation of the drug or the vehicle is necessary. By manipulating their physicochemical properties, or by making use of penetration enhancers, the transdermal absorption through the skin can be increased (Park *et al*., 2000:109).

Chemical penetration enhancers use different mechanisms of action to increase permeation across the skin (Moser *et al*., 2001:110). When chemical enhancers are used in combination, a synergistic action between these enhancers offers a method of overcoming limitations being experienced when single chemical enhancers are used in improving transdermal drug delivery (Williams & Barry, 2004:604). As mentioned, both the choice of vehicle and the physicochemical properties of the permeant and vehicle should be considered during drug skin distribution studies, as well as the mode of application (finite or infinite dose).

When consumers use commercial formulations to treat topical skin conditions, the vehicles applied are in varying doses lower than 30 mg/cm^2, depending on the application. In clinical situations, the formulation being applied depends on the body surface area being treated, i.e. the larger the surface area, the lower the amount of vehicle applied.

When a cold sore is treated, for example, an average amount of 20 mg/cm^2 of the vehicle is applied to the infected area, with the treated surface area generally being very small (Trottet *et al*., 2004:214). When sunscreens are applied to the skin, a very large surface area is treated and an average amount of only 0.5 mg/cm^2 is applied (Azurdia *et al*., 1999:255; Bech & Wulf, 1992:242). It is thus clear that the transdermal absorption of an active compound depends on the concentration being applied and the surface area treated. Considering the above parameters is thus of high significance when *in vitro* transdermal diffusion tests are performed.

Risk assessment studies comprise another important area in which clinical relevant dose plays a significant role when data on transdermal absorption of a substance is produced.

During *in vitro* diffusion studies to determine the permeability profile of an active compound, an "infinite dose(s)" (> 150 µl) of the vehicle is applied to the membrane. One shortcoming of the infinite dose application is that in some instances it may fail to imitate the levels of active compound being applied to the skin when commercial formulations are applied. It may also fail to imitate exposure levels to toxic chemicals. Results from *in vitro* studies would differ from those obtained during *in vivo* studies, if the clinically applied concentrations are not taken into account.

The aims of this study were to determine the penetration enhancement effects of different penetration enhancer vehicles on the permeation of lipophilic ibuprofen through synthetic Carbosil® membrane, when used individually and in multi-component solvent mixtures, as well as to determine the effects of finite (< 150 µl) dose applications of these enhancers on the delivery of ibuprofen.

In order to achieve the aims of this study, the objectives were to determine the permeation of lipophilic ibuprofen through Carbosil® membrane by using:

- Water and propylene glycol as penetration enhancer vehicles in combinations of 0/100 (v/v), 20/80 (v/v), 50/50 (v/v), 80/20 (v/v) and 100/0 (v/v);
- Mineral oil and Miglyol® as penetration enhancer vehicles in combinations of 0/100 (v/v), 20/80 (v/v), 50/50 (v/v), 80/20 (v/v) and 100/0 (v/v); and ;
- These penetration enhancer vehicles individually and in multi-component mixtures at different finite and infinite volumes, i.e. 2 µl, 5 µl, 10 µl, 20 µl, 50 µl, 150 µl, 250 µl, 500 µl and 1,000 µl; and to determine
- Which of the single or multi-component penetration enhancer vehicles would show the best transdermal delivery enhancement effect.

The solvents used all have different mechanisms of action by which they enhance penetration of drugs through skin. By using these solvents in combination, the expectation was that they would have a synergistic effect that would be higher than the penetration enhancement effect achieved with each individual solvent (Williams & Barry, 2004:604).

The outcomes of this study were as follows: The results for infinite dose applications of water and propylene glycol clearly showed that the best penetration enhancement of ibuprofen was achieved with 100% propylene glycol as the delivery vehicle. Contrary, water showed very little penetration enhancement properties for this drug. Results from this study also showed that the penetration enhancement effect of propylene glycol increased as the percentage of the propylene glycol in the solvent vehicle increase. This could have been as a result of the mechanism of action of propylene glycol to partition into the membrane and to increase the solubility of the permeant in and diffusion through the membrane (Squillante *et al.,* 1998:266). Chen *et al.* (2011:224) suggest that the higher the applied volume, the larger the surface area being covered by the vehicle and the thicker the layer of the vehicle solvent on the surface of the membrane, which would result in an increase in the hydration of the membrane, which in turn would increase the permeability of the membrane for the drug.

Application of finite doses of the single penetration enhancer solvent, propylene glycol, achieved the highest penetration enhancement effect, with the ibuprofen concentration of diffused ibuprofen being the highest with this solvent. The concentration of diffused ibuprofen that had been delivered from application of a finite dose of the water delivery vehicle could not be measured, due to the lipophilic nature of ibuprofen (log $P_{o/w}$ of 3.6) (Beetge *et al*., 2000:164) and the low solubility of ibuprofen in water, resulting in permeation concentrations that were immeasurable.

Results for the infinite dose applications of the lipophilic, single phase solvents of 100% mineral oil and 100% Miglyol®, showed the lowest penetration enhancement effects, compared to all multi-component combinations of these two enhancer vehicles. Multi-component mixtures of these solvents also showed very similar permeation profiles for ibuprofen. This could have been as a result of synergistic action between the two penetration enhancer solvents if used in combination. According to Moser *et al*. (2001:106), Miglyol® is known to modify the intercellular lipids of the stratum corneum, causing disruption of the barrier properties thereof and hence an increase in diffusivity through the membrane. Since Carbosil® membrane and human epidermis share a common solubility-diffusion mechanism of drug transport, it can be hypothesised that the Miglyol® would change the polar structure of the membrane and as a result enhance the permeability of substances through the membrane. Mineral oil is a lipophilic solvent and while Miglyol® modifies the heteropolar structure of the membrane to make it more viable to

penetration, mineral oil would carry the active to the lipophilic section of the membrane and as a result enhance the permeation of the lipophilic drug, ibuprofen (Hori *et al.,* 1991:33).

Results for finite dose applications of these solvents clearly showed that the 20/80 (v/v) mineral oil/Miglyol® combination achieved the best penetration enhancement effect for ibuprofen, compared to all other mineral oil and Miglyol® solvents, individually and in combination. Solvents and solvent mixtures containing 100% Miglyol®, 50/50 (v/v) and 80/20 (v/v) mineral oil/Miglyol® all showed similar penetration enhancement effects with finite dose applications. With solvent type permeant preparations applied to a membrane, three types of penetration influencing parameters should be taken into account, i.e. (a) thermodynamic effects resulting from different permeant solubilities in the different vehicles, (b) penetration enhancing effects between the vehicle and the membrane, (c) permeant depletion in the vehicle in the case of finite dose conditions. The extent of permeant depletion in the vehicle depends on the thickness of the applied solvent layer on the surface of the membrane (Leopold, 1998:167).

The results from this study confirmed the observations by Williams and Barry (2004:605) that:

1. Penetration enhancer properties appear to be drug specific (permeants with similar physico-chemical properties).
2. Penetration enhancers tend to work well with co-solvents, such as propylene glycol.
3. Most penetration enhancers have a complex concentration dependent effect.
4. Potential mechanisms of action of penetration enhancer solvents are different and can range from direct effects on the skin to modification of the formulation.

The outcomes of this study showed that increased levels of a penetration enhancer solvent, like propylene glycol in the delivery vehicle, not only increases the penetration of the active through the membrane, but it also improves penetration of the active through the membrane from finite dose applications.

The permeation profiles of the lipophilic, single phase mineral oil and Miglyol®, and combinations thereof, showed that permeation of the lipophilic ibuprofen was higher with small application volumes of these delivery vehicles. Chen *et al*. (2011:224) report that with an infinite dose application, the donor compartment is filled with a thick liquid layer covering the surface of the membrane, having a height of 1.6 mm, while the finite dose application forms only a thin layer of 0.1 mm. As a result, the hydration levels of the membrane are higher with infinite dose applications, which facilitate higher permeability of the membrane (Chen *et al*., 2011:224). Increased membrane hydration appears to increase the diffusion of both hydrophilic and low lipophilic compounds, due to the partitioning of the active into the membrane (Williams & Barry, 2004:605). This hydration effect on the membrane makes penetration of hydrophilic

compounds through the membrane easier, whilst making it more difficult for strongly lipophilic compounds (log P >2) to partition into the hydrated membrane (Zhang *et al*., 2010:895). Ibuprofen is a strongly lipophilic drug (log P = 3.6) (Beetge *et al*., 2000:164), hence the lower permeation results for infinite dose applications. Except for mineral oil that showed higher permeation levels with larger volumes, as a result of the lipophilic nature of both mineral oil and ibuprofen, the permeability of the lipophilic active increased as the membrane became more hydrated with the lipophilic solvent when larger volumes were applied.

From the findings in this study it has become evident that:

- The lipophilic/hydrophilic nature of the solvent and the permeant play a significant role in the absorption of a permeant through the membrane. This is an important factor in risk assessment studies, especially;
- If the membrane is hydrated with a lipophilic delivery vehicle while carrying a lipophilic toxic permeant, the effect may be more harmful at lower levels of exposure; Lipophilic ibuprofen showed higher permeation levels with small application volumes of Miglyol® and of lipophilic mineral oil/Miglyol® combination delivery vehicles.
- When a lipophilic toxic permeant comes in contact with a hydrophilic delivery vehicle, like propylene glycol and water, the effect may not be as significant even with high levels of exposure; and
- The nature of the delivery vehicle and the permeant, as well as the level of exposure or application, play enormous roles in the prediction of permeant absorption through Carbosil® membrane or the skin.

Keywords: Transdermal, Penetration enhancers, Finite dose, Infinite dose, Carbosil® membrane.

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UITTREKSEL

Die akkurate voorspelling van transdermale absorbsie van ‘n topikaal-aangewende substans en van ongewensde chemikalië in die omgewing, soos in die werksplek, is van uiterste belang vir beide formuleringsontwikkeling en risiko-analise (Gre’goire *et al*., 2009:80).

Die transdermale roete vir geneesmiddelaflewering hou voordele bo ander roetes van toediening in, deurdat dit die hepatiese, eerste deurgangsmetabolisme vermy, wat tot beter terapeutiese effektiwiteit, beter pasiëntnakoming, asook minder sistemiese newe-effekte kan aanleiding gee (Kydoneieus & Berner, 1987:69). . Een nadeel van hierdie roete van toediening is die swak aflewering van geneesmiddels deur die vel. Die intersellulêre lipiedstruktuur van die stratum korneum veroorsaak dat hierdie membraan uitstekende weerstand teen eksterne faktore bied, wat oorkom moet word ten einde geneesmiddelaflewering deur die vel te kan bevorder.

Faktore wat die verspreiding van geneesmiddels in en deur die vel beïnvloed is die fisies-chemiese eienskappe van die geneesmiddel, die tipe afleweringsisteem wat gebruik word en die wyse waarop die geneesmiddel aangewend word (klein en hoë dosisse) (Chen *et al*., 2011:224). Die deurlaatbaarheid van die vel word dus deur die fisies-chemiese eienskappe van beide die aktiewe bestanddeel en die penetrasie-bevorderaar / afleweringsisteem / deurlaatbaarheidsvoertuig beïnvloed (Dias *et al*., 2007:65).

Een van die wyses waarop hierdie skansfunksie van die vel oorkom kan word is om penetrasie-bevorderings-chemikalieë in topikale aanwendings in te sluit. Hierdie penetrasie-bevorderaars beweeg tot in die stratum korneum, waar dit met die intersellulêre lipiede interaksie het, wat dan ‘n tydelike en omkeerbare verlaging in die skansfunksie van die vel veroorsaak. Hierdie verlaagde weerstandsfunksie van die vel gee daartoe aanleiding dat geneesmiddels makliker deur die vel kan absorbeer (Magnusson *et al*., 2001:206). Wanneer ‘n geneesmiddel of afleweringsisteem nie oor hierdie ideale fisies-chemiese eienskappe beskik nie, is dit moeilik vir ‘n geneesmiddel om deur die vel te beweeg en is manipulasie van hierdie eienskappe dan nodig. Transdermale absorbsie deur die vel kan deur manipulasie van die fisies-chemiese eienskappe bevorder word, asook met die gebruik van penetrasie-bevorderaars (Park *et al*., 2000:109).

Chemiese penetrasie-bevorderaars maak van verskillende meganismes van werking gebruik om penetrasie deur die membraan te bevorder (Moser *et al*., 2001:110). Wanneer chemiese bevorderaars in kombinasie met mekaar gebruik word, veroorsaak dit ‘n sinergistiese effek wat die beperkings wat met die gebruik van individuele bevorderaars ervaar word, oorkom (Williams & Barry, 2004:604). Soos genoem, is dit belangrik om die keuse van die afleweringsisteem,

sowel as die fisies-chemiese eienskappe van die aktiewe bestanddeel en die metode van aflewering (klein of groot dosisse), tydens transdermale studies in ag te neem.
Kommersiële formulerings wat deur verbruikers gebruik word om velkondisies te behandel, word in verskeie dosisse laer as 30 mg/cm^2, afhangend van die aanwending, toegedien. In kliniese situasies hang die aangewende dosis van die grootte van die liggaamsoppervlak wat behandel word af, naamlik, hoe groter die liggaamsoppervlak, hoe minder van die produk word aangewend.

Wanneer ‘n koorsblaar byvoorbeeld behandel word, is die gemiddelde hoeveelheid van die aangewende formulasie op die geaffekteerde area, wat ‘n klein oppervlak behels, 20 mg/cm^2 (Trottet *et al*., 2004:214). Met die aanwending van sonskermformulerings op die vel is die geaffekteerde oppervlak baie groot en word gemiddeld 0.5 mg/cm^2 aangewend (Azurdia *et al*., 1999:255; Bech & Wulf, 1992:242). Dit is dus duidelik dat die transdermale absorbsie van ‘n aktiewe bestanddeel van die konsentrasie van die aangewende formulasie, asook van die grootte van die geaffekteerde oppervlak afhang. Oorweging van die bogenoemde faktore is dus van kardinale belang wanneer *in vitro*, transdermale diffusie-toetse uitgevoer word.

Risiko-analise studies is ‘n ander baie belangrike veld waarin die klinies-relevante-dosis ‘n baie belangrike rol speel wanneer data op transdermale absorbsie van ‘n substans geproduseer word.

Tydens die uitvoer van *in vitro* diffusie-studies om die deurlaatbaarheidsprofiel van ‘n membraan te bepaal, word hoë dosisse van die afleweringsisteem op die membraan aangewend. Een tekortkoming van hierdie hoë dosisse is dat dit in sommige gevalle mag faal om die klinies-aangewende dosis van kommersiële formulasies na te boots. Dit mag ook faal om konsentrasies na blootstelling aan toksiese chemikalieë na te maak. Resultate wat deur *in vitro* studies genereer word sal dus van die data van *in vivo* studies verskil, indien die kliniese konsentrasies nie in ag geneem word nie.

Die doel van hierdie studie was om die penetrasie-bevorderingsvermoë van verskillende afleweringsvoertuie vir die lipofiele ibuprofen, deur die sintetiese Carbosil® membraan te bepaal, wanneer dit in kombinasie met mekaar of afsonderlik gebruik word, asook om die effek van klein (< 150 µl) dosis toedienings te bepaal.

Ten einde hierdie doel te bereik, was die doelstellings om die beweging van lipofiele ibuprofen deur die Carbosil® membrane te bepaal, deur van die volgende gebruik te maak:

- Water en propileenglikool as penetrasie-bevorderaars in die kombinasies 0:100 (v/v), 20:80 (v/v), 50:50 (v/v), 80:20 (v/v) and 100:0 (v/v);

- Mineraalolie and Miglyol® as penetrasie-bevorderaars in die kombinasies 0:100 (v/v), 20:80 (v/v), 50:50 (v/v), 80:20 (v/v) and 100:0 (v/v);
- Hierdie penetrasie-bevorderaar-oplosmiddels, individueel en in kombinasie met mekaar in verskillende klein en hoë volumes, naamlik 2 µl, 5 µl, 10 µl, 20 µl, 50 µl, 150 µl, 250 µl, 500 µl and 1000 µl; en om te bepaal; en
- Watter van die individuele of kombinasie-penetrasie-bevorderaaroplossings sal die beste transdermale afleweringseffek toon.

Al die oplosmiddels wat in hierdie studie gebruik is, het verskillende meganismes waardeur hulle penetrasie van ‘n geneesmiddel deur ‘n membraan bevorder. Deur hierdie oplosmiddels in kombinasie met mekaar te gebruik, is verwag dat die sinergistiese effek van die kombinasie groter as die effek van die individuele afleweringsvoertuig sou wees (Williams & Barry, 2004:604).

Die uitkomstes vir hierdie studie was as volg: Die resultate wat met die aanwending van water en propileenglikool in hoë dosisse verkry is, het duidelik getoon dat 100% propileenglikool die beste penetrasie-bevorderingseffek vir ibuprofen gelewer het. Daarteenoor het water byna geen penetrasie-bevorderingvermoë vir die aktief getoon nie. Die resultate het voorts aangedui dat ‘n verhoging van propileenglikool in die oplosmiddel die penetrasie-bevorderingsvermoë vir ibuprofen verhoog het.

‘n Moontlike verduideliking hiervoor kan die meganisme wees waardeur propileenglikool in die membraan inbeweeg om die oplosbaarheid van die aktiewe bestanddeel en deurlaatbaarheid deur die membraan te bevorder (Squillante *et al.,* 1998:266).

Chen *et al.* (2011:224) stel voor dat hoe groter die aangewende volume van die oplosmiddel is, hoe groter die oppervlak wat deur die oplosmiddel bedek word en hoe dikker die laag wat die oplosmiddel op die oppervlak van die membraan vorm, wat tot gevolg het dat die membraan meer gehidreer word. ‘n Verhoging in die hidrasie-vlak van die membraan sal die deurlaatbaarheid van die membraan verhoog.

Die aanwending van klein dosisse van die enkel afleweringsvoertuig, propileenglikool, het die hoogste penetrasie-bevorderingsresultaat gelewer, deurdat die konsentrasie van gediffundeerde ibuprofen die hoogste vir hierdie oplosmiddel was. Die konsentrasie van die gediffundeerde ibuprofen wat met aanwending van ‘n klein dosis van water as afleweringsvoertuig gelewer is, kon nie gemeet word nie, weens die lipofiele aard van ibuprofen (log $P_{o/w}$ of 3.6) (Beetge *et al*., 2000:164) en die lae oplosbaarheid van ibuprofen in water, wat tot lae deurgelate konsentrasies, wat onmeetbaar was, gelei het.

Resultate vir die hoë dosisaanwendings van die lipofiele, enkelfase oplosmiddels van 100% mineraalolie en 100% Miglyol®, het die laagste penetrasie-bevorderingseffekte getoon, vergeleke met al die dubbele kombinasie-afleweringsvoertuie bestaande uit hierdie twee middels. Multi-komponent mengsels van hierdie twee oplosmiddels het voorts baie soortgelyke diffusie-profiele vir ibuprofen getoon. Dit kon aan die moontlike sinergistiese effek tussen die twee oplosmiddels in kombinasie toegeskryf gewees het.

Volgens Moser *et al*. (2001:106), is Miglyol® daarvoor bekend om die intersellulêre lipiede van die stratum korneum te modifiseer wat daartoe lei dat die weerstand van die stratum korneum afneem en die deurlaatbaarheid van die membraan toeneem. Aangesien Carbosil® membraan en die menslike vel dieselfde oplosbaarheids-diffusie-meganisme besit, kan dit aanvaar word dat Miglyol® die polêre struktuur van die membraan sal verander en sodoende die deurlaatbaarheid bevorder.

Mineraalolie is ‘n lipofiele oplosmiddel wat die aktiewe bestanddeel na die lipofiele deel van die membraan sal dra, terwyl Miglyol® die heteropolêre struktuur van die membraan wysig om dit meer deurlaatbaar te maak (Hori *et al.,* 1991:33).

Resultate vir klein dosisaanwendings van hierdie oplosmiddels het duidelik getoon dat die 20/80 (v/v) mineraalolie/Miglyol® kombinasie die beste verhoging in die konsentrasie van die gediffundeerde ibuprofen gelewer het, vergeleke met al die ander mineraalolie en Miglyol® oplosmiddels, enkel en in kombinasie. Oplosmiddels en mengsels daarvan, bestaande uit 100% Miglyol®, 50/50 (v/v) and 80/20 (v/v) mineral oil/Miglyol® het almal soortgelyke, verbeterde diffusie-resultate met klein dosistoedienings getoon.

Drie tipes penetrasie-beïnvloedings-parameters moet in ag geneem word met oplosmiddel-tipe voorbereiding van ‘n geneesmiddel, naamlik (a) die termodinamiese effek van verskillende oplosbaarhede van die aktiewe bestanddeel in die oplosmiddel, (b) penetrasie-bevorderingseffek tussen die oplosmiddel en die membraan, (c) die uitputting van die geneesmiddel in die oplosmiddel wanneer klein volumes aangewend word. Die mate van uitputting van die aktiewe bestanddeel in die oplosmiddel hang van die dikte van die laag wat die oplosmiddel op die oppervlak van die membraan vorm, af (Leopold, 1998:167).

Die resultate van hierdie studie het die bevindings wat deur Williams and Barry (2004:605) gemaak is bevestig, naamlik dat:

1. Penetrasie-bevorderingseienskappe spesifiek is tot ‘n aktiewe bestanddeel (aktiewe bestanddele met dieselfde fisies-chemiese eienskappe).
2. Penetrasie-bevorderaars neig om goed te werk in die teenwoordigheid van mede-oplosmiddels, soos propileenglikool byvoorbeeld.

3. Meerste penetrasie-bevorderaars het ‘n komplekse konsentrasie-afhanklike effek.
4. Die potensiële meganisme van werking van penetrasie-bevorderaaroplosmiddels is verskillend en kan wissel van ‘n direkte effek op die vel tot wysiging van die afleweringsisteem (Williams & Barry,.

Die resultate van hierdie studie het aangetoon dat verhoogde vlakke van ‘n diffusie-bevorderaar, soos propileenglikool in die afleweringsvoertuig, beide die penetrasie van die aktief deur die membraan verhoog, asook penetrasie van die aktief deur die membraan met klein dosisaanwendings verbeter.

Die deurlaatbaarheidsprofiel van die lipofiele, enkelfase mineraalolie en Miglyol®, asook kombinasies daarvan, het aangetoon dat die diffusie van die lipofiele ibuprofen hoër vir klein toedienings van hierdie afleweringsvoertuie was. Chen *et al*. (2011:224) rapporteer dat met ‘n hoë dosisaanwending, word die donorkomponent met ‘n dik vloeistoflaag gevul wat die membraan se oppervlakte heeltemal bedek, met ‘n hoogte van 1.6 mm, terwyl die klein dosisaanwending slegs ‘n dun lag van 0.1 mm vorm.

Gevolglik is die hidrasie-vlakke van die membraan hoër vir hoë dosisaanwendings, en fasiliteer dit die verhoogde deurlaatbaarheid van die membraan (Chen *et al*., 2011:224). Verhoogde hidrasie-vlakke van die membraan blyk die diffusie van beide die hidrofiele en lae lipofiele komponente te verhoog, weens die beweging van die aktief in die membraan in (Williams & Barry, 2004:605). Hierdie hidrasie-effek van die membraan sal penetrasie van hidrofiele komponente deur die membraan vergemaklik, terwyl meer lipofiele komponente (log P >2) dit moeilik sal vind om deur ‘n gehidreerde membraan te beweeg (Zhang *et al*., 2010:895). Ibuprofen is ‘n sterk lipofiele geneesmiddel (log P = 3.6) (Beetge et al., 2000:164), wat die lae deurlaatbaarheidsresultate in groter toedieningsvolumes verklaar. Buiten mineraalolie, wat hoër penetrasie-vlakke vir groter aanwendingsvolumes getoon het, weens die lipofiele natuur van beide mineraalolie en ibuprofen, het die deurlaatbaarheid van die lipofiele aktiewe bestanddeel verhoog soos wat die membraan meer gehidreer geraak het met die lipofiele oplosmiddel van groter aanwendingsvolumes.

Volgens die bevindings in hierdie studie het dit duidelik geword dat:

- Die lipofiele/hidrofiele aard van die oplosmiddel en die aktiewe bestanddeel ‘n groot rol in die absorbsie van die aktiewe bestanddeel deur die membraan speel. Hierdie is ‘n baie belangrike faktor in risiko-analise studies;
- Wanneer die membraan met ‘n lipofiele oplosmiddel in die teenwoordigheid van ‘n lipofiele aktiewe bestanddeel gehidreer is, die toksiese effek meer gevaarlik kan wees met klein aanwendingsvolumes. Ibuprofen het groter penetrasie-konsentrasies getoon vir Miglyol® en mengels van Miglyol® en lipofiele mineraalolie na aanwending van klein volumes getoon;

- Wanneer ‘n lipofiele, toksiese aktief in kontak met ‘n hidrofiele afleweringsisteem kom, soos propileenglikool en water, mag dit wees dat die effek nie so noemenswaardig is nie, selfs met hoë vlakke van blootstelling; en
- Die aard van die afleweringsisteem, die aktiewe bestanddeel, sowel as die graad van blootstelling of aanwending speel ‘n groot rol in die voorspelling van die transdermale absorbsie van ‘n aktiewe bestanddeel deur ‘n membraan of die vel.

Sleutelwoorde: Transdermaal, Penetrasie-bevorderaars, klein dosisse, hoë dosisse, Carbosil® membraan.

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CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

1.1 Introduction

Pharmaceutically, the transdermal route of drug delivery offers advantages over other routes of administration. Avoidance of the first-pass metabolism is the biggest benefit, whilst other advantages, such as smaller fluctuations in plasma drug levels for repeated dosing and good patient compliance also contribute to a preference for this route of drug delivery (Brown *et al.,* 2006:178).

Despite the benefits, there are many active pharmaceutical ingredients (APIs) that cannot be delivered *via* this route of administration, because of the barrier function of the skin. The skin is the largest organ of the human body and covers a surface area of 1.5 - 2.0 m^2. It consists of three layers, which include the stratum corneum having a thickness of 10 - 20 µm, the viable epidermis (50 - 100 µm) and the dermis (1 - 2 mm). The structure of the stratum corneum is described as a "brick-and-mortar" assembly, with the corneocytes representing the bricks and the intercellular lipids the mortar. It is mainly this "brick-and-mortar" structure and its lipophilic nature that are responsible for the barrier properties of the skin (Elias, 1983:45). The main reasons for this barrier characteristic of the skin are to protect the human body from the external environment, while maintaining body fluids within the system and keeping harmful substances out (Yamashita & Hashida, 2003:1185). Factors influencing the drug/skin distribution and altering of the barrier properties of the skin include the physicochemical properties of the permeant, the choice of the delivery vehicle and the application mode used (Chen *et al.,* 2011:223).

The accurate prediction of dermal absorption of a topically applied substance and of unwanted chemicals in the environment, such as in the workplace, are of utmost importance for both formulation development and risk assessment (Gre'goire *et al*., 2009:80)

During the past decades, numerous techniques have been employed to overcome the barrier function of the stratum corneum in an attempt to improve transdermal drug delivery, one of which is the employment of penetration enhancer chemicals. These chemicals offer the potential to overcome the skin barrier and to enhance the transport of molecules across the skin. When used individually, chemicals are limited in their efficacy to disrupt the skin barrier at low concentrations, whilst often causing skin irritation at high concentrations. One method of overcoming these limitations is to use multi-component mixtures of two or more chemicals, which have shown to effectively result in high skin permeation with less skin irritation. The

components of such mixtures work synergistically to give an increased pharmaceutical effect, compared to the chemicals individually, with the benefit that less of the chemicals need to be applied to the skin, which in turn causes less irritation (Karande & Mitragotri, 2009:2362).

When developing a topical therapeutic product, it is important to consider the transdermal effects of chemicals when used individually, as well as in combination with another(s). Equally important is consideration of the effects that these chemicals may have when carrying toxic substances and the risks to the individual applying the topical product, following dermal exposure to other toxic chemicals. Little has been found in the literature on the effect of the dose of the penetration enhancer on permeant penetration, despite guidelines suggesting that *in vitro* skin permeation studies should be performed using the clinical intended dosage (Diembeck *et al.,* 1999:191).

The potential of chemicals to cross the skin is a topic of growing interest, although currently, very little is still known about the contribution of dermal exposure to the overall risk to the general population and to occupationally exposed workers. Toxic substances are present in workplaces and in the environment and come into contact with the skin in several forms, depending on their physicochemical properties (Sartorelli *et al.,* 2000:133). Other factors that may contribute to the effects of such exposure to the individual include the presence of other chemicals that may enhance dermal absorption of a substance, as well as the level of exposure.

The two aims of this study were to determine the following through synthetic Carbosil® membrane:

1. The influences of different penetration enhancer vehicles, when used individually and as multi-component solvents, on the permeation of lipophilic ibuprofen.
2. The effects of finite dose applications of these penetration enhancer vehicles on the penetration of ibuprofen.

In order to achieve the first aim, the objectives were to determine the permeation of ibuprofen by:

- Using water and propylene glycol as penetration enhancer vehicles individually and in combinations of 0/100 (v/v), 20/80 (v/v), 50/50 (v/v), 80/20 (v/v) and 100/0 (v/v);
- Using mineral oil and Miglyol® as penetration enhancer vehicles individually and in combinations of 0/100 (v/v), 20/80 (v/v), 50/50 (v/v), 80/20 (v/v) and 100/0 (v/v);
- Applying these penetration enhancer vehicles at different infinite volumes, i.e. 250 µl, 500 µl and 1,000 µl; and
- Determining what vehicle(s) would have the best enhancement effect.

The solvents used all have different mechanisms of action by which they enhance penetration through the membrane. By using these solvents in combination, the expectation was that they would have a synergistic effect that would be higher than the penetration enhancement effect achieved with each individual solvent (Williams & Barry, 2004).

In order to achieve the second aim, the objectives were to determine the permeability of ibuprofen with finite dose applications (2 µl, 5 µl, 10 µl, 20 µl, 50 µl, and 150 µl) by making use of:

- Water and propylene glycol as delivery vehicles individually and in combinations of 0/100 (v/v), 20/80 (v/v), 50/50 (v/v), 80/20 (v/v) and 100/0 (v/v); and
- Mineral oil and Miglyol® as delivery vehicles individually and in combinations of 0/100 (v/v), 20/80 (v/v), 50/50 (v/v), 80/20 (v/v) and 100/0 (v/v).

Part of the above objectives was also to hypothesise what influence(s) the obtained outcomes would have on risk assessment studies.

Most quantitative structure/penetration relationships (QSAR's) for dermal absorption predict the permeability coefficient, K_p, of molecules in infinite dose conditions. In practice, however, dermal exposure to a toxic chemical mostly occurs under finite dose conditions (Buist *et al.,* 2010:200). Both finite and infinite dose conditions were investigated during this study. For the purpose of this study, finite dose refers to the volumes <150 µl, i.e. 2 µl, 5 µl, 10 µl, 20 µl, 50 µl and 150 µl. Infinite volume are the volumes >150 µl, i.e. 250 µl, 500 µl and 1,000 µl. These volumes were applied to the Carbosil® membrane in saturated solutions of the solvents, as listed above. The concentration (µg/cm^2) of ibuprofen was determined every hour until 6 hours, in order to establish the extent to which ibuprofen had crossed the membrane over time.

The chapters in this thesis are arranged as follows:

- The literature review on the influences of penetration enhancer vehicles on the delivery of ibuprofen through Carbosil® membrane is discussed in Chapter 2.
- The effects of application volumes, which include finite and infinite dose applications, are discussed in Chapter 3.
- Chapter 4 comprises an article that is intended for submission for publication in the *Journal of Drug Delivery*, focusing on the effects of single and binary phase penetration enhancer vehicles on transdermal delivery.
- Chapter 5 is also an article intended for submission for publication in the *International Journal of Pharmaceutics*, focusing on finite dosing.
- The outcomes of this study are finally summarised in Chapter 6.

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CHAPTER 2

PENETRATION ENHANCEMENT TECHNIQUES

2.1 Introduction

Transdermal drug delivery has the advantage of avoiding the hepatic, first-pass metabolism that would result in better therapeutic efficacy, better patient medication compliance and reduced systemic side effects (Kydoneieus & Berner, 1987:69). One disadvantage of this mode of drug delivery is the generally poor penetration of drugs through the stratum corneum, consisting of keratin rich dead cells, embedded in a very elegant, but complex lipid matrix. This intercellular lipid structure forms an excellent penetration barrier, which must be breached to enhance drug penetration through the skin.

One way of overcoming this barrier function of the skin is to include penetration enhancer chemicals in the topical application. These penetration enhancers partition into the stratum corneum and interact with the intercellular lipids, causing a temporary and reversible decrease in the skin barrier function. The physicochemical properties of both the permeant and the penetration enhancer influence the permeability of the skin (Dias *et al*., 2007:65). By manipulating these physicochemical properties and by making use of penetration enhancers, transdermal absorption through the skin can be increased (Park *et al*., 2000:109).

2.2 Definition of penetration enhancers

Penetration enhancers are substances that can partition into the skin and interact with the intercellular lipid lamellae and therefore result in a temporary and reversible decrease of the skin barrier function. When the skin barrier function is reduced, drug transport through the skin increases (Magnusson *et al*., 2001:206). In physicochemical terms, enhancement can be achieved by increasing:

- The saturation levels of the active compound in the solvent vehicle;
- The drug solubility in the stratum corneum; and
- The drugs' ability to diffuse through the barrier, i.e. its diffusivity.

Some enhancers may act in one or more of the above modes of action (Moser *et al*., 2001:110).

2.3 Penetration enhancement techniques

Penetration of a drug through the stratum corneum is described by Fick's first law (Equation 2.1). Drug permeation is a passive diffusion process from an area of high concentration of a drug (on the surface of the stratum corneum) to an area of low concentration of that drug (within the skin).

$$J = K_P.\triangle C = (K.D/h).\triangle C \qquad \text{Equation 2.1}$$

The steady state flux (J) is related to the diffusion coefficient (D) in the stratum corneum over an area available for diffusion, or membrane thickness (h), as well as to the partition coefficient (K_P) between the stratum corneum and the vehicle, and the applied drug concentration (C), which is assumed to be constant (Benson, 2005:25). Equation 2.1 is thus used to identify the ideal parameters for the diffusion of drugs across the skin. Figure 2.1 summarises the techniques used to enhance penetration through the skin, whereafter the different techniques are discussed.

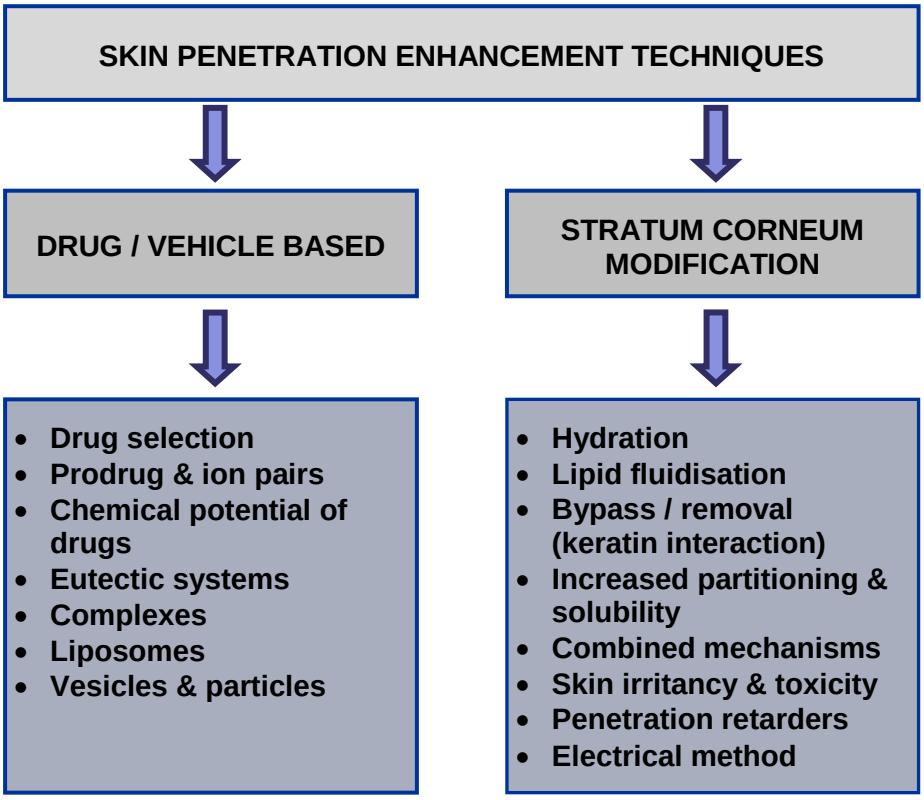

Figure 2.1: Techniques used to enhance drug penetration through the skin (Benson, 2005:25).

2.3.1 Penetration enhancement through optimisation of drug and vehicle properties

2.3.1.1 Drug selection

The highest permeability through the stratum corneum is attained at a log P (octanol/water partition coefficient) value of 2.5, whereas optimal permeability across the stratum corneum is related to a low molecular size (Pots & Guy, 1992:665) of ideally < 500 Da (Bos & Meinardi, 2000:165). The low molecular size influences the diffusion coefficient, as well as a low melting point that is related to solubility. Katz and Poulsen (1971:135) studied the effects of solubility and the partition coefficient on the diffusion of drugs across the stratum corneum. They found that when a drug had a partition coefficient (log $P_{octanol/water}$) of 1 – 3, the solubility of the drug in the lipid domain of the stratum corneum was sufficient to break the barrier function of the stratum corneum to move across the stratum corneum into the lipid domain. The hydrophilic nature of the drug was also sufficient to allow the drug to partition into the viable epidermis (Katz & Poulsen, 1971:134). An example of this is the parabolic relationship between skin permeability and partition coefficient for a series of salicylates and non-steroidal, anti-inflammatory drugs (Benson, 2005:26).

When a drug complies with all of these ideal characteristics (as in the case of nicotine and nitroglycerine), penetration through the skin is feasible. However, when a drug does not have the ideal physicochemical properties, penetration through the skin is difficult and manipulation of the drug or the vehicle is necessary.

2.3.1.2 Prodrugs and ion pairs

The prodrug approach is used when a drug has an unfavourable partition coefficient (Sloan, 1992:313). The prodrug technique involves the addition of a promoiety to increase the partition coefficient and solubility, and hence the transport of the drug in the lipid rich stratum corneum. The prodrug technique also releases the drug into the viable epidermis by hydrolysis and thereby optimises the solubility of the drug in the aqueous epidermis (Benson, 2005:26).

Charged drug molecules do not partition into, nor permeate through the skin easily. This technique involves the addition of oppositely charged species to the charged molecule, resulting in a neutrally charged ion pair to form, that enables the molecules to permeate the stratum corneum more readily. The ion pair then releases the charged drug into the aqueous viable epidermis (Benson, 2005:26). Sarveiya *et al*. (2004:718) report on a sixteen-fold increase in the steady-state flux of ibuprofen ion pairs across a lipophilic membrane.

2.3.1.3 Chemical potential of drug in vehicle: saturated and supersaturated solutions

With supersaturated solutions, the thermodynamic activity of a drug is at its highest and the skin penetration rate at its maximum. Supersaturated solutions can occur due to evaporation of the solvent, or by mixing of the co-solvents. In clinically applied situations, the most common mechanism of evaporation of the solvent is through evaporation from the warm skin surface, as is the case with most topically applied formulations. These supersaturated solutions are very unstable and by incorporating anti-nucleating agents, the stability of the solutions improves (Santos *et al*., 2011:72).

Magreb *et al*. (1995) report that the flux of oestradiol from an eighteen times saturated system increased eighteen-fold across the human stratum corneum, compared to thirteen-fold in silastic membrane. The authors in this article suggest that the complex combination of fatty acids in the stratum corneum may provide an anti-nucleating effect that stabilises the super saturated system (Magreb *et al*., 1995:279).

2.3.1.4 Eutectic systems

The melting point of a drug influences its solubility and hence its skin penetration ability. The lower the melting point, the more soluble the drug is in both the solvent and in the skin lipids. The melting point of a delivery system can be lowered by incorporating eutectic systems. A eutectic system is a mixture of two components, which, at a certain ratio, inhibits the crystalline processes of each other. The melting point of the mixture is lower than the melting point of each individual component in the mixture. Solubility of the drug or delivery system increases when the melting point of the drug or delivery system is lower than body temperature (Benson, 2005:26).

EMLA cream, a formulation consisting of a eutectic mixture of lignocaine and prilocaine, is a prime example of such a system, when applied under an occlusive film, to provide effective local anaesthesia (Benson, 2005:26).

2.3.1.5 Complexes

This enhancement technique includes complexation of drugs with cyclodextrin to enhance aqueous solubility and drug stability. Cyclodextrin for pharmaceutical use has a unique structure, containing either six, seven or eight dextrose molecules, bound in a 1,4-configuration to form rings of various diameters. Each ring has a hydrophilic exterior and lipophilic centre, resulting in increased aqueous solubility and chemical stability. Complexation with cyclodextrin

has, however, been reported to both increase and decrease skin penetration, causing it to remain a controversial topic (Benson, 2005:26).

2.3.1.6 Liposomes and vesicles

Many cosmetic products contain ingredients that are encapsulated in vesicles. These ingredients include humectants, sunscreens, enzymes and tanning agents. Encapsulating systems include liposomes, transfersomes, ethosomes and niosomes.

Liposomes are colloidal particles, formed as concentric biomolecular layers that are capable of encapsulating drugs. The mechanism of drug delivery is associated with accumulation of the liposome and the drug in the stratum corneum, as well as in the upper layers of the skin, with minimal penetration of the drug into the deeper layers of the skin and the systemic circulation (Foldvari, 1994:1595). The most effective liposomes are those that consist of lipids similar to those in the stratum corneum (Egbaria *et al*., 1990:107). Phosphatidylcholine from soybean or egg yolk is the most common example (Benson, 2005:27).

Transfersomes are vesicles composed of phospholipids, ethanol and a surfactant. The surfactant molecule is less than one tenth of the diameter of the transfersome and enables the transfersome to penetrate through channels in the stratum corneum (Cevc, 1996:258). Transfersomes penetrate *via* the pores in the stratum corneum and reach the viable epidermis where they are systemically absorbed (Benson, 2005:26). The skin penetration of estradiol was enhanced nine-fold by transfersomes, compared to traditional liposomes (Benson, 2005:27).

Ethosomes are liposomes with a high alcohol content, capable of enhancing penetration into the deeper layers of the skin and into the systemic circulation (Biana & Touitou, 2003:65).

Niosomes are vesicles that consist of non-ionic surfactants that can act as carriers for drugs and cosmetic applications (Shahiwala & Misra, 2002:223).

2.3.1.7 Solid lipid nanoparticles

Solid lipid nanoparticles act as carriers for sunscreens and vitamins A and E, and enhance skin delivery of these molecules. The mechanism of action to enhance skin penetration is through skin hydration, caused by the occlusive film that forms on the skin surface (Wissing & Muller, 2003:65). Wissing and Muller (2003:65), found a 31% increase in skin hydration after four weeks of application of SLN-enriched cream.

2.3.2 Penetration enhancement by stratum corneum modification

2.3.2.1 Hydration

The water content in the stratum corneum is around 15 - 20% of the dry weight, but can vary according to the humidity level in the external environment. An increase in the stratum corneum water content increases the permeant solubility and modifies partitioning from the vehicle into the membrane. Another alternative is swelling of the intercellular lipids, due to the high levels of hydration that cause the stratum corneum structure to open, with a subsequent increase in drug penetration through the skin (Benson, 2005:28). A commercial example of this is the use of an occlusive dressing to enhance skin penetration of lignocaine and prilocane from EMLA cream to provide sufficient local anaesthesia (Benson, 2005:28).

2.3.2.2 Fluidisation by chemical penetration enhancers

Many penetration enhancers increase penetration through the skin by disordering, or "fluidising" the lipid structure of the stratum corneum. The diffusion coefficient (D in Equation 3.1) increases as the enhancer molecules disrupt the lipid bilayers. Such penetration enhancers include Azone®, dimethylsulphoxide (DMSO), alcohols, fatty acids and terpenes (Benson, 2005:29).

2.3.2.3 Interaction with keratin

Chemicals, such as DMSO, decylmethylsulphoxide, urea and surfactants not only have an effect on the stratum corneum lipids, but also interact with keratin in the corneosytes (Benson, 2005:29). Penetration enhancers affect both the stratum corneum lipids and interact with keratin in the corneocytes. When a surfactant penetrates the stratum corneum, it interacts and binds with the keratin filaments, resulting in a disruption within the corneocyte, which in turn causes an increase in the diffusion coefficient, as well as an increase in permeability (Benson, 2005:29).

2.3.2.4 Increased partitioning and solubility in the stratum corneum

Solvents, such as ethanol and propylene glycol increase partitioning of a permeant through the stratum corneum and its solubility in the stratum corneum, and therefore increase the partition coefficient of Fick's equation. The solubility parameter of skin lipids is about 10 $(cal/cm^3)^{1/2}$. If a permeant has a solubility parameter significantly different to 10 $(cal/cm^3)^{1/2}$, a solvent capable of altering the solubility parameter of the permeant will be closer to 10 $(cal/cm^3)^{1/2}$ and will therefore enhance distribution within the stratum corneum and increase flux (Sloan, 1992:179-

220). This has been demonstrated by the enhanced permeability of metronidazole in the presence of propylene glycol (Benson, 2005:29).

2.3.2.5 Combined mechanisms

Fick's law (Equation 3.1) indicates that a combination of enhancement effects would lead to a multiplicative result to enhance diffusivity (D) and partitioning (K). Synergistic effects have been observed with different combinations of penetration enhancers, such as Azone® and propylene glycol (Benson, 2005:29). An example of this mechanism is high concentrations of DMSO that disturb intercellular organisation and extract stratum corneum lipids as well, whilst interacting with keratin and facilitating lipid drug partitioning (Benson, 2005:30).

2.3.2.6 Skin irritancy and toxicity due to chemical penetration enhancers

Chemical penetration enhancers reversibly disrupt the physicochemical nature of the stratum corneum in order to increase its permeability, by reducing its diffusional resistance. One of the biggest disadvantages of chemical enhancers is that they cause irritancy of the skin (Benson, 2005:30). The highest levels of irritancy are caused by those vehicles of which the solubility parameter is equal to that of the skin. Solubility parameters are useful in predicting drug/vehicle/skin interactions and potential irritancy (Sloan, 1992:179). Chemical enhancers, such as essential oils, terpenes and polymeric enhancers have been investigated in recent years to determine the general safety of these chemicals (Benson, 2005:30).

2.3.2.7 Skin penetration retarders

Penetration retarders are useful in formulations where it is important to minimise systemic absorption, such as insect repellents and sunscreens (Benson, 2005:31). Some Azone® analogues are examples of penetration retarders (Hadgraft *et al*., 1996:23).

2.3.2.8 Other physical and electrical methods

Electrical methods of penetration enhancement include iontophoresis, phonophoresis, electroporation and photomechanical waves. A number of methods to bypass or remove the stratum corneum have also been used successfully, such as microneedles, jet-propelled particles and ablation of the stratum corneum. None of these were, however, of interest to this study.

2.4 Physicochemical characterisation of permeation enhancer solvents

The aqueous ionisation constant (pK_a) predicts the degree of ionisation of a molecule at a particular pH and influences the availability of a chemical to take part in physical, biological and chemical reactions. The pK_a affects the absorption, distribution and elimination of a substance. Normally, the unionised form of a substance is capable of entering and passively diffusing through the stratum corneum.

The log P describes the distribution of a molecule between the aqueous and the organic solutions, which is the ratio concentration of a molecule dissolved at equilibrium in the two phases. It can be used to predict the biological activity of drugs. Measuring the pK_a and log P is, however, difficult for substances that are poorly soluble in water (Thomas & Finnin, 2004:699).

2.5 Types of enhancers

2.5.1 Physical enhancers

Physical enhancers may provide an effective alternative, or offer a synergistic method to other methods for improved permeation (Purdon *et al*., 2004:119). Examples of physical enhancers are microneedle array, stratum corneum ablation, iontophoresis and ultrasound.

2.5.2 Chemical enhancers

After years of research, several chemicals are known to interact with the skin and disrupt the highly ordered lipid bilayer of the stratum corneum that comprises the primary barrier to diffusion through the skin. Currently, more than 300 chemicals have been investigated for their penetration enhancement benefits in carrying active molecules across the skin. Chemical enhancers may consist of different chemical functional groups that enable them to act through a variety of different mechanisms to enhance the transport of chemicals through the skin. These chemical enhancers may also offer benefits in addition to their skin permeability functions, such as improving drug solubility, improving aesthetic aspects, such as odour, colour and texture, and in some cases they can act as emulsifiers, preservatives and fillers (Karande & Mitragotri, 2009:2363). In this study, only the penetration enhancement effects of chemical enhancers were investigated.

The chemical enhancers that were investigated during this study were propylene glycol, water, mineral oil and Miglyol®. Propylene glycol and water were used as single phase solvents, as well as in binary phase combinations in 50/50 (v/v), 20/80 (v/v) and 80/20 (v/v) combinations. The same was done with mineral oil and Miglyol®.

2.5.2.1 Mechanism of action of chemical enhancers

Enhancers can act by one or more of the following modes of action:

- Interaction with intercellular lipids;
- Interaction with intercellular keratin;
- The penetration of high amounts of penetration enhancers or co-solvents into the stratum corneum to increase the dissolving capacity of the barrier for drugs and co-solvents; and
- By achieving a synergistic effect of a co-enhancer (binary component) system of two enhancers that work differently. One enhancer can increase the delivery of the other, as well as that of the drug to give a multiplicative effect (Williams & Barry, 2004:606).

Interactions between penetration enhancers and intercellular lipids cause fluidisation of the lipids. Polar molecules interact with the polar head groups of the lipids *via* hydrogen bonds and ionic forces. This influences the arrangement of the lipids and the degree of hydration of the stratum corneum. Lipophilic compounds interact with the cholesterol enforced lipids and increase the diffusion of the penetration enhancer by rearranging the lipid lamellae with increased fluidity (Bach & Lippold, 1998:4).

According to the lipid/protein partitioning theory, these mechanisms of action would fall into any of the following categories:

- Disruption of the lipid matrix of the stratum corneum;
- Interaction with intracellular protein;
- Improvement in partitioning of a substance into the stratum corneum;
- Disruption of the corneocyte envelope;
- Manipulation of protein junctions, such as desmosomes; and
- Changing the partitioning between the stratum corneum components and the diffusion pathway lipids (Kanikkannan *et al*., 2006:18; Barry, 2006:9).

2.5.2.2 Types of chemical enhancers

2.5.2.2.1 Water

Water is the most natural penetration enhancer (Roberts & Walker, 1993:3). Hydration of the stratum corneum is a very important aspect of determining the penetration enhancement of an active compound. The human stratum corneum typically contains between 15 - 20% of the tissue dry weight, depending on the humidity of the environment. By soaking the membrane in

water, the humidity within the membrane increases. Another way of increasing the humidity is by occlusion to prevent transepidermal water loss. Many clinically effective preparations and products, like ointments and patches, enhance penetration through the skin by the occlusion effect, due to the modification of the stratum corneum water content (Williams & Barry, 2004:606). An increase in stratum corneum tissue hydration increases transdermal delivery of both hydrophilic and lipophilic permeants. Due to the nature of the stratum corneum, the water within the membrane is either "bound" to some structural elements within the membrane, or "free" and available to act as a solvent for polar permeants in the membrane (Walkley, 1972:225). This free water within the stratum corneum solubilises the permeant in the stratum corneum and modifies partitioning from the permeant vehicle into the membrane. This explains the mechanism of transdermal penetration of hydrophilic permeants. Since the stratum corneum lipids are mainly responsible for the barrier to transdermal delivery, high water content can cause the lipids to swell and result in disruption of this lipid structure between the corneocytes, as well as disruption of the bilayer packing. This explains the mechanism of transdermal delivery of lipophilic permeants (Williams & Barry, 2004:607). When water is used as a penetration enhancer vehicle, it thus acts in two ways to enhance penetration through the membrane, i.e. by acting as a solvent to the permeant and by disrupting the lipid bilayer structure by swelling of the polar heads.

2.5.2.2.2 Sulphoxides and related chemicals

One of the most widely studied penetration enhancers is dimethylsulphoxide (DMSO). It is a colourless, odourless, hygroscopic compound that is widely used in many pharmaceutical formulations as a "universal solvent". This powerful aprotic solvent forms hydrogen bonds with itself rather than with water and it is known to enhance penetration of both hydrophilic and lipophilic permeants. DMSO is used in commercial applications of idoxuridine, for the treatment of severe herpetic infections of the skin. Because of the rapid penetration enhancement effect of DMSO, it has some disadvantages. When the chemical comes into contact with the skin, it can be tasted in the mouth within seconds. Another disadvantage is that the effect of enhancement is concentration dependent and levels of > 60% are needed for an optimum enhancement effect. Such high levels of DMSO can cause erythema and wheals. A further problem with the use of DMSO as a penetration enhancer is the metabolic dimethylsulphide that is produced from the solvent, which causes a foul odour of breath (Williams & Barry, 2004:607).

Because of the disadvantages of DMSO as a penetration enhancement solvent, continued research had been done in order to find other solvents similar to DMSO, but with less disadvantages. Dimethylacetamide (DMAC) and dimetylformamide (DMF) are known to be similar in structure to DMSO and both are powerful aprotic solvents with a broad range of penetration enhancement activities (Southwell & Barry, 1983:510). Although the disadvantages

of DMAC and DMF are less than those of DMSO, they also have negative properties. DMF, for example, causes irreversible damage to the skin membrane. Another compound that is similar in structure to DMSO is decylmethylsulphoxide (DCMS). The negative effect of DCMS on human skin is reversible and it is known to be an excellent enhancer for hydrophilic permeants, but less effective in enhancing the permeation of lipophilic compounds. The mechanism of action of sulphoxide penetration enhancers is very complex and requires more research for it to be fully understood (Williams & Barry, 2004:608).

2.5.2.2.3 Azone®

The first molecule that was designed to specifically promote penetration through the skin was Azone® (1-dodecylazacycloheptan-2-one or laurocapram). Azone® is considered a hybrid of a cyclic amide with an alkylsuphoxide, but without the aprotic sulphoxide group that is responsible for the disadvantages of DMSO. It is a colourless and odourless compound with a melting point of -7°C, and when included in a formulation, has a smooth, oily, but non-greasy feel. Azone® is a lipophilic compound with a log P of 6.2 and it is soluble in and compatible with organic solvents, like propylene glycol.

Azone® is known to promote the penetration enhancement effect of both lipophilic and hydrophilic permeants, with its enhancement effect being strongly concentration dependent, whilst also being influenced by the vehicle in which it is applied. Usage levels of Azone® are between 0.1 - 5%. Its mechanism of action is its interaction with the lipid domains of the stratum corneum. It partitions into the bilayer lipids and disrupts their packing arrangements. As a result, Azone® molecules exist dispersed within the barrier lipids, which in turn lowers the barrier function of the stratum corneum (Williams & Barry, 2004:608).

2.5.2.2.4 Pyrrolidones

Pyrrolidones is another group of chemicals that can be considered as penetration enhancers. As for the above penetration enhancers being discussed, they have a better effect on enhancing the penetration of hydrophilic permeants than of lipophilic compounds. N-methyl-2-pyrrolidone and 2-pyrrolidone are the two most known examples of pyrrolidones. N-methyl-2-pyrrolidone is a polar aprotic solvent and is a clear liquid at room temperature that is compatible with most solvents, including water and alcohols. 2-Pyrrolidone, like n-methyl-2-pyrrolidone, is also compatible with most solvents and is in liquid form at 25°C. Commercially, it is used as a solvent in the production of oil, sugar, iodine and polymers. Similar to the other penetration enhancer solvents, pyrrolidones enhance the penetration of hydrophilic molecules (Park *et al.*, 2001:980). The mechanism of action is that pyrrolidones partition into the stratum corneum and act by modifying the solvent nature of the membrane and the pyrrolidone to generate

"reservoirs" within the skin. The reservoir effect leads to the sustained release of the permeant over a period of time. In some instances, adverse reactions with regards to erythema and irritation have been reported (Williams & Barry, 2004:609).

2.5.2.2.5 Fatty acids

Long chain fatty acids have also been investigated for increased transdermal absorption, of which the most popular is oleic acid. From investigations done by Aungst *et al*. (1986:227), it is clear that saturated alkyl chain lengths of C_{10}-C_{12}, attached to a polar head group, are the best penetration enhancers. Penetration enhancers containing an unsaturated alkyl chain do not have good penetration enhancement properties, unless the *cis* configuration is used. The *cis* configuration is expected to interact with the intercellular lipids in the stratum corneum to increase penetration through the skin (Aungst *et al*., 1986:225). Similar to Azone®, oleic acid is effective at low concentrations and can work synergistically with other solvents, like propylene glycol. The mechanism of action of fatty acids is that it interacts and modifies the lipid domain of the stratum corneum to decrease the barrier function of the stratum corneum (Williams & Barry, 2004:610).

Caprylic- and capric triglycerides comprise a specific fraction of the class of coconut oil fatty acids and are more stable, with positive effects on the skin. Caprylic- and capric fatty acids are responsible for the dry powdery feel of the oil. The trade name for these triglycerides is Miglyol®. The main components of Miglyol®are caprylic acid (~ 50 - 60%), capric acid (30 - 45%), caproic acid, lauric acid and myristic acid (the last three acids make up 6 - 10%). In this study, the penetration enhancement effect of Miglyol®was investigated. Miglyol® is a vegetable alternative to mineral oil. Fatty acids have been used to improve transdermal drug delivery of both lipophilic and hydrophilic permeants. Miglyol®'s main mechanism of action is to modify the intercellular lipids of the stratum corneum in order to disrupt the barrier of the stratum corneum and increase the diffusivity through the membrane (Moser *et al*., 2001:105).

2.5.2.2.6 Amines

Primary, secondary and tertiary, cyclic- and acyclic amines have been studied and found successful penetration enhancers for a variety of drugs. The mechanism by which they enhance penetration through the skin may be because of their permeation into the lipid bilayers of the stratum corneum, or due to their partitioning into the skin (Karande & Mitragotri, 2009:2364).

2.5.2.2.7 Alcohols, fatty alcohols and glycols

Ethanol is commonly used in transdermal applications and is it the solvent of choice for patch applications. Ethanol is often used in combination with water. When using the ethanol/water co-solvent vehicle, the enhancement effect of ethanol is concentration dependent. High ethanol levels dehydrate the stratum corneum membrane and reduce penetration through the skin. A few mechanisms of action, by which ethanol enhances penetration through the skin, however, exist. Firstly, as a solvent it increases the solubility of the drug in the vehicle (Pershing *et al*., 1990:173). Secondly, ethanol permeates into the stratum corneum and modifies the solubility properties of the tissue and consequently improves the drug partitioning into the membrane (Megrab *et al*., 1995:105). Furthermore, the rapid penetration of ethanol, or the loss of this solvent due to evaporation, changes the thermodynamic properties of the permeant in the formulation. This mechanism of action is more prominent when a finite dose is applied. As the amount of ethanol decreases (due to evaporation), the drug concentration increases and a supersaturated state of the permeant acts as a driving force for permeation (Morimoto *et al*., 2002:135). Another mechanism of action is that because of the rapid permeation of ethanol across the skin, a "solvent vehicle" carries the permeant into the tissue (Williams & Barry, 2004:611).

Fatty alcohols also act as penetration enhancers and they are applied to the skin in co-solvents (often propylene glycol) at concentrations between 1 - 10%. Propylene glycol is a very popular choice for a penetration enhancement solvent and is widely used as a vehicle to enhance penetration of active compounds through the skin. Propylene glycol can be used on its own, whilst it also shows good synergistic action when used in combination with other penetration enhancement solvents. Propylene glycol's mode of action is very similar to that of water. Firstly, it acts as a solvent and solubilises the permeant in the vehicle. Furthermore, permeation of propylene glycol into the stratum corneum enhances the solubility properties of the tissue and consequently enhances drug partitioning into the membrane. Permeation of propylene glycol through the stratum corneum could change the thermodynamic activity of the drug in the vehicle, resulting in a modification of the driving force of diffusion through minor disruption of the intercellular lipid packing within the stratum corneum (Williams & Barry, 2004:611).

2.5.2.2.8 Surfactants

Surfactants are commonly found in therapeutic and cosmetic formulations, for the purpose of solubilising the lipophilic active ingredients. Because of this function, surfactants can also solubilise lipids in the stratum corneum. Anionic surfactants, such as sodium lauryl sulphate, have the potential of damaging human skin. Both anionic and cationic surfactants cause the intercellular lipids to swell and interact with keratin. Both anionic and cationic surfactants are

also irritants to the skin and cause trans-epidermal water loss. Non-ionic surfactants are regarded as safe and they have less of an irritancy effect on the skin. Anionic surfactants have a low penetration enhancement effect that increases over time, whereas non-ionic surfactants have a minor penetration enhancement effect that remains unchanged (Williams & Barry, 2004:612).

2.5.2.2.9 Urea

Normally, urea is applied in water in oil vehicles as a hydrating agent for the treatment of psoriasis and other hyperkeratoxic skin conditions. Urea on its own and also in combination with ammonium lactate causes significant stratum corneum hydration and increases penetration of a permeant through the skin (Gloor *et al*., 2001:812).

2.5.2.2.10 Essential oils, terpenes and terpenoids

Terpenes are found in essential oils and consist of carbon, hydrogen and oxygen atoms that can be used as fragrances, flavourants and medicines. The most effective penetration enhancer essential oil is eucalyptus oil. The use of terpenes as penetration enhancers continues to be a popular choice. The mechanism of action of terpenes is to modify the solvent nature of the stratum corneum and improving penetration through the skin. The smaller terpenes tend to be more effective penetration enhancers than the larger sequiterpenes. The hydrocarbon, “non-polar group containing” terpenes, such as limonene, increases penetration of lipophilic compounds. In contrast, the “polar containing group” terpenes increases penetration of hydrophilic compounds (Williams & Barry, 2004:614).

2.5.2.2.11 Hydrocarbons

Hydrocarbons include alkanes, alkenes, halogenated alkanes, squalane and mineral oil. The mechanism by which hydrocarbons enhance penetration through the skin is by partitioning chemicals into the stratum corneum, where these chemicals then disrupt the ordered lipid bilayer structure and decrease the barrier function of the stratum corneum (Hori *et al*., 1991:33). Mineral oil forms part of a wide range of skin care formulations. It is incorporated into formulations with the purpose of increasing the emollient properties and to add moisturising benefits to a product. More importantly, mineral oil is also used for its penetration enhancement effects of carrying active compounds through the skin. In this study, mineral oil was investigated for its penetration enhancement effect.

2.5.2.2.12 Phospholipids

In many formulations, phospholipids are used to carry drugs through the skin. The mechanism by which phospholipids increase penetration through the skin is by the effect of occlusion on the skin surface. As a result of the occlusion effect, the stratum corneum becomes more hydrated, causing penetration of drugs through the skin to increase. If applied as vesicles (liposomes), it can interact with the stratum corneum lipids and increase drug penetration through the skin (Williams & Barry, 2004:614).

2.5.2.2.13 Miscellaneous

In addition to the chemicals discussed above, other chemicals that had been studied and found to show penetration enhancement effects on the transport of drugs across the skin include amino acids, thioacyl derivatives of amino acids, alkyl amino esters and oxazolidinones. Enzymes comprise a fairly new class of chemicals that have been investigated and found to demonstrate penetration enhancement effects, in particular the papain and medicinal leech enzymes. Macrocyclic ketones with 12-carbon atoms or more have also demonstrated successful enhancing effects on drug penetration (Karande *et al*., 2009:2365). Lastly, metabolic intervention schemes that affect the synthesis of the stratum corneum components and its homeostasis have also been considered as methods for permeation enhancement (Williams & Barry, 1992:306).

2.5.2.3 Limitations of chemical enhancers

2.5.2.3.1 Efficacy

The most prominent limitation is that most of the chemical enhancers do not achieve the desired reversible skin barrier disruption, which limits drug transport through the skin (Karande *et al*., 2004:195). The second significant limitation of chemical enhancers is that a decrease in the enhancer concentration across the stratum corneum also reduces its activity (Chen & Langer, 1998:340). Investigation of the physicochemical parameters, such as polar forces, charge, partition coefficient, solubility and hydrogen binding, should enable the development of a quantitative structure activity correlation (QSAR). This method would have to take into account the chemical structure of the penetration enhancer and its skin disruption potential. Based on such data, a new, more efficient and more effective penetration enhancer could be developed (Chen & Langer, 1998:340). Two types of enhancers could be developed, namely one that works as a lipid extractor and another that works as a lipid fluidiser. Lipid extractor enhancers are very effective, but not very safe, because of the protein denaturisation that causes irritation. Lipid fluidiser enhancers have a high partition coefficient, but because of their poor solubility in

aqueous formulations, their activity in penetration enhancement is very limited (Karande *et al*., 2005:4689).

2.5.2.3.2 Safety

Another challenge in using chemical enhancers is that they can potentially cause skin irritation. The more effective the penetration enhancer, the more disruption to the skin barrier structure occurs, with subsequently more skin irritation. This also happens when too high levels of penetration enhancers are used (Karande *et al*., 2005:4692).

2.5.2.4 Synergistic mixtures of chemical penetration enhancers

One way of overcoming the limitations of chemical penetration enhancers is to use different enhancers in combination with each other in different mixtures. The synergistic effects of such enhancer mixtures would offer a more effective penetration enhancement effect, compared to single phase enhancers, which, if used in high quantities to achieve the desired penetration enhancement effect, may cause skin irritation. By combining two chemical enhancers, the risk of skin irritation is thus reduced, whilst the penetration enhancement effect is increased. A good example would be the combination of one chemical enhancer that acts on the lipids in the stratum corneum and another that acts on the corneocytes. The net effect would be the opening of the intercellular hydrophobic and hydrophilic pathways for penetration of an active substance. Similarly, one component of a mixture could increase partitioning of an active compound in the stratum corneum, whilst the other could create diffusion pathways, resulting in the net penetration enhancement effect to increase (Chen & Langer, 1998:340).

2.6 Summary

It has become clear that many different potential mechanisms of action exist through which penetration enhancers operate, ranging from direct interaction with the skin to modification of the formulation. Penetration enhancers that act directly on the skin have different modes of action by which they enhance penetration through the skin, i.e. by:

- Acting on the stratum corneum intracellular keratin, by modifying the conformation through swelling and through increased hydration;
- Affecting the demosomes between the corneocytes;
- Disrupting the intercellular lipid structure to reduce the barrier resistance of the bilayer lipids; and

- Altering the solvent nature in the stratum corneum to modify partitioning of the drug or the co-solvent into the tissue and increasing the amount of permeant within the skin.

Penetration enhancers can also act indirectly on the skin by making use of the following modes of action:

- Modification of the thermodynamic activity of the vehicle through rapid permeation of the solvent into the stratum corneum that leaves the permeant in a saturated state, as if the solvent were present; and
- Solubilising the permeant in the donor. Especially where a permeant has a low aqueous solubility, the depletion effect is reduced and drug permeation is prolonged (Williams & Barry, 2004:616).

The field of penetration enhancement is the most widely studied field in transdermal delivery, for the one reason of ensuring optimum delivery of an active through the skin. Special care should be taken when choosing a penetration enhancer for delivering optimum quantities of the active pharmaceutical ingredient by ensuring effective and safe delivery.

The aim of this literature study was to establish a better understanding of the mechanisms by which penetration through the membrane can be enhanced.

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CHAPTER 3

THE EFFECT OF FINITE DOSE APPLICATION ON TRANSDERMAL AND TOPICAL DRUG DELIVERY

3.1 Introduction

When consumers use commercial formulations to treat topical skin conditions, the vehicles applied are in varying doses lower than 30 mg/cm^2, depending on the application. In clinical situations, the formulation applied depends on the body surface area being treated, i.e. the larger the surface area, the lower the amount of vehicle applied.

When a cold sore is treated, for example, an average amount of 20 mg/cm^2 of the vehicle is applied to the infected area, with the treated surface area generally being very small (Trottet *et al*., 2004:214). Based on their review of the dose being applied with topical formulations, Surber and Davis (2002:481) report that when topical skin diseases are treated, higher body surface areas are involved, whilst the amount of vehicle applied varies from as little as 0.7 mg/cm^2 to 4.0 mg/cm^2. When sunscreens are applied to the skin, obviously a very large surface area is treated and an average amount of only 0.5 mg/cm^2 is applied (Azurdia *et al*., 1999:255; Bech & Wulf, 1992:242).

The transdermal absorption of an active compound depends on the concentration being applied and the treated surface area. Considering the aforementioned parameters is thus of high significance when *in vitro* experiments are performed.

Risk assessment studies comprise another important field in which clinical relevant dose plays a significant role to be taken into account when data on transdermal absorption of a substance is produced. Human exposure to toxic substances may pose a serious health risk, since when the skin comes into contact with a chemical it has the potential for absorption, either locally, or systemically.

During *in vitro* diffusion studies to determine the permeability profile of an active compound, “infinite dose” of the vehicle is applied to the membrane. One shortcoming of the infinite dose application is that in some instances it may fail to imitate the levels of active compound being applied to the skin when commercial formulations are applied. It may also fail to imitate exposure levels to toxic chemicals. Results from *in vitro* studies would differ from those obtained during *in vivo* studies, if the clinically applied concentrations are not taken into account.

For the purpose of this study, *finite dose* is defined as the application of a very small amount of formulation (< 150 µl), whereas *infinite dose* is defined as the application of bigger amounts of formulation (> 150 µl).

The skin is the largest organ of the human body and has a surface area of 1.5 - 2.0 m^2. It consists of three layers, including the stratum corneum, having a thickness of 10 - 20 µm, the viable epidermis (50 - 100 µm) and the dermis (1 - 2 mm). It is mainly the unique brick-and-mortar structure of the stratum corneum and the lipophilic nature of the membrane that are responsible for the barrier properties of the skin (Elias, 1983:45). Fick's (as cited in Williams, 2005:199) law describes the mechanism by which chemicals cross this barrier, either by diffusion, or by the rate of permeation. Fick's law states that the rate of permeation is proportional to the concentration gradient (Fick, as cited in Williams, 2005:199). The accurate prediction of dermal uptake and exposure to topically applied chemicals is relevant to both formulation development and risk assessment (Gre'Goire *et al*., 2009:80).

In order to investigate the transdermal penetration of a molecule, *in vivo* experiments on humans are required. These experiments have in the past shown disadvantages, as they often are morally undesirable, expensive and time consuming, whilst high inter- and intra-individual variations are found in the data. *In vitro* studies, using human or animal skin, offer alternatives to *in vivo* studies. Such methods of testing, however, also have many disadvantages, such as difficulty in obtaining the skins from cosmetic surgical procedures, high subject variations with regards to age, race, sex and general health of the donor, whereas extreme care is needed to prepare the human skin for *in vitro* studies. The use of synthetic membranes eliminates the problems of biological inter- and intra-individual variations of skin, as experienced when using real skin.

3.2 The use of synthetic membranes instead of skin in permeation studies

Pharmaceutically the skin offers advantages to other routes of administration, such as the avoidance of the first-pass metabolism, smaller fluctuations in plasma drug levels for repeated dosing and good patient compliance (Brown *et al*., 2006:178). Despite these advantages, a large amount of pharmaceutical active ingredients are unsuitable for this mode of administration, because of the barrier function of the skin and the physicochemical properties of the permeant. The stratum corneum is also known to show selective permeability to certain types of diffusing molecules. Factors influencing the drug-skin distribution include the physicochemical characteristics of the drug, the choice of the vehicle and the application mode applied (Chen *et al*., 2011:223).

The use of synthetic membranes in studying the diffusion of active compounds is very attractive for a number of reasons. Compared to skin, these polymeric membranes are readily available, homogeneous, chemically pure and easier to handle (Feldstein *et al*., 1998:26). The main reason why synthetic membranes are so popular is that they provide predictive information about *in vivo* transdermal drug delivery through the stratum corneum.

Over the past years, a range of artificial membranes had been developed and studied as models to imitate the skin barrier function of the stratum corneum, for investigation of the cutaneous permeation of transdermal applied active compounds. As a result of these studies, a number of artificial membranes are available today for use as skin imitating barriers in transdermal absorption studies.

Silicone membrane is a non-porous, hydrophobic, relatively inert and reproducible barrier. This membrane consists of an elastomeric nature, making it useful to apply as a barrier, when factors, such as drug concentration on permeation, are evaluated (Zadeh *et al*., 2008:633). Combining different polymers, such as silicone and cellulose acetate, had also been investigated as models to imitate the hydrophilic and lipophilic domains of the stratum corneum. A silicone-cellulose, acetate, multi-laminate is reported to deliver reproducible data on stratum corneum permeation (Nastruzzi *et al*., 1993:45). Other hydrophobic membranes, such as polyethylene, had also proven suitable imitations of the stratum corneum barrier function. Because of the partitioning characteristics of both silicone and Carbosil® membranes, the diffusional barrier is significantly lower when compared to polyethylene. Feldstein *et al*. (1998:25) report that the permeation of drugs through skin and Carbosil® membrane uses the same process of solubility-diffusion. This fact allows for Carbosil® membrane to be a proper replacement barrier for skin in permeation studies. The difference between permeation rates is related to the structure and physicochemical properties of the membranes.

Carbosil® is a polydimethylsiloxane (PDMS), polycarbonate (PC) block, copolymer membrane, acting as a skin imitating, permeation barrier (Feldstein *et al*., 1998:3). It is produced by "Medpoymer", using heterophase polycondensation of oligo-dioxiarylcarbonates with oligo-bis-chlorformate alkyl siloxanes (Feldstein *et al*., 1998:20; Listvoib, 1992:177).

Carbosil® membrane and human skin share the same heterophasic and heteropolar structures, with a common solubility-diffusion mechanism of drug transport (Feldstein *et al*., 1998:20). This membrane differs from the stratum corneum in that it is a rather amphiphilic, or moderately hydrophilic membrane, whereas the stratum corneum is a lipophilic permeation barrier (Zadeh *et al*., 2008:636). Results obtained from the study by Zadeh *et al*. (2008:636) show that the diffusion coefficient of the active compound was three times lower when Carbosil® membrane was used, compared to the results obtained with silicone membrane, which was closer to what had been the expected results from the use of skin was as a membrane. This was due to the

rigid nature of the polymer, which makes this membrane a good choice for use as a skin barrier imitating model in transdermal absorption studies (Zadeh *et al*., 2008:637).

3.3 Infinite dose application compared to clinically applied dose

In order to understand transdermal absorption and to make it relevant to consumer use, it is important to consider the rate and extent of absorption of the active compound for both finite and infinite dosages. Finite and infinite dosing are both influenced by the amount of vehicle applied and the concentration of the active compound in the vehicle (Franz, 1983a:500; Wester & Maibach, 1976:518), as well as by the composition of the vehicle in which the active compound is dissolved (Barry, 1983:127; Franz, 1983b:70). When very small amounts of a formulation (finite dose) are applied to the skin, the net effect is very different from when infinite dose applications are used. Both the rate of absorption and the amount of active compound being absorbed differ (Franz, 1975:215). The two physicochemical determinants that can be manipulated to increase the absorption of a drug are the concentration of the diffused substance, and the permeability coefficient. When finite dose applications are used, steady state rate is seldom found and therefore the permeability coefficient cannot be calculated (Franz *et al*., 1993:213). In this study, ibuprofen was used as a model drug to investigate the differences between finite (similar to clinical dose) and infinite dose applications.

3.4 Ibuprofen

Ibuprofen (2-(4-isobutylphenyl) propionic acid) belongs to a class of non-steroidal, anti-inflammatory drugs (NSAID's), known as the propionic acid derivates, or profens. All profens are organic acids and form water soluble salts with alkaline reagents. Profens are ionised at physiological pH and are more lipophilic than other NSAID's (Jablonowska & Bilewc, 2007:3963). Because of the lipophilicity (log P of 3.6) of ibuprofen, it has the ability to form a reservoir in the stratum corneum, where it is exposed to enzymatic breakdown. The effect of enzymatic breakdown is more significant for ibuprofen, compared to other NSAID's, because of its short half life of 2.2 hours and as a result, the plasma concentration levels of ibuprofen is very low (Beetge *et al*., 2000:262). All of these above constrains were considered in selecting ibuprofen as the model drug in this study. These constrains offered research opportunities to investigate transdermal delivery in clinical situations, but also to develop methods that would enhance delivery of this drug that is known for not easily permeating through the skin.

Non-steroidal, anti-inflammatory drugs (NSAID's) have been used for decades in the treatment of inflammatory or rheumatic disorders and as common pain killers. All NSAID's inhibit cyclooxygenase and reduce the production of mucosal prostaglandins, both of which are

inflammation mediators. The oral administration of NSAID's has some disadvantages, including gastric mucosal damage, mucosal secretion inhibition, structural and viscosity modification, and the reduction of surface hydrophobicity. Ibuprofen consists of two enantiomers, of which S-ibuprofen is a potent cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inhibitor and as a result, this drug presents with less gastric mucosal irritation compared to other NSAID's.

3.5 Risk assessment after dermal exposure to toxic substances

Various studies have been conducted to investigate the absorption of chemicals through the skin. Although interest in the field of developing methods that would improve the absorption of active pharmaceutical ingredients (API's) through the skin has escalated, very little is known about the risks and effects of dermal exposure to chemicals by the general population and by occupationally exposed workers. As toxic substances can be present in the workplace and in the environment, these substances can come into contact with the skin in different ways, depending on the physicochemical properties of the substance, such as vapour deposition, liquid contact, through contaminated water, or during solid contact, such as through contaminated soil.

Transdermal absorption has thus become increasingly important in environmental medicines. Although the absorption of substances through the skin has been studied for years and despite much being known about dermal penetration of pharmaceutical and cosmetic substances, little is known about dermal penetration resulting from exposure to industrial substances. The same technology used to study transdermal penetration of pharmaceutical and cosmetic substances can be used for researching toxic compounds. In order to predict the systemic absorption of toxic substances, it is important to take into account the rate and amount of transdermal penetration. There is thus a need for a reliable *in vitro* method to measure the penetration rate, by generating reproducible results, relevant to human exposure. In order to do a toxicological risk assessment of chemical substances, dermal absorption data is required to estimate the systemic dose after dermal exposure. This systemic dose could then be compared to a systemic limit value to assess the safety risk of the dermal exposure (Buist *et al*., 2010:200).

According to the European Technical Guidance (as cited in Buist *et al*., 2010:200) on risk assessment, 100% absorption is assumed to occur in the absence of experimental data. However, a 10% absorption is assumed, if the risk of exposure is to a chemical having a molecular weight of > 500 and a log K_{ow} (octanol/water partition coefficient) < -1 or > 4 (European Technical Guidance, as cited in Buist *et al*., 2010:200). Since most industrial substances are lipophilic, they are more favourable to penetrate the skin. This fact adds to the importance of being able to accurately measure exposure levels, as well as to developing a standardised *in vitro* test method, which in turn could be compared with *in vivo* data. The

following important characteristics of the applied substance should be considered when real exposure conditions are investigated:

- Concentration;
- Vehicle;
- Time of exposure;
- Amount applied (finite or infinite); and
- Diffusion area (Sartorelli, 2000:138).

3.6 Predictive models for transdermal absorption of active compounds

The accurate prediction of dermal absorption of a topically applied substance and of unwanted chemicals in the environment, such as in the workplace, are of utmost importance for both formulation development and risk assessment (Gre'goire *et al*., 2009:80). A number of different models and approaches have been developed over recent years in an attempt to predict clinical situations from *in vitro* experimental data. The quantitative structure-activity relationships (QSAR) modelling system is an example of such an approach. *In vitro* transdermal data includes the assessment of the permeability coefficient, flux and steady state. By using the QSAR modelling system, predictions can be made for *in vivo* situations (Gre'Goire *et al*., 2009:80). Potts and Guy (1992:663) used QSAR to predict the permeability coefficient (K_p) of a compound, which was applied to the skin in an aqueous solution under infinite conditions. The permeation coefficient (K_p) predicts the ability of a chemical to pass through the skin, which includes the amount completely transported through the skin, as well as the amount present in the tissue (Potts & Guy, 1992:663). In clinical applications, the dermal exposure mostly occurs under finite dose conditions. A simple model that can predict finite dose dermal absorption from infinite dose data was hence required and the stratum corneum/water partition coefficient was developed by Buist *et al*. (2010:200). This QSAR for the stratum corneum/water partition coefficient use infinite K_p and lag time data to estimate dermal absorption for finite dose exposure scenarios (Buist *et al*., 2010:208).

According to the European Union (EU) Risk Assessment Procedure, the dermal absorption, calculated from *in vitro* experiments, involves adding the amount of chemical that has penetrated the skin to the amount of chemical present in the skin. This is the worst approach, since lipophilic substances may be retained in the stratum corneum and may never be available for systemic absorption. The method by Buist *et al*. (2010:201) thus offers an advantage over the risk assessment approach, in that only the K_p and lag time are determined under infinite conditions, in order to predict absorption under a whole range of exposure scenarios, including different exposure times, exposure concentrations, dermal loading and exposed skin area. With

this method, the need to perform many dermal absorption experiments under different scenarios is thus eliminated (Buist *et al*., 2010:208).

Another finite dose diffusion model approach was developed by Boix *et al*. (2010:94). This model is useful in diffusion studies during which the solutions of drugs with a low solubility/permeability balance are used, or when the drug used in the donor formulation has a low concentration in relation to its solubility (Boix *et al*., 2005:94).

Most cosmetic and dermatological applications, intended for topical use, consist of a number of ingredients and combinations of ingredients, which most of the time differ largely from aqueous solutions. The Potts and Guy (1992:663) approach should be used with care for application vehicles that are not single aqueous solutions. An alternative model has been developed for more complex vehicles, which is based on the physicochemical descriptor, i.e. the Relative Polarity Index. This approach enables suitable selection of an emollient that would ensure penetration of the active though the skin (Wiechers *et al*., 2004:174). The equation from Bunge *et al*. (1995:88) is an algorithm that is recommended by the United States (US) Environmental Protection Agency for the assessment of dermal exposure (Brounaugh *et al.*, 1992). This equation calculates the cumulative mass being absorbed as a function of time when a chemical comes into contact with the skin and includes the effect of an epidermal resistance on the permeation of lipophilic chemicals.

The available finite dose prediction models are constantly under investigation in an attempt to provide useful and cost effective dermal absorption data that can be used in risk assessment studies.

3.7 Mode of application of delivery vehicles

The mode of application (finite or infinite dose application) directly impacts on the drug skin penetration and deposition, but also affects the degree of hydration of the stratum corneum (Chen *et al*., 2011:231). Increased stratum corneum hydration leads to increased transdermal delivery of both low lipophilic and hydrophilic compounds, due to an increase in drug partitioning into the skin (Williams & Barry, 2004:606). Increased hydration of the stratum corneum enhances the penetration of hydrophilic drugs, whereas the penetration of highly lipophilic drugs becomes more difficult (Zhang *et al*., 2010:895). Hydrophilic and lipophilic drugs have a different molecular mechanism by which they diffuse through the stratum corneum. Because of the lipophilic nature of the stratum corneum, the limiting step for skin penetration of hydrophilic drugs is its partitioning into the stratum corneum. The limiting step for lipophilic drugs is their partitioning into the less lipophilic epidermis. With an infinite dose application, modification of the micro-structure of the stratum corneum could be reinforced to compare to a finite dose

application. This is due to more hydration of the stratum corneum with an infinite dose application, compared to the lower hydration with a finite dose application. This modification of the micro-structure of the stratum corneum by an infinite dose does not have the same penetration enhancement effect on hydrophilic and lipophilic substances (Chen *et al*., 2011:233).

3.7.1 Infinite dose technique

The infinite dose technique has been the most frequently used *in vitro* method for studying the absorption of active compounds. By using this technique, the skin or membrane is mounted as a barrier between two compartments, i.e. the donor and receptor compartments. The active compound is applied to the membrane in the donor compartment as part of a saturated solution in volumes > 150 µl. The absorption of the active compound through the membrane is analysed by assaying the receptor fluid. If the infinite dose technique is used as a predictive model for transdermal penetration, several objections can be raised:

1. The skin is exposed to an aqueous solution on both the receptor and donor sides, which enhances hydration of the skin and as a result increases penetration of the active through the skin. This is different from real conditions where the stratum corneum is exposed to a dry environment.
2. Hydrophobic substances do not come into contact with the skin in aqueous solutions (Sartorelli, 2000:136).

The application of an infinite dose in the donor compartment forms a thick liquid layer on the surface of the membrane having a thickness of 1.6 mm, which would result in higher hydration conditions of the stratum corneum with an infinite dose application. This increase in skin hydration would cause higher penetration of the permeant through the skin (Chen *et al*., 2011:231). Zhang *et al*. (2010:895) suggest that the hydration of the stratum corneum would increase the penetration of hydrophilic drugs, whilst making the penetration of lipophilic compounds into the hydrated stratum corneum more difficult, which would result in a reduction in the permeability capacity of lipophilic compounds through the stratum corneum.

By using infinite dose applications in diffusion studies, it is important to focus on three characteristic parameters of the compound, i.e. the steady state rate of absorption, the permeability coefficient and lag time (Franz, 1975:213). Results from infinite dose applications provide characterisation information of the compound (i.e. by defining the steady state absorption rate, permeability coefficient and lag time), which can be used for comparison between one material with others having the same physical/chemical properties. In other words, the prolonged exposure to infinite doses of cosmetic or pharmaceutical vehicles do not

mimic actual clinical conditions, but are these experiments conducted in an attempt to determine the effect of the chosen vehicle on the skin permeation parameters.

2.7.1.1 Effect of vehicle saturation on infinite dose applications

Santos *et al*. (2010:70) used supersaturation as a method to improve permeation of a drug through the skin. The thermodynamic activity in a solution is at its maximum when the solution is saturated. Supersaturated solutions are obtained when the concentration of the drug in the solvent is higher than its solubility in the solvent (Hadgraft, 2004:292). Due to the thermodynamically unstable nature of supersaturated solutions, drug crystallisation in the solution occurs over time, decreasing the degree of saturation (Santos *et al*., 2010:70). As a result of the decrease in the saturation levels of a solvent, the absorption through the membrane is reduced. Santos *et al*. (2011:158) conclude that when infinite dose applications are used, the permeation across the skin increases with increasing levels of saturation of the vehicle, confirming that supersaturated solutions enhance penetration through the skin with infinite applications. The results obtained by Santos *et al*. (2011:158) demonstrate that the first layers of the stratum corneum have the ability to support supersaturated states. Pellet *et al*. (1997:91) suggest that the lipid combination of the first layer of the stratum corneum has nucleating properties that enables the stratum corneum to maintain supersaturated states. Santos *et al*. (2011:158) conclude that when infinite doses were applied to the skin, the absorption from supersaturated and saturated solutions was proportional to the degree of saturation of the solvent vehicle (Santos *et al*., 2011:158).

3.7.2 Finite dose

Experiments done by using finite dose applications of an active compound are probably more relevant to human exposure, or clinical conditions (Franz *et al*., 1993:213). Very little is known about the effects of finite dose and how it compares to *in vivo* study results. Because this mode of application imitates clinically applied conditions, it is important to take into account environmental variables, such as temperature, wind and clothing that can lead to evaporation and depletion of the active and the formulation, as well as to consider mechanisms of enhancing penetration through the skin, by using finite dosing. The application of a finite dose in the donor compartment forms a thin layer of only 0.1 mm of liquid formulation on the surface of the membrane, compared to the 1.6 mm layer achieved with infinite applications. Because of the thin application layer at the surface of the membrane, the extent of stratum corneum hydration is less and as a result the interaction between the permeant and the stratum corneum intercellular lipids would decrease, whilst the penetration through the skin would also decrease

(Chen *et al*., 2011:231). The thin application layer on the surface of the membrane/skin is thus more relevant to clinical conditions.

3.7.2.1 Effect of vehicle saturation on finite dose applications

Santos *et al*. (2010:67) found that when finite dose applications were used, the flux of the permeant through the skin was the same for saturated and supersaturated solvent vehicles. This outcome was due to drug crystallisation, as a result of solvent depletion. The solvent depletion at the skin surface (32 °C) occurs due to evaporation or permeation of the solvent. Drug crystallisation of the permeant results in a reduced penetration enhancement when supersaturated solutions are applied (Santos *et al*., 2010:70). When finite dose applications are applied to the skin, the drug partition and diffusion coefficient vary during the diffusion, whilst the permeation flux decreases over time. The lack of penetration enhancement with supersaturation, when finite dose applications are used, is due to the depletion of the solvent vehicle from the donor compartment, as well as from the crystallisation of the permeant on and in the membrane (Santos *et al*., 2011:159).

3.7.2.2 Permeant depletion in the vehicle of finite dose conditions

The permeant depletion from the donor solution may result from a high thermodynamic activity of the permeant in the vehicle and/or from rapid penetration enhancement caused by the vehicle (Leopold, 1998:165). The extent of permeant depletion in the solvent vehicle depends on the thickness of the applied solvent layer. In the case where a thin film (finite dose) is applied to the skin, reduced permeant penetration should be considered, because of rapid depletion of the permanent in the applied solvent. Permeant depletion occurs because of crystallisation or rapid penetration of the solvent, but not of the permeant. When permeants with low solubility in the vehicle or vehicles with penetration enhancement properties are used, permeant depletion should be considered as a possibility of decreased penetration through the skin. This problem can be avoided by the application of a suspension type formulation, or by using vehicles with high dissolving capacity (Leopold, 1998:173).

3.8 Summary

The skin is the largest organ of the human body and consequently comprises the organ that is mostly exposed to chemical substances and various compounds. Some of these substances pass the skin barrier function and are systemically absorbed, affecting the metabolism and health of humans. Understanding the mechanism of skin penetration and the toxicity and irritancy of substances would enable researchers to enhance transdermal drug delivery and to

increase the reliability of risk assessment on dermal exposure to toxic substances (Barry, 2007:580). Permeation of chemicals through the skin is not only dependent on the chemical structure and properties of the permeant, but it is also affected by the chemical structure and properties of the mixture of chemicals present in the applied formulation or in the environment.

Solvents and combinations of solvents can change the permeation profile of a chemical substance in various ways. The solvent or mixture of solvents can enhance penetration through the skin by changing the properties of the stratum corneum lipid and protein domains, and/or by increasing the solubility of the permeant and therefore the thermodynamic activity of the permeant in the solvent mixture that would cause the permeant to partition from the solvent mixture into the stratum corneum (Ghafourian, 2010:28). It is hence important that during risk assessment investigations, or during the development of transdermal commercial products, that the physicochemical properties of both the active compound and of the applied vehicle are taken into account. For some active compounds, like insect repellents and sunscreens, permeation through the skin is undesired and special care should be taken when a solvent vehicle is chosen, in order to prevent these substances from penetrating the skin.

From the insights of this chapter, it is evident that not only the mode of application, but also the mixture of chemicals applied or exposed to the skin, would influence the positive or negative effects of a substance penetrating the skin. The literature showed that the mode of application will have a direct impact on the drug deposition and penetration through the membrane. For this reason diffusion studies on Carbosil® membrane have been conducted to determine the impact of the mode of application on permeation of ibuprofen as the model drug.

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CHAPTER 4

ARTICLE FOR PUBLICATION IN THE JOURNAL OF DRUG DELIVERY

Chapter 4 is in article format, in preparation for submission for publication in the Journal of Drug Delivery. Guidance for authors is outlined in Appendix C and from there it is advised that the author writes in concise US English. Note that the general formatting and style have for the purpose of inclusion in this chapter been adjusted in accordance with the rest of this thesis, whereas the reference style was mainly kept as per the article requirements.

The penetration enhancement effect of single and binary phase combinations of hydrophilic and lipophilic vehicles

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4.1 Abstract

Context: Ibuprofen, used as the model drug, is a potent, non-steroidal, anti-inflammatory drug that is often used in long-term therapeutic treatments. Transdermal delivery may thus be more effective in reducing the well-known side-effects of this drug, while maintaining its therapeutic blood concentration. It is, however, difficult to maintain effective blood concentrations by transdermal delivery of ibuprofen, due to its poor skin permeability. **Objective:** The aim of this study was to establish which penetration enhancers and / or combinations thereof, would best deliver ibuprofen through Carbosil® membranes, by using infinite modes of application. **Materials and method:** Single and binary phase penetration enhancement vehicles were used in Franz cell diffusion studies. The vehicles used were hydrophilic propylene glycol and water individually and in combinations of 20/80 (v/v), 50/50 (v/v) and 80/20 (v/v), as well as lipophilic

mineral oil and Miglyol® individually and in the same combinations as above. These permeation studies were conducted over 6 hour periods in order to determine the penetration enhancement effect of each vehicle, when using infinite application volumes. **Results and Discussion:** The highest concentration of diffused ibuprofen through Carbosil® membrane was measured from the binary vehicle comprising 20/80 (v/v) mineral oil/Miglyol®. This related to the synergistic mechanism of action of the two solvent vehicles in combination. Diffused ibuprofen levels from the propylene glycol/water vehicles were lower, but increased with an increase in the level of propylene glycol. **Conclusion:** Results from this study showed that when penetration enhancer solvents are used in combination, the synergistic effect between the two enhancers may lead to an improved penetration enhancement effect of the active compound through Carbosil® membranes.

4.2 Introduction

The transdermal route of drug delivery is frequently used to deliver potent therapeutic agents with low molecular weight, as this route of drug delivery offers several advantages over conventional dosage forms, like tablets and injections, including avoidance of the first-pass metabolism, minimizing pain and the possible sustained release of drugs (Qing *et al.,* 2006). Ibuprofen was used as a model drug in this study. Because of its lipophilic nature, much difficulty was experienced with employing the transdermal route of administration for delivering this active ingredient. The administration of non-steroidal, anti-inflammatory drugs (NSAIDs) *via* the dermal route has been adopted in order to bypass the disadvantages of the oral route, such as irritation and ulceration of the gastro-intestine (Cordero *et al.,* 1997), despite the limitations of utilising the transdermal delivery path, due to the generally low drug penetration across the skin.

The main barrier to the transport of drugs across the skin is known to be the stratum corneum. Since it comprises dead tissue, the transport of substances through this skin layer involves a process of passive diffusion down a concentration gradient (Williams, 2005). It hence is extremely difficult and in some cases highly unlikely for a formulator to deliver therapeutic levels of a drug at the desired site of action, when using single or binary delivery vehicles. Furthermore, the physicochemical properties of the drug also largely influence the delivery of the drug to the target site. It is therefore often necessary to increase the amount and rate of dermal delivery in order to achieve the required therapeutic levels. Factors influencing the drug-skin distribution include the physicochemical properties of the drug, the choice of the delivery vehicle and the application mode used (Chen *et al*., 2011).

For the investigations being performed during this study, Carbosil® membrane was used as the barrier membrane, instead of the stratum corneum. Carbosil® membrane is a synthetic membrane that shares a common solubility-diffusion mechanism of drug transport with human

epidermis. This membrane can be used for the quantitative prediction of transdermal drug delivery rate, as well as in serving as a skin imitating standard membrane in *in vitro* drug delivery studies (Feldstein *et al*., 1998).

The majority of studies focusing on penetration enhancement are concerned with delivery vehicles that penetrate the membrane and modify the structure of the stratum corneum in such a way that it is more favourable to allow substances through. The concentration of the diffused substance can increase if the enhancer induces modification of the polarity of the skin, such as an increase in the solubility of the drug in the skin. The use of a co-enhancer (binary component) system has also proven very effective (Williams & Barry, 2004). A multiplicative, synergistic effect is achieved through which one enhancer increases the delivery of the other, as well as the drug. What is clear is that the choice of the enhancement vehicle will depend on the physicochemical properties of the permeant (Williams & Barry, 2004).

The aim of this study was to establish which penetration enhancers and / or combinations thereof, would best deliver ibuprofen through Carbosil® membranes, by using different modes of application. For this purpose the penetration enhancement effects of selected vehicles having different properties were examined, and their influences on the permeation of ibuprofen, when applied in infinite doses (volumes >150 µl, i.e. 250 µl, 500 µl and 1,000 µl), were investigated. Franz cell permeation studies were conducted in order to assess permeation, whilst the concentrations of the ibuprofen having diffused across the skin were analyzed by employing high performance liquid chromatography analyses (HPLC).

The skin is the first line of defense of an organism and also forms the last barrier for protecting the organism from its hostile environment of external aggressors, such as viruses, pathogens and toxins. Because of its functions, the skin naturally offers a very low permeability that prevents the active transport of foreign molecules across. Chemicals acting as penetration enhancers offer the potential of overcoming the skin barrier and as such enhance the transport of molecules. Individual chemicals are limited in their efficacy to overcome the barrier function at low concentrations, whilst they usually cause skin irritation at high concentrations. The use of multi-component mixtures can overcome this problem and have shown to provide higher skin penetration properties compared to individual chemicals, without causing skin irritation.

Chemical penetration enhancers were normally classified based on their chemical structures, rather than their mechanisms of action to enhance penetration through the skin. Karande and Mitragotri (2009) found that chemicals belonging to the same group can act on skin through different mechanisms, depending on their individual physicochemical properties. Water is the most natural penetration enhancer. By increasing the hydration state of the membrane will lead to enhanced penetration through the membrane (Roberts & Walker, 1993).

Hydrocarbons, including mineral oils, are used as vehicles or penetration enhancers that increase the permeation of drugs across the skin. They work by improving drug partitioning into the stratum corneum and by disrupting the ordered lipid bilayer structure of the skin. Like hydrocarbons and mineral oils, alcohols are also frequently used as vehicles, solvents or penetration enhancers in improving the transdermal delivery of drugs. Such alcohols include glycols, for example propylene glycol, and enhance skin permeation through a variety of mechanisms, such as the extraction of lipids and proteins, the swelling of the stratum corneum, or by improving drug partitioning into the skin or the solubility of the drug in the formulation (Loth, 1991). Another category of popular penetration enhancer solvents are acids, of which fatty acids are the most commonly used. These chemicals enhance the transport of molecules across the skin either by partitioning into the lipid bilayer and by disrupting their ordered domains, or by improving penetration through the skin by forming lipophilic complexes with drugs (Komata *et al.,* 1992). Primary, secondary and tertiary, cyclic and acyclic amines have also successfully been used to enhance skin permeation by drug partitioning into the lipid bilayer and by improving drug partitioning into the skin. Cyclic and acyclic amides, such as Azone®, form another significant class of chemicals that can be employed as penetration enhancers, by acting as solvents to enhance the activity of the drug in the solvent, and by improving drug partitioning in the skin (Loth, 1991). Esters of fatty acids, particularly isopropyl myristate, are the most widely studied ester for transdermal penetration enhancement. The mechanism of enhancing drug penetration is through partitioning into the ordered lipid domain itself and by enhancing the permeability of the stratum corneum (Loth, 1991). Surfactants are usually used with vehicle or solvent systems and their activities depend on their hydrophilic to lipophilic balance, charge and lipid tail length (Karande & Mitragotri, 2009). Terpenes, terpenoids and essential oils are further popular choices of penetration enhancers and their effects on the skin will depend on their physicochemical properties and in particular their lipophilicity. Sulfoxides, lipids and various other chemicals (e.g. amino acids, thioacyl derivatives of amino acids, alkyl amino esters, oxazolidinones, enzymes and acrocyclic ketones with 12-carbon atoms or more) are other skin penetration enhancer chemicals that are widely used and studied (Karande & Mitragotri, 2009).

A number of studies have also shown that when chemicals are used in combination, the mechanism of action by which they enhance permeation through the membrane would be synergistically, causing higher levels of penetration enhancement than when used individually (Mollgaard, 1993). The mechanism by which such systems increase transdermal flux may include (a) change in the thermodynamic activity (increasing the degree of saturation in the solvent), or (b) specific interaction with the stratum corneum (either by increasing the drug solubility in the stratum corneum, or (c) by altering the different pathways to enhance transport through the skin) (Williams & Barry, 2004).

4.3 Materials and methods

4.3.1 Materials

The ibuprofen being used during this study was obtained from Albemarle Corporation (South Carolina, USA). Other ingredients included deionised HPLC grade water, prepared by the Milli-Q water purification system (Millipore, Milford, USA) and Carbosil® membranes (Medpolymer). Methanol (from Merck Laboratory Supplies, Midrand, South Africa) and Milli-Q water (70/30) were used as the HPLC mobile phase. The phosphate buffer solution (PBS) consisted of sodium chloride, disodium orthophosphate dehydrate and sodium dihydrogen dehydrate (from Merck Laboratory Supplies, Midrand, South Africa) and Milli-Q water.

Single and binary phase penetration enhancer solvents were used as delivery vehicles. Propylene glycol (from Merck Laboratory Supplies, Midrand, South Africa) and HPLC grade water (Millipore, Milford, USA) were used. A second combination of solvents was used as penetration enhancer delivery vehicles, which comprised of mineral oil (from Sigma-Aldrich, Johannesburg, South Africa) and Miglyol® (from Merck Laboratory Supplies, Midrand, South Africa). These solvents were used individually and in combination in ratios of 20/80 (v/v), 50/50 (v/v) and 80/20 (v/v). Different infinite volumes of application were used when applying the saturated vehicles to the Carbosil® membrane, i.e. 250 µl, 500 µl and 1,000 µl.

4.3.2 Analysis of ibuprofen

4.3.2.1 Preparation of standard solution for calibration curve

A stock solution was prepared by accurately weighing 50 mg of ibuprofen into a 100 ml volumetric flask. The flask was then filled to volume with methanol/Milli-Q water (50/50) and sonificated to ensure a homogenous solution. From this stock solution, 2, 5, 6, 8 and 10 ml samples were transferred into separate 100 ml volumetric flasks each and filled to volume with methanol/Milli-Q water (50/50). HPLC vials were filled with samples from each volumetric flask and analysed in duplicate on the HPLC.

4.3.2.2 Method of analysis

The HPLC method used during this study had been previously validated at the Analytical Technology Laboratory of the School of Pharmacy, at the Potchefstroom Campus of the North West University (South Africa), whilst HPLC analyses were conducted under controlled laboratory conditions of 25°C. All reagents were HPLC grade.The HP1100 series HPLC, equipped with a pump, auto sampler, UV detector and Chemstation Rev. A.06.02 data acquisition and analysis software, or equivalent, were used for the analysis of ibuprofen

concentrations. The Luna C_{18}-2 column, 150 x 4.6 mm, 5 µm, 100 Å pores, 17.8% carbon load, endcapped, Phenomenex, Torrance, CA was used. Detection took was at 240 nm. The mobile phase consisted of a filtered and degassed mixture of Milli-Q water/methanol (70/30). The mobile phase flow rate was 1.0 ml/min with an injection volume of 100 µl. The retention time of ibuprofen was 5 minutes and the sample run time 7 minutes.

4.3.3 Solubility studies and preparation of the donor phase

Solubility studies were conducted in an in an attempt to determine the saturated ibuprofen levels of each solvent used. Excess drug was added to each solvent vehicle and stirred using a magnetic stirrer for 24 hours (to attain equilibrium) in a water bath maintained at 32°C. The solubility of ibuprofen was determined at 32°C, like all diffusion experiments, as human skin temperature is 32°C. A 5 ml syringe and a filter were also pre-heated to 32°C. The supernatant was then filtered and diluted with methanol (1/10) (supernatant/methanol). The samples were assayed on HPLC. Assays were performed in triplicate to determine the solubility of the drug in the solvent. For each subsequent test, ibuprofen was added to each solvent vehicle in concentrations as determined by the solubility study, in order to ensure saturated solutions of ibuprofen during this study.

4.3.4 Preparation of the receptor phase

Phosphate buffered solution (PBS) (pH 7.4) was used in the receptor phase and was prepared by dissolving 4.4 g of sodium chloride, 9.2 g of disodium orthophosphate dehydrate and 2.1 g of sodium dihydrogen dehydrate in 1,000 ml freshly prepared Milli-Q water.

4.3.5 Membrane permeation studies

Twenty-four vertical glass Franz diffusion cells, with a diffusion area of 1.075 cm^2 and receptor capacity of approximately 2 ml were used during this study. Carbosil® membrane was cut into circles big enough to cover the area of the Franz cell that is available for diffusion. Prior to each test, all Carbosil® membrane circles were pre-soaked for 12 hours at 32°C in the relevant delivery vehicles (excluding the active) in order to allow saturation thereof. When the membrane is saturated with the solvent vehicle, the permeant partitions into the membrane at a much faster rate than when the membrane is unsaturated. After 12 hours the membrane circles were removed from the soaking solution and dried with tissue paper to remove excess solution on the membrane surfaces. Each membrane circle was mounted on the receptor compartment of the Franz cell to cover the diffusion area. Each Franz cell assembly was then sealed with Dow Corning, high vacuum grease and secured with a horseshoe clamp. All cells were placed

on a submersible Variomag® stirrer plate and maintained at 37°C, taking care not to allow bubbles in the receptor compartment. Saturated solutions of ibuprofen in the different solvent vehicles were prepared an hour before onset of each test. The saturated solutions were kept at 32°C prior to application to the membrane. Different application volumes were applied onto each membrane, using a calibrated pipette. A separate test was conducted for each solvent vehicle at each of the specified volumes. Of the twenty-four Franz cells that were used per diffusion experiment, the receptor phase of the first four cells were extracted after 1 hour and not refilled. Likewise, after 2 hours the second four cells were extracted. The extraction of four cells each continued hourly, until 6 hours have passed and all of the receptor compartments were sampled. This method was followed in an attempt to keep the diffusion area consistent for finite volumes that did not cover the entire area of the Franz cell, available for diffusion. When the Franz cell was left stagnant during the experiment, the droplet remained in one place on the membrane and the area for diffusion could be kept constant and could be measured. Because of the design of this experiment, however, it was impossible to use cumulative concentrations or to calculate flux.

4.3.6 Data analysis

Before analyzing the samples obtained from the diffusion studies, a standard solution was prepared and its linearity determined. In this study, as was mentioned, it was impossible to calculate cumulative amounts of ibuprofen per area (flux), since the receptor compartments of the Franz cells could not be refilled after each hourly sampling interval. The concentration ($\mu g/cm^2$) of the ibuprofen that had diffused through the Carbosil® membrane per sampling interval was calculated.

4.4 Results and discussion

4.4.1 Solubility of ibuprofen in selected solvents and binary mixtures thereof

Ibuprofen was dissolved in the different solvent vehicles of propylene glycol and water individually and in ~~the~~ different combinations thereof, and similarly in Miglyol® and mineral oil and in combinations thereof. Table 4.1 summarizes the solubility of ibuprofen in the different solvent vehicles. The racemic, crystalline ibuprofen consists of rigid molecules that pair off as hydrogen-bonded (R)-(S) dimers (Iervolino *et al.,* 2001). From the results obtained it was clear that the solubility of ibuprofen increased from 0.94 - 323.14 mg/ml, as the percentage of propylene glycol increased from 0 – 100% in the solvent. As ibuprofen molecules may form hydrogen bonds with solvents through the carboxylic group of the molecule, it is suggested that

ibuprofen could then interact with propylene glycol (Iervolino *et al.,* 2001) and hence increase the solubility of the drug, as may have occurred during this study.

Table 4.1 also lists the solubility of ibuprofen in the solvent vehicle combinations of mineral oil and Miglyol®. When comparing the solubility of ibuprofen in Miglyol® and mineral oil, it is apparent that ibuprofen was more soluble in Miglyol® (31.99 mg/ml) than in mineral oil (10.69 mg/ml) and most soluble in a 50/50 (v/v) mixture of the two solvents (1696.01 mg/ml). It is likely that ibuprofen could itself associate through intermolecular bonds to form dimers (Iervolino *et al.,* 2001). The carboxylic acid (-COOH) ends of the molecule are polar, but if 'protected', it gives a non-polar dimer (Iervolino *et al.,* 2001). For this reason, ibuprofen showed good solubility in both solvents and in a 50/50 mixture of the two solvents its solubility was at its highest.

4.4.2 The effect of single and binary phase solvents of propylene glycol, water, mineral oil and Miglyol® and the diffusion of ibuprofen through Carbosil® membrane

The concentrations (µg/cm^2) of ibuprofen that had diffused through the Carbosil® membranes in the different **propylene glycol** and **water** vehicles are graphically illustrated in Figure 4.1. Each bar in these graphs depicts the average total concentration (calculated from the four diffusion cells being extracted at each sampling interval) of ibuprofen that had diffused through the Carbosil® membrane at each sampling interval (i.e. at 1, 2, 3, 4, 5 and 6 hours). .

The concentration of ibuprofen (µg/cm^2) that had diffused through the Carbosil® membrane using the single phase solvent, **water** (Figure 4.1.A) in varying infinite volumes, increased as the amount of solvent applied increased. The highest concentration of diffused ibuprofen from the water delivery vehicle was measured for the 1,000 µl application (0.22 µg/cm^2), followed by the 500 µl application (0.11 µg/cm^2) and last by the 250 µl application (0.01 µg/cm^2). The 250 µl application showed permeation of the active across the membrane only after 3 hours.

Figure 4.1.B shows the concentration of ibuprofen (µg/cm^2) that had diffused through the Carbosil® membrane using the binary phase solvent **20/80** (v/v) **propylene glycol/water** in different infinite dose volumes. The results showed that the quantity of ibuprofen that had moved across the membrane in the 1,000 µl application (0.22 µg/cm^2) was lower than in the 500 µl application (0.35 µg/cm^2), but higher than in the 250 µl application (0.1 µg/cm^2).

When the binary solvent **50/50** (v/v) **propylene glycol/water** was employed as the delivery vehicle (Figure 4.1.C), the amount of ibuprofen that had diffused through the membrane in the 500 µl application was extremely low (0.02 µg/cm^2), while the amounts of diffused ibuprofen in

the 250 µl and 1,000 µl applications were ten times higher, with concentrations of 0.21 µg/cm^2 and 0.26 µg/cm^2, respectively, thus showing very small differences in the absorption levels between these two volumes.

The amounts of ibuprofen that had diffused through the Carbosil® membrane, using various application volumes of the **80/20** (v/v) **propylene glycol/water** vehicle, showed very similar results, as illustrated in Figure 4.1.D. The concentration of diffused ibuprofen in the 1,000 µl application was 1.16 µg/cm^2, followed by the 250 µl application (0.99 µg/cm^2) and the 500 µl application (0.96 µg/cm^2).

Figure 4.1.E clearly illustrates that with the application of the single phase solvent, **propylene glycol**, the highest level of permeation was measured for the 500 µl application (2.34 µg/cm^2), while the 250 µl (1.79 µg/cm^2) and 1,000 µl (1.71 µg/cm^2) applications showed very similar results

Overall, with regards to the permeation results obtained with the **propylene glycol and water** vehicles, Figure 4.1 (A - E) illustrates that the higher levels of diffusion of the active through the Carbosil® membrane was measured when infinite dose volumes of 100% propylene glycol as the carrier vehicle were applied. The results further showed that higher volumes of 100% propylene glycol as the delivery solvent resulted in more ibuprofen diffusing across the membrane. This would have been caused by the mechanism of action of propylene glycol to partition into the membrane and increase permeant solubility in, and diffusion through the membrane (Squillante *et al.,* 1998). A higher volume applied increases the surface area covered by the vehicle, whilst the layer of vehicle solvent on the surface of the membrane would be thicker, resulting in improved hydration of the membrane (Chen *et al.,* 2011).

Water is the most natural penetration enhancer (Roberts & Walker, 1993) and by soaking the membrane in water, the hydrophilicity within the membrane will increase. As a result of the increased hydration levels of the membrane, its permeability will also increase. The lipophilic nature of ibuprofen will cause it to penetrate through highly hydrated membranes with difficulty (Beetge *et al.,* 2000). This would explain the low permeation concentrations measured for water as the delivery vehicle. The addition of propylene glycol would overcome some penetration difficulties, due to the nature of the Carbosil® membrane and the mechanism of action of propylene glycol. Like human skin, Carbosil® membrane consists of a hydrophilic and lipophilic structure (Feldstein *et al*., 1998). The propylene glycol will carry the ibuprofen, a lipophilic drug, into the lipophilic section of the membrane and increase the solubility of the drug, resulting in higher levels of diffused ibuprofen. The more propylene glycol available for this action, the better the effect, which would explain the increase in permeation concentrations as the level of propylene glycol in the combination vehicles increased.

In summary, Figure 4.1 (A - E) shows that the best penetration enhancement effect was observed with a 100% propylene glycol delivery vehicle, followed by combinations of propylene glycol, while water showed to have very little penetration enhancement properties for the permeant, ibuprofen.

Figure 4.2 illustrates the concentration of the ibuprofen that had diffused through the membrane from **mineral oil** and **Miglyol®** penetration enhancement vehicles. Figure 4.2.A illustrates that for the application of 500 µl of the single phase solvent, **Miglyol®**, to the Carbosil® membrane, the level of diffused ibuprofen measured (0.81 µg/cm^2) was slightly higher than that measured for 250 µl (0.75 µg/cm^2), followed by the ibuprofen level measured for a 1,000 µl (0.65 µg/cm^2) application.

Figure 4.2.B shows the permeation concentrations of ibuprofen when **20/80** (v/v) **mineral oil/Miglyol®** was used as the delivery vehicle. The permeation results for the 500 µl (3.78 µg/cm^2) and 1,000 µl (3.72 µg/cm^2) applications were very similar and were slightly higher than the result obtained for the 250 µl (2.83 µg/cm^2) application.

With the **50/50** (v/v) **mineral oil/Miglyol®** mixture as delivery vehicle of the active through the Carbosil® membrane, the best result was obtained after 6 hours for the 250 µl (3.09 µg/cm^2) application, followed by very similar results for the 1,000 µl (2.88 µg/cm^2) and 500 µl (2.65 µg/cm^2) applications, as illustrated by Figure 4.2.C.

Figure 4.2.D illustrates the concentration of ibuprofen that had diffused through the membrane when **80/20** (v/v) **mineral oil/Miglyol®** vehicles were employed to deliver the active. The permeation concentrations measured for the different volumes of solvent applied were very similar, i.e. 1,000 µl (3.19 µg/cm^2), followed by the 250 µl (2.93 µg/cm^2) and 500 µl (2.38 µg/cm^2) applications.

Figure 4.2.E illustrates the penetration outcomes for the single phase **mineral oil** delivery vehicle. The results showed that the amount of ibuprofen that had diffused through the membrane increased as the applied vehicle volume increased. The 1,000 µl (0.86 µg/cm^2) application showed the highest permeation results, followed by the 500 µl (0.53 µg/cm^2) application and lastly the 250 µl (0.51 µg/cm^2) application.

Overall, Figure 4.2 (A - E) illustrates that the single phase solvents of 100% mineral oil and 100% Miglyol® showed the lowest penetration enhancement effects, followed by the other single phase vehicles. The mineral oil/Miglyol® combination vehicles gave the best ibuprofen diffusion results, possibly due to a synergistic action between the combined penetration enhancer solvents The three multi-component solvents further showed very similar permeation profiles(Table 4.1). . According to Moser *et al*. (2001), Miglyol® is known for modifying the

intercellular lipids of the stratum corneum and to disrupt the barrier function of this skin layer, which causes the diffusivity of chemicals through the membrane to increase. Since Carbosil® membrane and human epidermis share a common solubility-diffusion mechanism of drug transport, it could be hypothesised that Miglyol® would change the polar structure of the membrane and as a result enhance its permeability. Mineral oil is a lipophilic solvent and while Miglyol® modifies the heteropolar structure of the membrane to make it more viable, mineral oil would then carry the active to the lipophilic section of the membrane and as a result enhance the permeation of the lipophilic drug ibuprofen (Hori *et al.,* 1991). A further explanation for the successful penetration enhancement of these solvents may be their lipophilic nature. It is possible that when applied to the membrane, it may increase the lipophilic hydration levels of the membrane, resulting in an increase in drug transport across the membrane. It is for this reason that higher levels of the lipophilic drug (ibuprofen) could penetrate the membrane from these vehicles (Chen *et al.,* 2011).

4.5 Conclusion

Two groups of penetration enhancement vehicles were tested for their abilities to improve the permeation of the lipophilic drug, ibuprofen, through the Carbosil® membrane, similar in nature to the stratum corneum of human skin. The first group of solvents comprised of single phase Propylene glycol and water vehicles, as well as in three different combinations. The second group consisted of mineral oil and Miglyol®, individually and in three different combinations. Furthermore, each solvent was applied in three different infinite volumes (i.e. 250 µl, 500 µl and 1,000 µl) to the membrane.

From the outcomes of this study, it has become evident that the binary penetration enhancement solvents containing mineral oil and Miglyol®, showed the best diffusion enhancement effects for ibuprofen (see Figures 4.1 and 4.2). The 20/80 (v/v) mixture of mineral oil/Miglyol® increased the permeation of ibuprofen through the Carbosil® membrane to the highest level than any of the other vehicles of both groups, tested. This would have been due to the synergistic action of the two solvents as previously explained (see section 1.1).

Propylene glycol is widely used as a penetration enhancer, or as a vehicle for other penetration enhancers, or in combination with other penetration enhancers. When used with other penetration enhancers, it shows synergistic action. Previous studies on the efficacy of propylene glycol as a penetration enhancer showed mixed results. Evidence suggests that propylene glycol has a very mild enhancement effect on molecules, such as estradiol and 5-fluorouracil (Williams & Barry, 2004). Results from this study also showed that the penetration enhancement effect of propylene glycol increased as the percentage of the propylene glycol in a combination solvent vehicle increased, and as the application volume of a single phase vehicle increased. Its mechanism of action is to partition into the stratum corneum and to increase the

solubility of the permeant in the membrane, causing an increase in the flux of both the propylene glycol and the permeant (Trottet *et al.,* 2004). As Carbosil® membrane shares the same heteropolar structure as human stratum corneum, the same mechanism of action is applicable to the Carbosil® membrane.

The outcomes of this study further showed that the single and multi-component solvents containing water, delivered less of the active through the Carbosil® membrane, compared to the other solvents tested. Contrary to water, mineral oil and Miglyol®, propylene glycol individually showed good penetration enhancement properties.

The penetration enhancement potential of the four chemicals used in this study individually or in combination, was investigated in an attempt to identify chemicals that could in future be successfully employed for facilitating delivery of drugs through the human skin barrier. The results from this study confirmed the observations by Williams and Barry (2004):

a) Penetration enhancer properties appear to be drug specific (permeants with similar physicochemical properties).

b) Penetration enhancers tend to work well with co-solvents, such as propylene glycol.

c) Most penetration enhancers have a complex concentration dependent effect.

d) Potential mechanisms of action of penetration enhancer solvents are different, and can range from direct effects on the skin to modification of the formulation (Williams & Barry, 2004).

Mixtures of chemicals employing synergistic action may offer a solution to overcoming the limitations that were shown by chemicals that were applied individually in enhancing transdermal drug delivery.

In order to investigate the direct effects that these tested chemicals would have on human skin, further studies, using human skin itself, would be necessary.

Declaration of interest

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Tables

Table 4.1: Solubility of ibuprofen in propylene glycol and water as well as in mineral oil and Miglyol®

Vehicle	Solubility (mg/ml)
Propylene glycol : Water	
100 : 0	323.14
80 : 20	26.65
50 : 50	2.13
20 : 80	1.02
0 : 100	0.94
Mineral oil : Miglyol®	
100 : 0	10.69
80 : 20	167.63
50 : 50	1696.01
20 : 80	53.61
0 : 100	31.99

Figures

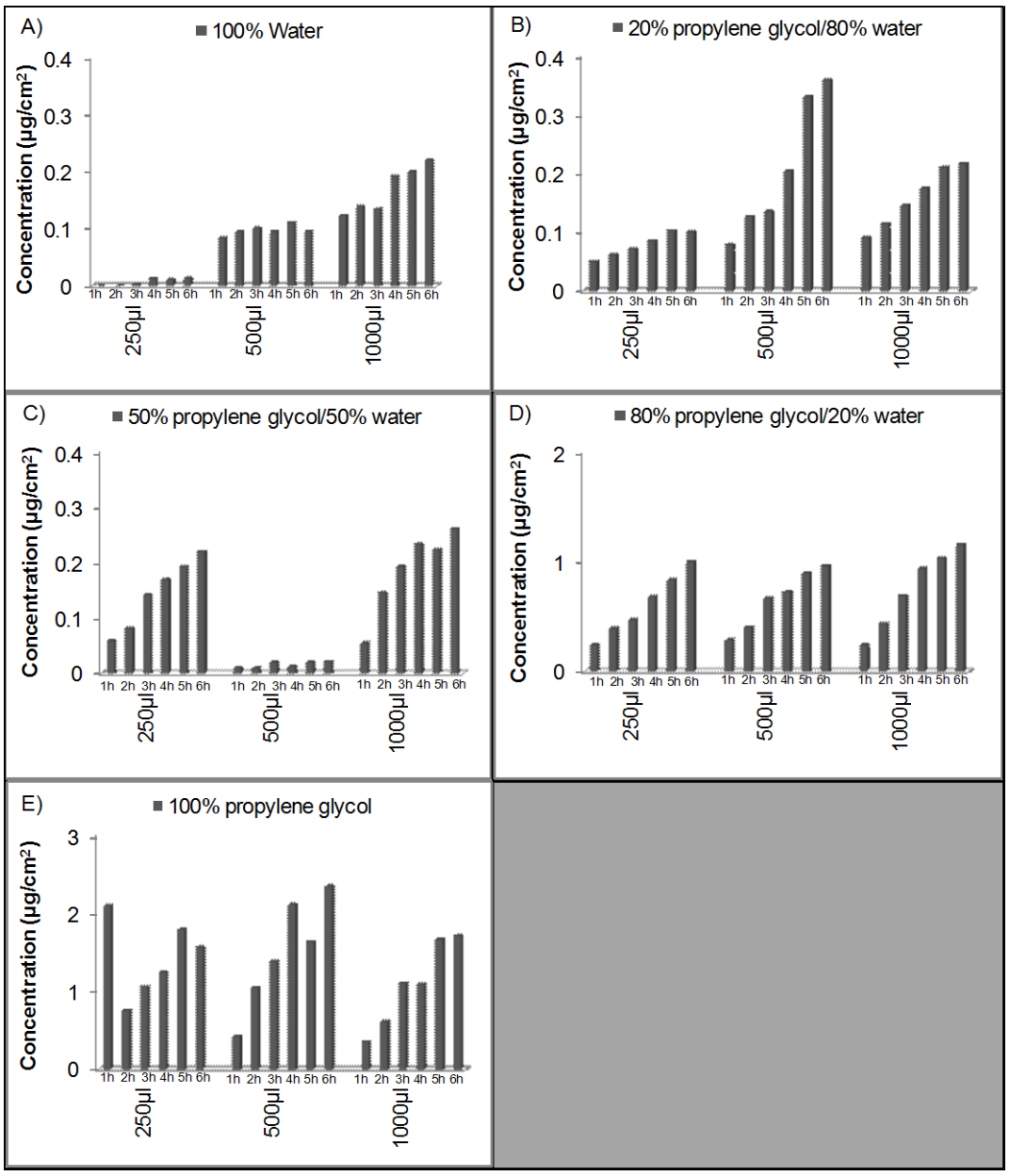

Figure 4.1: Ibuprofen concentration (µg/cm^2) that diffused over 6 hours through the Carbosil® membrane when infinite dose volumes were applied using single and binary phase solvents of propylene glycol and water as the delivery vehicles. The average of 4 cells (n=4) were used to calculate one data point. Standard deviation was in all instances less than 0.2.

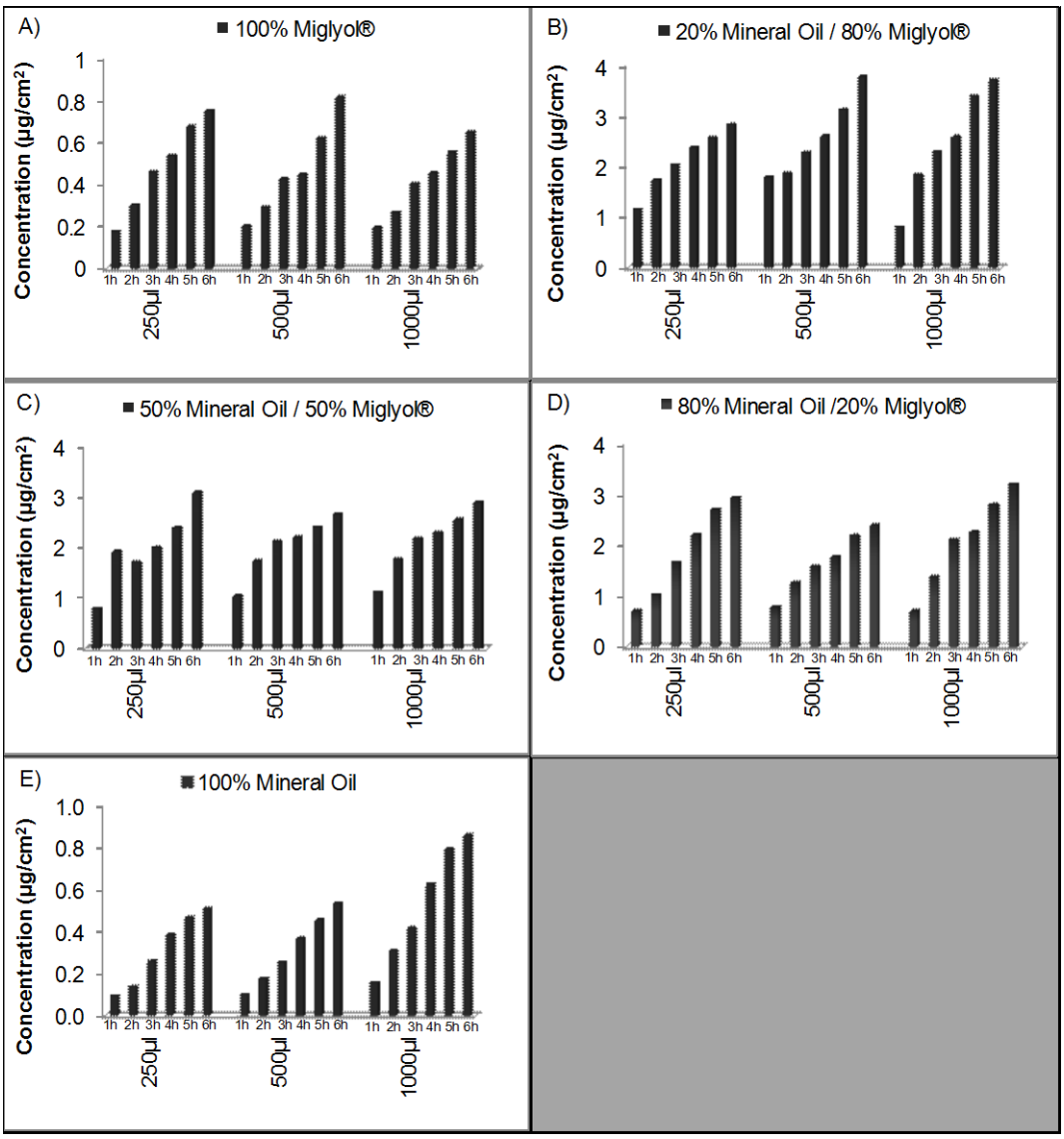

Figure 4.2: Ibuprofen concentration (µg/cm^2) that diffused over 6 hours through the Carbosil® membrane when infinite dose volumes were applied using single and binary phase solvents of mineral oil and Miglyol® as the delivery vehicles. The average of 4 cells (n=4) were used to calculate one data point. Standard deviation was in all instances less than 0.2.

CHAPTER 5

ARTICLE FOR PUBLICATION IN THE INTERNATIONAL JOURNAL OF PHARMACEUTICS

Chapter 5 is in article format, in preparation for submission for publication in the International Journal for Pharmaceutics and is written in US English. Guidance for authors is outlined in Appendix B. Note that the general formatting and style have for the purpose of inclusion in this chapter been adjusted in accordance with the rest of this thesis, whereas the reference style was mainly kept as per the article requirements.

DELIVERY OF FINITE DOSES OF IBUPROFEN THROUGH CARBOSIL® MEMBRANES

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5.1 Abstract

For the purpose of obtaining a mechanistic understanding of drug transport through skin with finite dose applications, the permeation of lipophilic drug ibuprofen through Carbosil® membrane has been studied, using Franz diffusion cells. For the purpose of this study, varying finite volumes of two groups of penetration enhancer vehicles were evaluated, i.e. propylene glycol and water, in different combinations and individually, and likewise, mineral oil and Miglyol®. The concentrations (µg/cm^2) of the ibuprofen that had penetrated the membrane hourly, over a period of 6 hours, were measured for each application of several finite doses.

In the manufacturing industry especially, such as a pharmaceutical or chemical plant, it is of outmost importance to obtain dermal absorption data for toxicological risk assessment of occupational exposure to chemical substances, in order to estimate internal dose after dermal exposure to such chemicals. The outcomes of this study showed that with exposure to finite dose levels (< 150 µl) of a substance, in the presence of a penetration enhancer, the

permeation of substances through the Carbosil® membrane may be significantly affected. Since the understanding of the problem of drug permeation, uptake and target site delivery in the finite dose situation is very complex, new strategies for risk assessment need to be considered.

Keywords: Transdermal delivery, Finite dose, Penetration enhancers, Ibuprofen.

5.2 Introduction

The accurate prediction of dermal absorption after topical exposure to a chemical compound is relevant to both formulation development and risk assessment. The skin is the largest organ in the human body and covers a surface area of 1.5 - 2.0 m^2. It consists of three layers, i.e. the stratum corneum, the viable epidermis and the dermis (Chen *et al*., 2011). It is mainly the "brick-and-mortar" structure and the lipophilic nature of the stratum corneum that are responsible for the barrier properties of the skin (Michaels *et al*., 1975). Not only is the skin the biggest organ in the human body, but also the organ that is mostly exposed to external factors in the environment, like chemicals and micro-organisms. While forming a barrier to influences from the external environment and keeping harmful substances out, the skin also maintains the body fluids within the system (Yamashita & Hashida, 2003). An understanding, therefore, of how the skin allows substances to penetrate it finds application in the pharmaceutical and cosmetic sciences, as well as in risk assessment studies in the workplace. When a topical pharmaceutical product is applied to the skin, maximum delivery of the active compound is expected, for it to achieve the desired clinical response, for which reason investigation of the delivery rate of the active compound is very important. It is equally essential to understand the penetration rate when a person is exposed to a toxic substance. Over the years, numerous techniques have been investigated in an attempt to overcome the barrier properties of the stratum corneum to improve dermal drug delivery. The majority of investigations to date have focused on *in vitro* experimental methods that have been unsuccessful in predicting the possible impact to human beings of exposure to small amounts of a harmful substance, after having applied a typically clinical topical product (Trottet *et al*., 2004).

Despite considerable successes in predicting the steady-state, dermal absorption rates of chemical compounds from large or infinite (> 150 µl) doses being applied to the skin, little progress has been made in predicting the absorption rate and the extent of absorption with small doses of topically applied compounds. *In vitro* studies of transdermal absorption often utilize such "infinite doses" of the compound being investigated, as a means to define its permeability profile. This experimental approach allows for the determination of three parameters, which may be used to characterize the permeation properties of a compound, i.e. the steady state rate of absorption, the permeability coefficient and the lag time (Franz, 1993).

Clearly, one shortcoming of the infinite dose technique is that it fails to mimic an actual dose being applied during topical treatment (typically ~ 2 mg/cm^2). The ultimate goal for any *in vitro* model system is to yield results in agreement with the more complicated *in vivo* process, which it mimics. The *in vivo* experimental method has some disadvantages, i.e. (a) *in vivo* experiments have significant ethical implications, (b) hence they are expensive and time consuming, whereas (c) interpretation of the results may be complicated by inter- and intra-subject variability. An alternative method to the *in vivo* method is the *in vitro* experimental method that utilizes human skin. However, like the *in vivo* method, it also has its disadvantages, i.e. (a) obtaining human skin can be difficult, (b) extreme care is required when preparing human skin, whilst (c) the length and method of skin storage may also introduce variability. One way of eliminating the difficulties of working with human skin is the use of skin-imitating synthetic membranes, like Carbosil® for evaluating the penetration of an active compound through skin (Houk & Guy, 1988). It has thus become normal practice to use polymeric membranes as skin-imitating barriers in the evaluation of drug delivery systems, because, in contrast to human tissue, polymeric membranes are readily available, have a uniform composition and good tensile strength (Feldstein *et al*., 1998).

Synthetic membranes provide results, which may be useful in facilitating the interpretation of data obtained *in vivo* when (a) the passive diffusional barrier imposed by the stratum corneum offers the major resistance to transport, (b) the drug of interest is known to be metabolically inert and not specifically bound in viable skin, (c) the formulation does not contain a permeability enhancer that can interact with skin but not the membrane, whilst (d) *in vivo* experiments of similar design have been or can be performed and correlated with the *in vitro* results (Feldstein *et al*., 1998). However, results obtained from experiments using synthetic membranes, are not directly comparable to excised skin, because the composition of such membranes does not replicate the complexity of natural skin.

In this study, the synthetic membrane, Carbosil®, a polydimethylsiloxane-polycarbonate (PDMS-PC) block copolymer, was used to mimic the barrier function of the stratum corneum. This PDMS-PC block copolymer is synthesised by Medpolymer *via* heterophase polycondensation of oligo-dioxiaryl carbonates with oligo-bis-chlorformate alkyl siloxanes (Raigorodskii *et al*., 1995; Listvoib, 1992). Carbosil® (0.04 mm in thickness) is produced by casting a 12 - 15 weight percentage block polymer solution in methylene chloride, followed by drying.

As for human skin epidermis, this synthetic membrane has a heterophasic and heteropolar structure and shares a common solubility-diffusion mechanism of drug transport, which provides a mechanically substantiated model for transdermal drug absorption (Feldstein *et al*., 1998). Carbosil® membranes are potentially useful for both the quantitative prediction of transdermal

drug delivery rate and as a skin-imitating standard membrane for use during *in vitro* drug delivery experiments (Feldstein *et al*., 1998).

For the purpose of this study, saturated solutions of the selected drug, ibuprofen ((±-2-(*p*-isobutylphenyl)propionic acid), in a range of single and binary phase penetration enhancer solvents, were prepared and applied to the membrane in volumes ranging between 2 - 150 µl. Ibuprofen is a potent lipophilic, non-steroidal, anti-inflammatory drug (NSAID) with pronounced analgesic properties. It is being used in the long-term treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis (Adams *et al*., 1975; Arendt-Nielsen *et al*., 1994). Ibuprofen has a log P of 3.6 and pKa of 5.3 and hence is expected to be substantially ionized at normal physiological pH (Beetge *et al*., 2000). Potent drugs, like ibuprofen, known for their negative side-effects, have contributed to an increase in the number of investigations into transdermal penetration enhancement. The majority of these investigations are concerned with delivery vehicles that penetrate the stratum corneum and interact with the intercellular barrier lipids. The concentration of the diffused substance can be increased, if the enhancer can induce modification to the polarity of the skin, such as an increase in the solubility of the drug in the skin. By using a co-enhancer (binary component), a synergistic effect, through which one enhancer increases the delivery of the other, as well as that of the drug, results in a multiplicative effect. What is clear is that the choice of the enhancement vehicle depends upon the physicochemical properties of the permeant (Williams & Barry, 2004).

5.3 Materials and methods

5.3.1 Materials

The Ibuprofen being used during this study was obtained from Albemarle Corporation (South Carolina, USA). Other ingredients included deionized HPLC (high performance liquid chromatography) grade water, prepared by the Milli-Q water purification system (Millipore, Milford, USA) and Carbosil® membranes (Medpolymer). Methanol (from Merck Laboratory Supplies, Midrand, South Africa) and HPLC grade Milli-Q water 70/30 were used as the HPLC mobile phase. Propylene glycol (from Merck Laboratory Supplies, Midrand, South Africa), mineral oil (from Sigma-Aldrich, Johannesburg, South Africa) and Miglyol® (from Merck Laboratory Supplies) were used as solvents in this study. The phosphate buffer solution (PBS) consisted of sodium chloride, disodium orthophosphate dehydrate and sodium dihydrogen dehydrate (from Merck Laboratory Supplies, Midrand, South Africa) and HPLC grade Milli-Q water.

5.3.1.1 Single and multi-component solvents

Two groups of single and binary phase penetration enhancer solvents were used as delivery vehicles for ibuprofen through Carbosil® membrane. Propylene glycol (from Merck Laboratory Supplies, Midrand, South Africa) and HPLC grade Milli-Q water were used individually and in 20/80 (v/v), 50/50 (v/v) and 80/20 (v/v) combinations. Likewise, mineral oil and Miglyol® were used individually and in combinations of 20/80 (v/v), 50/50 (v/v) and 80/20 (v/v).

A range of finite dose volumes of saturated solutions of each of these vehicles was applied to membranes, i.e. 2 µl, 5 µl, 10 µl, 20 µl, 50 µl and 150 µl, using a calibrated pipette. Special care was taken to prevent the sample droplet from adhering to the walls of the Franz cell, in order to keep the diffusion area per volume applied constant. A separate experiment was conducted for each volume applied per donor phase vehicle for a specific volume. The diameter of each droplet was measured for each of the applied volumes below 150 µl. The volumes above 150 µl covered the total diffusion area of the Franz cell (1.075 cm^2). For volumes below 150 µl, only a fraction of the area available for diffusion was covered by the droplet, hence each droplet per specific volume and per enhancer vehicle was measured, in order to calculate the diffusion area for each.

5.3.2 Methods

5.3.2.1 Preparation of phosphate buffered solution (pH 7.4)

Phosphate buffered solution (PBS) (pH 7.4) was prepared by dissolving 4.4 g of sodium chloride, 9.2 g of disodium orthophosphate dehydrate and 2.1 g of sodium dihydrogen dehydrate in 1,000 ml freshly prepared HPLC grade Milli-Q water.

5.3.2.2 Analysis of ibuprofen

5.3.2.2.1 Preparation of standard solution for calibration curve

A stock solution was prepared by accurately weighing 50 mg of ibuprofen into a 100 ml volumetric flask. The flask was then filled to volume with 50/50 methanol/Milli-Q water and sonicated to ensure a homogenous solution. From this stock solution 2, 5, 6, 8 and 10 ml samples were transferred into separate volumetric flasks each and filled to 100 ml with methanol/Milli-Q water (50/50). HPLC vials were filled with samples from each volumetric flask and analyzed in duplicate on the HPLC.

5.3.2.2.2 High performance liquid chromatography method

The HPLC method used during this study had been previously validated and performed at the Analytical Technology Laboratory of the School of Pharmacy, at the Potchefstroom Campus of the North West University (South Africa), whilst HPLC analyses were conducted under controlled laboratory conditions of 25°C. A HP1100 series HPLC, equipped with a pump, auto sampler, UV detector and Chemstation Rev. A.06.02 data acquisition and analysis software, or equivalent, was used for the analysis of ibuprofen concentrations. All reagents were HPLC grade. The Luna C_{18}-2 column, 150 x 4.6 mm, 5 µm, 100 Å pores, 17.8% carbon load, endcapped, Phenomenex, Torrance, CA was used. Detection was at 240 nm. The mobile phase consisted of a filtered and degassed mixture of HPLC grade water/methanol (70/30). The mobile phase flow rate was 1.0 ml/min with an injection volume of 100 µl. The retention time of ibuprofen was 5 minutes and the sample run time was 7 minutes.

5.3.2.3 Solubility study

Solubility studies were conducted in an attempt to determine the saturated ibuprofen levels of each solvent used. Excess drug was added to each solvent vehicle and stirred using a magnetic stirrer for 24 hours (to attain equilibrium) in a water bath maintained at 32°C. The solubility of ibuprofen was determined at 32°C, like all diffusion experiments, as human skin temperature is 32°C. A 5 ml syringe and a filter were also pre-heated to 32°C. The supernatant was then filtered and diluted with methanol (1/10) (supernatant/methanol). The samples were analyzed on HPLC. Assays were performed in triplicate to determine the solubility of the drug in each solvent. For each subsequent test, ibuprofen was added to the solvent vehicle in concentrations as determined by the solubility study, in order to consistently ensure saturated solutions of ibuprofen during permeation testing.

5.3.2.4 Permeation studies

5.3.2.4.1 Preparation of donor solutions

Saturated solutions of ibuprofen and the solvents were prepared one hour before the start of each test. The saturated ibuprofen/solvent vehicles were each stirred for one hour at 32°C, before commencing each test. Each prepared solution was applied to the Carbosil® membrane as the donor phase, after confirming that no undissolved particles were present. Depending on the finite dose application being investigated, the applied donor volume varied.

5.3.2.4.2 Membrane permeation

Twenty-four vertical glass Franz diffusion cells, with a diffusion area of 1.075 cm^2 and receptor capacity of approximately 2 ml were used during this study. Carbosil® membrane was cut into circles large enough to cover the area of the Franz cell that is available for diffusion. Prior to each test, all Carbosil® membrane circles were pre-soaked for 12 hours at 32°C in the relevant delivery vehicles (excluding the active) in order to allow saturation thereof. The membranes were removed from the soaking solution and dried with tissue paper to remove excess donor solution on the membrane surfaces. Each membrane was mounted on the receptor compartment of the Franz cell to cover the diffusion area. Each Franz cell assembly was then sealed with Dow Corning, high vacuum grease and secured with a horseshoe clamp. All cells were placed on a submersible Variomag® stirrer plate.

The receptor phase was filled with PBS (pH 7.4) and maintained at 37°C, taking care not to allow bubbles in the receptor compartment. Of the twenty-four Franz cells that were used per diffusion experiment, the receptor phases of the first four cells were extracted after 1 hour and not refilled, and the next four cells after 2 hours. The extraction of four cells each continued hourly until 6 hours have passed and all of the receptor compartments were sampled. The reason for this method was to keep the cells as static as possible for the duration of the experiment in an attempt to keep the diffusion area for finite volumes as constant as possible. Were the receptor volumes refilled, the cell would have had to be turned upside down, which could have caused the droplet to adhere to the walls of the cell and hence be unavailable for optimum diffusion.

5.3.3 Data analysis

Before analyzing the samples from the diffusion studies, a standard solution was prepared and its linearity determined. In this study, as was mentioned, it was impossible to calculate cumulative amounts of ibuprofen per area (flux), since the receptor compartments of the Franz cells could not be refilled after sampling, in order to prevent possible adhesion of the droplets in the donor compartment to the walls of the Franz cells and thus inconsistent diffusion areas. The concentration (µg/cm^2) of ibuprofen that had diffused through the Carbosil® membrane per sampling interval was calculated.

5.4 Results and discussion

5.4.1 Solubility of ibuprofen in selected solvents and binary mixtures thereof

Ibuprofen was dissolved in the different solvent vehicles individually (propylene glycol, Miglyol®, mineral oil and water) and in combinations thereof (see section 5.3.1.1).

Table 5.1: Solubility of ibuprofen in propylene glycol and water, and in mineral oil and Miglyol®

The solubility of ibuprofen in single and multi-component solvents of propylene glycol and water, and similarly in mineral oil and Miglyol®, are summarized in Table 5.1. Ibuprofen has a very low aqueous solubility (0.94 mg/ml) in water. With increasing amounts of propylene glycol in the mixture, the solubility of ibuprofen increased exponentially. Table 5.1 clearly shows that ibuprofen was more soluble in Miglyol® (31.99 mg/ml) than in mineral oil (10.69 mg/ml) and the most soluble in a 50/50 combination of the two solvents (169.01 mg/ml). It is suggested that ibuprofen itself associates through intermolecular bonds to form dimers. The carboxylic acid (-COOH) ends of the molecule are polar, but if 'protected', gives a non-polar dimer (Iervolino *et al*., 2001).

5.4.2 Membrane permeation

5.4.2.1 Permeation with single and binary delivery vehicles of propylene glycol and water with finite volume applications

The concentration (µg/cm^2) of ibuprofen that had permeated the Carbosil® membrane with application of the single phase **water** solvent in finite doses (Figure 5.1.A) could not be measured, due to the lipophilic nature and low solubility of ibuprofen in water. Consequently, the concentration of the applied active with finite dose volumes was extremely low and the quantity of ibuprofen that had crossed the membrane could not be detected by HPLC.

The amount of ibuprofen that had permeated the membrane with finite dose applications of the combination solvent of **propylene glycol/water (20/80)** (v/v) is illustrated in Figure 5.1.B. Only the 150 µl application of this penetration vehicle showed measurable permeation concentrations (0.13 µg/cm^2). Volumes lower than 150 µl could not be detected by HPLC, due to these low application concentrations.

Figure 5.1.C demonstrates the results obtained with the **50/50** (v/v) **propylene glycol/water** applications. Diffusion concentrations of ibuprofen could be measured for the 50 µl application (0.15 µg/cm^2). The concentration of the applied active was, however, too low to measure

penetration through the membrane for volumes smaller than 50 µl. The concentration of diffused ibuprofen being measured for the 150 µl application was higher (0.24 µg/cm^2) than with the 50 µl application.

The results obtained with the application of the binary solvent **propylene glycol/water (80/20)** (v/v) are illustrated in Figure 5.1.D. With the majority (> 50%) of the solvent being propylene glycol, the successful penetration enhancement effect of this solvent was evident. The concentration of ibuprofen having diffused with a 10 µl application was 4.23 µg/cm^2, with the highest levels of the permeant showing after 6 hours. Permeation concentrations for 2 µl (3.08 µg/cm^2), 20 µl (3.24 µg/cm^2) and 50 µl (3.56 µg/cm^2) applications were lower than for the 10 µl application after 6 hours, with insignificant differences among the three volumes. The 150 µl application showed very little permeation of the drug through the membrane (0.16 µg/cm^2).

The concentrations of the ibuprofen that had penetrated the membrane with varying finite doses of **100% propylene glycol** as the delivery solvent are illustrated in Figure 5.1.E. The highest concentration (7.96 µg/cm^2) of diffused ibuprofen was measured for an application of 20 µl of the saturated delivery vehicle. The permeation concentrations being measured for the applied volumes of 5 µl (5.69 µg/cm^2), 10 µl (5.09 µg/cm^2) and 50 µl (5.10 µg/cm2) showed very similar results. The viscosity of the solvent was as such that when the 150 µl vehicle was applied to the membrane, the donor volume adhered to the walls of the Franz cell, preventing the diffusion area to be kept consistent throughout the duration of the experiment, resulting in the test data not being used, because of the significant variations.
From Figure 5.1 (A – E) it is evident that the amount of ibuprofen that had been delivered from the **water** penetration enhancer vehicle, with finite dose applications, was too low to be measured, due to the high lipophilic nature of ibuprofen (log $P_{o/w}$ of 3.6) and the low solubility of ibuprofen in water (0.94 mg/ml) (Beetge *et al*., 2000). The low solubility of ibuprofen in water had led to very low donor concentrations, which resulted in the permeation concentrations being immeasurable.

Understandably, **propylene glycol** as the sole solvent showed better results (7.96 µg/cm^2) than the 80/20 (v/v) propylene glycol/water combination (4.23 µg/cm^2), followed by the 50/50 (v/v) propylene glycol/water solvent (0.24 µg/cm^2), and lastly by the 20/80 (v/v) propylene glycol/water combination (0.13 µg/cm^2). As the percentage of the propylene glycol increased in the solvent vehicle, the solubility of ibuprofen increased, causing the concentration of the active in the donor phase to be sufficiently high to show permeation through the membrane. The single phase propylene glycol hence showed the best penetration enhancement effect with finite dose applications than any other enhancer vehicle, with the highest concentration of permeated ibuprofen being measured for the 20 µl application.

The **80/20** (v/v) **propylene glycol/water** vehicle also showed good permeation concentrations for ibuprofen, but lower (4.23 µg/cm^2) than with 100% propylene glycol. This could be explained by the mechanism of action of propylene glycol to partition into the membrane and to increase the permeant solubility in and diffusion through the membrane (Squillante *et al*., 1998). Like human skin epidermis, Carbosil® membrane has a lipophilic and hydrophilic structure and shares a common solubility-diffusion mechanism of drug transport, which provides a mechanically substantiated model for transdermal drug absorption (Feldstein *et al*., 1998). It is hypothesized that propylene glycol would carry the lipophilic drug, ibuprofen, into the lipophilic area of the membrane and increase its solubility and as a result enhance the penetration of the drug in and through the membrane.

5.4.2.2 Permeation with single and binary delivery vehicles of Miglyol® and mineral oil with finite volume applications

The permeation profile of ibuprofen through the Carbosil® membrane, with the application of finite doses of **100% Miglyol®** is illustrated in Figure 5.2.A. The 10 µl application showed the highest concentration of diffused ibuprofen (8.35 µg/cm^2) after 6 hours, followed by applications in the order 20 µl (6.51 µg/cm^2), 2 µl (6.05 µg/cm^2), 5 µl (2.56 µg/cm^2), 50 µl (1.83 µg/cm^2) and lastly 150 µl (0.58 µg/cm^2).

Figure 5.2.B shows the permeation of ibuprofen through the membrane with finite dose applications of the **20/80** (v/v) **mineral oil/Miglyol®** delivery vehicles. The 2 µl application delivered the highest concentration (20.85 µg/cm^2) of diffused ibuprofen , whilst the concentration of diffused ibuprofen decreased as the finite dose volume being applied increased, with the 150 µl application showing the lowest permeated ibuprofen (2.71 µg/cm^2).

The 2 µl application of the **50/50** (v/v) **mineral oil/Miglyol®** delivery solvents showed the best results, as illustrated in Figure 5.2.C. The concentrations of the ibuprofen that had permeated the membrane from these finite dose applications were in the order 2 µl (6.99 µg/cm^2), 10 µl (5.49 µg/cm^2), 5 µl (5.34 µg/cm^2), 20 µl (4.77 µg/cm^2), 50 µl (4.73 µg/cm^2) and lastly 150 µl (2.10 µg/cm^2).

Figure 5.2.D illustrates the outcomes for the finite dose applications of the **80/20** (v/v) **mineral oil/Miglyol®** delivery vehicles. Permeation data from the 10 µl and 20 µl applications could not be used, because of significant variations following inconsistent diffusion areas. The droplet sizes were as such that they had adhered to the walls of the Franz cells and could not be kept consistent for the duration of these tests. Permeant absorption results for the 2 µl (9.29 µg/cm^2) and 5 µl (6.98 µg/cm^2) applications were much higher than for the 50 µl (4.15 µg/cm^2) and 150

µl (2.74 µg/cm^2) applications. Of all measurable volumes, therefore, the 2 µl application showed the highest permeant absorption and the 150 µl vehicle the lowest.

Figure 5.2.E shows the generally low permeation concentrations of ibuprofen through Carbosil® membrane when finite dose volumes of the **100% mineral oil** solvent vehicle were used. No results were generated for the 2 µl and 5 µl applications, because of the diffusion concentrations being too low to be detected by HPLC. The results in Figure 5.2.E illustrate that the concentration of diffused ibuprofen being measured for the 10 µl application (0.23 µg/cm^2) was the lowest, with the highest concentration being measured for the 150 µl application (0.44 µg/cm^2). The diffused ibuprofen concentrations per application volumes were in the order 150 µl (0.44 µg/cm^2), 20 µl (0.34 µg/cm^2), 50 µl (0.29 µg/cm^2) and 10 µl (0.23 µg/cm^2).

Figure 5.2 (A – E) illustrates that the **20/80** (v/v) **mineral oil/Miglyol®** solvents showed the best penetration enhancement effects, compared to all other mineral oil and Miglyol® solvents. Single and multi-component solvents, containing 100% Miglyol®, and 50/50 (v/v) and 80/20 (v/v) mineral oil/Miglyol®, all showed similar penetration enhancement capabilities. Penetration enhancement effects being measured for the single phase mineral oil solvent showed the lowest results of all these solvents. Although the solubility of ibuprofen in the 20/80 (v/v) mineral oil/Miglyol® vehicle was not high (maximum of 1 µg/cm^2), the highest level of diffusion through the membrane was measured after application of this solvent in finite doses. This could be explained by a synergistic effect of penetration enhancement between the two solvents in a suitable combination. Since the solubility of ibuprofen in mineral oil is very low, the measured concentration of the active having diffused through the membrane was not more than 1 µg/cm^2. Mineral oil, however, was the only solvent being studied during this research project, that showed an increase in the permeation concentration of ibuprofen, as the finite dose volume increased. With all the other penetration enhancer vehicles being studied, the opposite effect was measured.

With solvent type permeant preparations being applied to a membrane, three types of penetration influencing parameters should be taken into account, i.e. (a) thermodynamic effects resulting from different permeant solubilities in the different vehicles, (b) penetration enhancing effects between the vehicle and the membrane and (c) permeant depletion in the vehicle in the case of finite dose conditions. The extent of permeant depletion in the vehicle depends on the thickness of the applied solvent layer on the surface of the membrane (Leopold, 1998). Mineral oil does not show good penetration enhancement effects with finite dose applications. The reason for the decrease in ibuprofen absorption through Carbosil® membrane with finite dose conditions could have been caused by the thin application layer and as a result permeant depletion in the solvent.

5.5 Conclusions

Two groups of penetration enhancement vehicles were tested for their abilities to improve the permeation of the lipophilic drug, ibuprofen, through the Carbosil® membrane, similar in nature to the stratum corneum of human skin. The first group of solvents comprised of single phase propylene glycol and water vehicles, as well as in three different combinations. The second group consisted of mineral oil and Miglyol®, individually and in three different combinations. Furthermore, each solvent was applied in different finite volumes (i.e. 2 µl, 5 µl, 10 µl, 20 µl, 50 µl and 150 µl) to the membrane.

Figure 5.1 summarizes the permeation profiles of the lipophilic drug, ibuprofen, with hydrophilic penetration enhancer solvents, i.e. propylene glycol and water individually and in combinations. Figure 5.1 clearly demonstrates that with increased levels of propylene glycol as the penetration enhancer solvent in the delivery vehicle, not only would the penetration of ibuprofen through the Carbosil® membrane increase, but would it also enhance the penetration of the active through the membrane with finite dose applications.

Figure 5.2 summarizes the ibuprofen permeation profiles through Carbosil® membrane with mineral oil and Miglyol® solvents and solvent mixtures. It is evident that permeation of lipophilic ibuprofen was higher with small application volumes when lipophilic solvents were used as delivery vehicles. Chen *et al*. (2011) found that with an infinite dose application, the donor compartment would be filled with a thick liquid formulation layer covering the surface of the membrane, having a height of 1.6 mm, while the finite dose application would form a thin layer on the surface of the membrane of 0.1 mm. As a result, the hydration levels of the membrane would increase with infinite dose applications. Although infinite dose applications are not part of this article, it is worth considering that the effect of hydration with a 150 µl application would be more significant than with a 2 µl application. The 150 µl application covers the entire area available for diffusion of the Franz cell, whereas the smaller volumes only cover a fraction of the available diffusion area. This increase in the hydration levels of the membrane would hence facilitate higher permeability of the membrane (Chen *et al*., 2011). Increased membrane hydration appears to increase the diffusion of both hydrophilic and low lipophilic compounds, due to the partitioning of the active into the membrane (Williams & Barry, 2004). This hydration effect on the membrane makes penetration of hydrophilic compounds through the membrane easier, but makes it more difficult for highly lipophilic compounds (log P >2) to partition into the hydrated membrane (Zhang *et al*., 2010). Ibuprofen is a highly lipophilic drug with a log P of 3.6 (Beetge *et al*., 2000). It is thus very difficult for this drug to partition into a hydrated membrane, which explains the low concentration of ibuprofen that had permeated through the membrane with the 150 µl applications. The high levels of permeation through the membrane from the lower finite dose volumes may have been as a result of less membrane hydration, as well as

evaporation of the solvent vehicle, that may have resulted in super-saturated donor solvents, which caused higher diffusion levels of the active compound.

Mineral oil showed higher permeation levels with larger finite volume applications, because of the lipophilic nature of both mineral oil and ibuprofen. As a result, the permeability of the lipophilic active increased as the membrane hydrated more with the lipophilic solvent, as larger volumes were applied.

From the findings in this study it became evident that the lipophilic/hydrophilic nature of both the solvent and the permeant would significantly impact on the absorption of a permeant through Carbosil® membrane. If the membrane is hydrated with a lipophilic delivery vehicle while carrying a lipophilic toxic permeant, the effect may be even more harmful at lower levels of exposure. Ibuprofen showed higher permeation levels with small application volumes of 100% Miglyol® and mixtures of Miglyol® and mineral oil (lipophilic) delivery vehicles. When a lipophilic toxic permeant is present in a hydrophilic delivery vehicle, like propylene glycol and water, the effect may be less significant when the level of exposure is larger.

It is recommended that the effects of finite dose conditions compared to infinite dose conditions should be explored further on human skin, in order to more accurately predict what the clinical effects would be.

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Figures

Table

Table 5.1: Solubility of ibuprofen in propylene glycol and water, and in mineral oil and Miglyol®

Vehicle	Solubility (mg/ml)
Propylene glycol : Water	
100 : 0	323.14
80 : 20	26.65
50 : 50	2.13
20 : 80	1.02
0 : 100	0.94
Mineral oil : Miglyol®	
100 : 0	10.69
80 : 20	167.63
50 : 50	1696.01
20 : 80	53.61
0 : 100	31.99

Figures

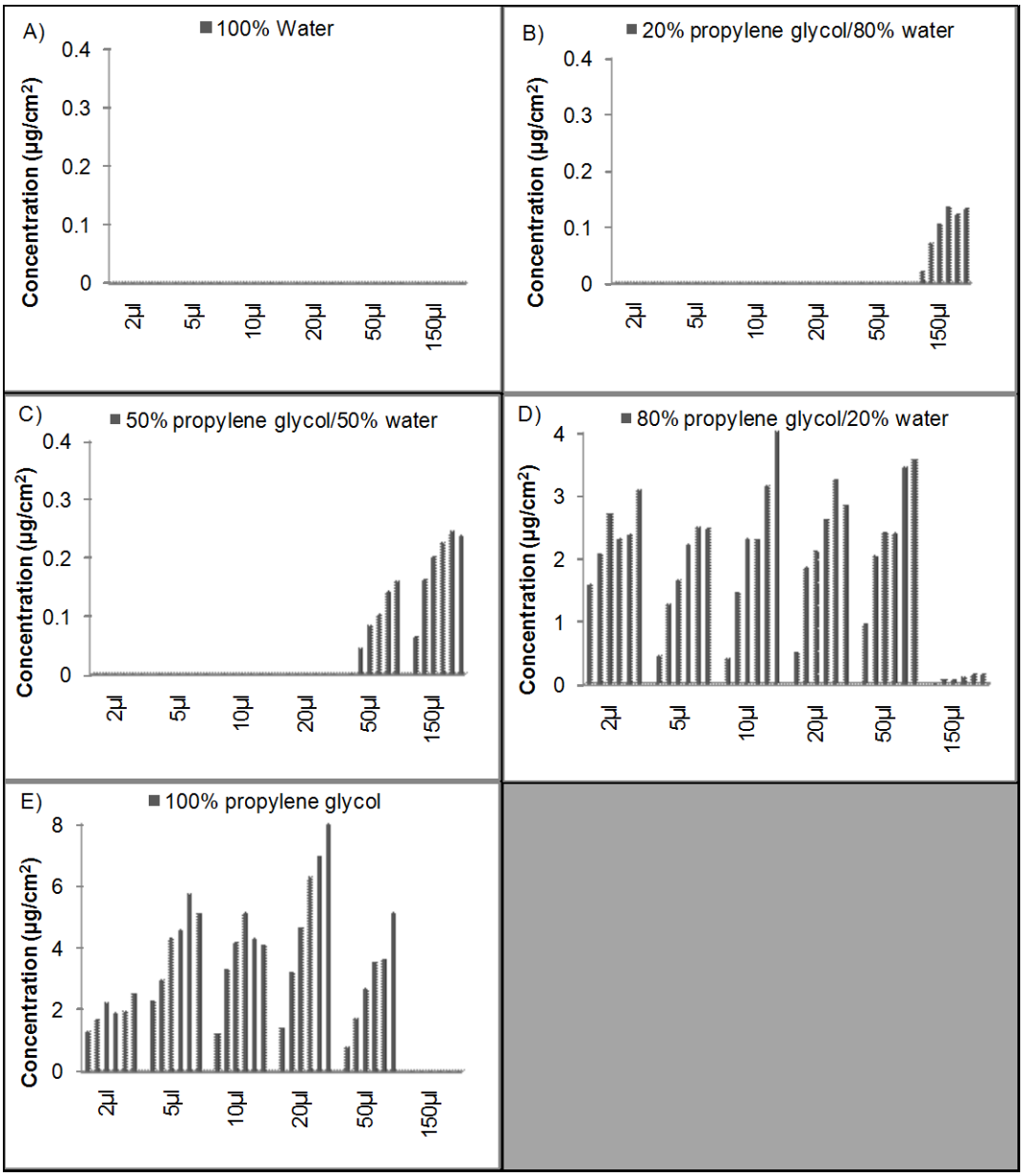

Figure 5.1: Concentrations (µg/cm^2) of ibuprofen that had diffused over a period of 6 hours through the Carbosil® membrane with the application of finite dose volumes, using single and binary phase solvents of propylene glycol and water as the delivery vehicles. Each bar illustrates a data point per sampling interval (1, 2, 3, 4, 5 and 6 hours). Data from four cells (n=4) were used to calculate one data point. Standard deviation was in all instances less than 1.0.

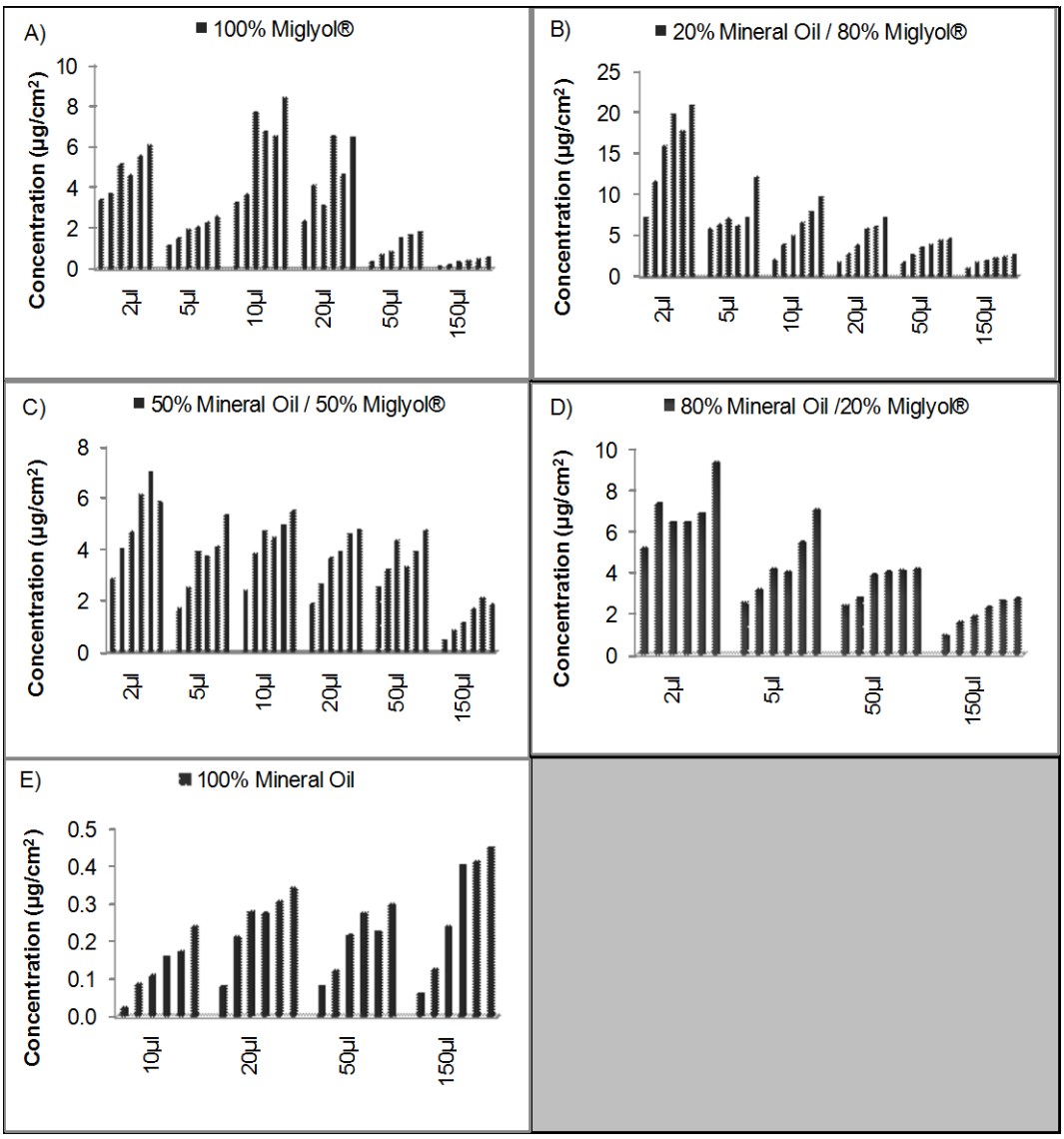

Figure 5.2: Concentrations (µg/cm^2) of ibuprofenthat had diffused over a period of 6 hours through the Carbosil® membrane with the application of finite dose volumes, using single and binary phase solvents of mineral oil and Miglyol® as the delivery vehicles. Each bar illustrates a data point per sampling interval (1, 2, 3, 4, 5 and 6 hours). Data from four cells (n=4) were used to calculate one data point. Standard deviation was in all instances less than 1.0.

CHAPTER 6

FINAL CONCLUSION AND FUTURE PROSPECTS

6.1 Penetration enhancer solvent vehicles

Over the past decade, numerous techniques have been investigated to overcome the barrier function of the stratum corneum in the skin. One method being used to improve transdermal drug delivery is the employment of penetration enhancer solvents as delivery vehicles. Penetration enhancement technology is a challenging development in the pharmaceutical industry that aims at increasing the number of drugs that are suitable for transdermal administration. The penetration of drugs through the skin can be enhanced by both chemical penetration enhancement and physical penetration enhancement (Williams & Barry, 2004:604). Although both techniques have been discussed in Chapter 2, the focus of this study was chemical enhancement and the probable mechanisms of action.

An ideal penetration enhancer reversibly reduces the barrier resistance offered by the stratum corneum, without damaging the viable cells (Pathan & Setty, 2009:175). Penetration enhancers should have the desired properties when acting within the skin, including:

1) Penetration enhancers should be non-toxic, non-irritating and non-allergenic.
2) The duration and effect of the enhancer should be both predictable and reproducible.
3) The enhancer should have no pharmacological activity within the body.
4) The enhancer should allow therapeutic agents into the body, whilst preventing the loss of endogenous materials from the body.
5) When removed from the skin, the barrier properties should return to normal rapidly and completely.
6) The enhancer should be cosmetically acceptable with acceptable skin feel (Pathan & Setty, 2009:175).

As it is very difficult to find an enhancer solvent that has all of these ideal properties, it is understandable that the research potential in the field of penetration enhancement is growing rapidly.

The mechanisms by which chemical penetration enhancers act can be divided into three main mechanisms, which include:

1. Disruption of the highly ordered structure of the stratum corneum lipids.

2. Interaction with the intercellular protein.

3. Improved partitioning of the drug, co-enhancer or solvent into the stratum corneum.

An enhancer acts through one of the above pathways and when used in combination with another, the combined effect may be synergistic, meaning that more than one of the above mechanisms of action are employed, with the effect being better, compared to that of a single solvent (Pathan & Setty, 2009:175).

The objectives for the penetration enhancement aspects of this study were to:

- Determine the permeation of ibuprofen across Carbosil® membrane by using water and propylene glycol as penetration enhancer vehicles in combinations of 0/100 (v/v), 20/80 (v/v), 50/50 (v/v), 80/20 (v/v) and 100/0 (v/v);
- Determine the permeation of ibuprofen across Carbosil® membrane by using mineral oil and Miglyol® as penetration enhancer vehicles in combinations of 0/100 (v/v), 20/80 (v/v), 50/50 (v/v), 80/20 (v/v) and 100/0 (v/v);
- Determine the permeation of ibuprofen across Carbosil® membrane by using these penetration enhancer vehicles individually and in multi- mixtures at different infinite volumes, i.e. 250 µl, 500 µl and 1,000 µl; and
- Determine which of the single or binary penetration enhancer vehicles would show the best transdermal delivery enhancement effect.

Results from this study demonstrated that the permeation of ibuprofen was enhanced by the vehicle that was used to deliver the permeant. Water, the most natural penetration enhancer, can increase drug penetration by the mechanism of membrane hydration. Roberts and Walker (1993:4) suggest that when the hydration state of the membrane increases, the penetration thought the membrane would also increase. In this study, however, probably because of the hydrophilic nature of water, it showed no penetration enhancement effect for the lipophilic drug ibuprofen. Propylene glycol is widely used as penetration enhancer or as a vehicle for penetration enhancers. When used as a single phase delivery vehicle during this study, it showed only moderate penetration enhancement properties. Combinations of the two lipophilic solvents, mineral oil and Miglyol®, enhanced the penetration of the lipophilic permeant, ibuprofen, across Carbosil® membrane. However, when used as single vehicles, both mineral oil and Miglyol® showed average penetration enhancement properties.

The outcomes of this study revealed that the binary 20/80 (v/v) mineral oil and Miglyol® combination showed the highest penetration enhancement effect for ibuprofen through Carbosil® membrane. This could have been as a result of synergistic action of the combined

enhancers. Miglyol® acts on disrupting the barrier function of the skin to increase diffusivity of a chemical, by modifying the intercellular lipids of the stratum corneum (Moser *et al*., 2001:105). As for human skin epidermis, Carbosil® membrane has a heterophasic and heteropolar structure and share a common solubility-diffusion mechanism of drug transport (Feldstein *et al*., 1998). Mineral oil, which is part of the hydrocarbon group, enhances penetration through the membrane by partitioning into the membrane and carrying the active through it (Hori *et al.,* 1991:33).

The penetration enhancement effects measured for the binary propylene glycol and water vehicles were generally lower than those of the mineral oil and Miglyol® combinations. Propylene glycol in combination with other penetration enhancers shows synergistic action (Williams & Barry, 2004:611). Results from this study also showed that the penetration enhancement effect of propylene glycol increased as the percentage of the propylene glycol in a combination solvent vehicle increased, and as the application volume of a single phase vehicle increased. The mechanism of action by which propylene glycol enhances penetration through the membrane is to partition into the membrane and to increase the solubility of the permeant in the membrane, which then increases the flux of both the propylene glycol and the permeant (Trottet *et al.,* 2004:213).

Future prospects and recommendations arising from this aspect of the study include:

- It would be beneficial to repeat the same experiments under clinical conditions on human skin; and
- It would be beneficial to investigate more solvent combinations, including ternary (composed of three enhancers) solvent combinations.

6.2 Mode of application: finite and infinite doses

The accurate prediction of dermal absorption of a topically applied substance and of unwanted chemicals in the environment, such as in the workplace, are of utmost importance for both formulation development and risk assessment (Gre'goire *et al*., 2009:80 The potential of a chemical substance to cross the skin is receiving growing interest. Very little is still known about the potential risk to the general population and to occupationally exposed workers, of dermal exposure to chemicals in the environment (Sartorelli *et al*., 2000:133). Many toxic substances, present in workplaces and in the general environment come into contact with the skin in several forms, depending on their physicochemical properties, such as vapour deposition, liquid contact to contaminated water for example, and contact to solids, such as contaminated soil (Sartorelli *et al*., 2000:133). Most prediction models for dermal absorption predict the permeability coefficient, K_p, of molecules in infinite (> 150 µl) dose conditions. In practice, however, dermal

exposure to a toxic chemical mostly occurs under finite (< 150 µl) dose conditions (Buist *et al.,* 2010:200). The transdermal absorption of a substance after dermal exposure is still a new research area of environmental medicine, with little data currently available. Data on the applied dose in clinical situations suggests that the quantity of the applied formulation depends on the body surface area being treated, i.e. the larger the surface area, the lesser the required active ingredient to be applied (Trottet *et al*., 2004:214). This significant factor should be taken into account when *in vitro*, transdermal diffusion studies are conducted. Most *in vitro* diffusion studies make use of infinite dose donor volumes, without considering the level of exposure in clinical situations, which usually comprises finite doses only.

The objectives for this aspect of the study were to:

- Determine the permeation of ibuprofen across Carbosil® membrane for finite dose applications (2 µl, 5 µl, 10 µl, 20 µl, 50 µl and 150 µl) of penetration enhancer vehicles, i.e. water and propylene glycol in combinations of 0/100 (v/v), 20/80 (v/v), 50/50 (v/v), 80/20 (v/v) and 100/0 (v/v);
- Determine the permeation of ibuprofen across Carbosil® membrane for finite dose applications (2 µl, 5 µl, 10 µl, 20 µl, 50 µl and 150 µl) of the penetration enhancer vehicles, i.e. mineral oil and Miglyol® in combinations of 0/100 (v/v), 20/80 (v/v), 50/50 (v/v), 80/20 (v/v) and 100/0 (v/v); and
- Hypothesise what influence such penetration enhancer vehicles applied in low volumes would have on risk assessment studies.

The outcomes of this study clearly demonstrated that the lipophilic mineral oil and Miglyol® delivery vehicles showed good penetration enhancement capabilities for lipophilic ibuprofen in finite dose applications, compared to the hydrophilic propylene glycol and water solvents. The above findings should, however, also be considered in the context of the quantity of the active being applied, or the level of exposure. The quantity of the applied solvent to the membrane influences the degree of hydration of the membrane (Chen *et al.,* 2011:224). Depending on the lipophilicity or hydrophilicity of both the permeant and the delivery vehicle, the effect of permeation may be positive or negative.

Another important factor that may significantly impact on the extent of the absorption of the active ingredient from finite dose applications is the diffusion area.

As mentioned, Trottet *et al.* (2004:214) suggest that in clinical situations, the quantity of the formulation to be applied depends on the body surface area being treated, i.e. the larger the surface area, the lesser the required active ingredient to be applied.

The exposure time to a small quantity of a chemical substance can significantly affect its transdermal absorption. From the finite dose exposure data it has become evident that permeation of a substance in finite conditions could have a higher permeation effect in the first hour, compared to the results after 6 hours.

Future prospects and recommendations arising from this aspect of the study include:

- The clinical effect of finite dose conditions, compared to infinite dose conditions should be explored further under clinical conditions on human skin; and
- Clinical risk assessment studies could be conducted on human skin and be compared to *in vitro* data.

6.3 Final conclusion

The higher permeation concentrations of lipophilic drug ibuprofen being measured with finite dose applications of lipophilic mineral oil and Miglyol®, compared to infinite dose applications, could be explained as being a result of the effect of hydration of the membrane by the lipophilic vehicle applied. The application of an infinite dose to the donor compartment of the Franz cell forms a thick solvent layer on the surface of the membrane, having a height of 1.6 mm, depending on the infinite volume applied, while a finite dose application forms a thin layer of only 0.1 mm, depending on the volume applied. As a result, much higher hydration conditions of the membrane occur with an infinite dose than with a finite dose (Chen *et al.,* 2011:231). Because of the lipophilic nature of mineral oil and Miglyol®, it was possible that when being applied to the membrane in infinite doses, these lipophilic solvents increased the hydration levels of the membrane, which increased the permeation of the lipophilic permeant, ibuprofen, into the non-polar layer of the membrane. Higher levels of the lipophilic drug could hence have penetrated the Carbosil® membrane with infinite and finite dose applications of mineral oil and Miglyol® as delivery vehicles, compared to when hydrophilic propylene glycol and water vehicles were used.

The general interest in the transdermal absorption of chemicals has increased over recent years. Very limited quantitative and qualitative data is currently available on the outcomes of dermal exposure to chemicals by occupationally exposed workers and by the general public. In order to accurately predict the systemic risk of certain chemicals on humans when absorbed transdermally, extensive data should in future be generated in similar transdermal studies regarding the rates and levels of exposure to environmental chemicals.

Through this study, a contribution has been made towards the importance of having to standardise *in vitro* diffusion experiments so that they relate to therapeutic (finite) doses or transdermal exposure levels. Although the penetration data in this study was done on skin-

imitating Carbosil® membrane, it has become evident that the level of application, or exposure of the membrane to a chemical, can show different results.

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APPENDIX A

FRANZ CELL DIFFUSION STUDIES

A.1 Introduction

The importance of accurately predicting dermal absorption after topical exposure to a chemical compound is relevant to both formulation development and risk assessment. The skin is the largest organ in the human body and covers a surface area of 1.5 - 2.0 m^2. It consists of three layers, i.e. the stratum corneum, the viable epidermis and the dermis (Chen *et al.,* 2011:223). The stratum corneum forms the main barrier to drug absorption, making it is very difficult to deliver drugs across the skin. Despite this obstacle to delivering drugs transdermally, this route of drug delivery still offers advantages over other routes of administration. Avoidance of the first-pass metabolism is the most important benefit, whilst advantages, such as smaller fluctuations in plasma drug levels for repeated dosing and good patient compliance also contribute to a preference for the transdermal delivery of a drug (Brown *et al.,* 2006:178). Because of these advantages, there have been investigations over the years into numerous techniques to overcome the barrier properties of the stratum corneum, in an attempt to improve transdermal drug delivery. It is thus important to develop methods that would deliver drugs efficiently across the skin. The permeation of a drug through the skin can be enhanced by both chemical penetration enhancement technology, as well as by physical enhancement methods. In this study, chemical penetration enhancement technology was investigated by using different modes of applications. Vertical Franz cell diffusion studies were performed to determine whether the model drug, i.e. lipophilic ibuprofen, could be successfully transported across the skin-imitating Carbosil®membrane.

A.2 Materials

Ibuprofen was obtained from Albemarle Corporation (South Carolina, USA). Other ingredients included deionised HPLC (high performance liquid chromatography) grade water prepared by the Milli-Q water purification system (Millipore, Milford, USA) and Carbosil® membranes (Medpolymer). Methanol (from Merck Laboratory Supplies, Midrand, South Africa) and Milli-Q water (70/30) were used as the HPLC mobile phase. The phosphate buffer solution (PBS) consisted of sodium chloride, disodium orthophosphate dehydrate and sodium dihydrogen dehydrate (from Merck Laboratory Supplies, Midrand, South Africa) and Milli-Q water.

A.2.1 Single and binary solvents

Single and binary phase penetration enhancer solvents were used as delivery vehicles. Propylene glycol (from Merck Laboratory Supplies, Midrand, South Africa) and Milli-Q water were used separately and in 20/80 (v/v), 50/50 (v/v) and 80/20 (v/v) combinations. The second group of penetration enhancer solvents being used were mineral oil (from Sigma-Aldrich) and Miglyol® (from Merck Laboratory Supplies), also separately and in combinations of 20/80 (v/v), 50/50 (v/v) and 80/20 (v/v).

A.2.2 Synthetic membrane

In this study a synthetic membrane was used to mimic the barrier function of the stratum corneumi.e. Carbosil® membrane, a polydimethylsiloxane-polycarbonate (PDMS-PC) block copolymer. These PDMS-PC block copolymers are synthesised at Medpolymer by making use of heterophase polycondensation of oligo-dioxiaryl carbonates with oligo-bis-chlorformate alkyl siloxanes (Listvoib, 1992:177; Raigorodskii *et al.,* 1995:447). Carbosil® (0.04 mm in thickness) is produced by casting of 12 - 15 wt percentage block polymer solution in methylene chloride, followed by drying.

Like human skin epidermis, this synthetic membrane has a heterophase and heteropolar structure and share a common solubility-diffusion mechanism of drug transport which provides a mechanically substantiated model for transdermal drug absorption (Feldstein *et al.,* 1998:3). Carbosil® membranes are useful for both the quantitative prediction of transdermal drug delivery rate and as a skin-imitating standard membrane in *in vitro* drug delivery experiments (Feldstein *et al.,* 1998:1).

A.3 Methods

A.3.1 High performance liquid chromatography analysis

Validation is a crucial step in determining whether the substance being used satisfies certain criteria (Karnes *et al*., 1991:421). Validation establishes whether the method used to analyse a substance is reliable and sensitive enough to determine the amount and recovery of a formulated drug. The HPLC method that was used in this study had been previously validated at the Analytical Technology Laboratory of the School of Pharmacy, at the Potchefstroom Campus of the North West University (South Africa) under regulated laboratory conditions of 25°C.

The validation process usually consists of the following steps:

1. System qualification: This step determines whether an instrument is reliable and suitable for the anticipated analysis.
2. Sampling: This ensures that the selected sample represents the material as a whole.
3. Sample preparation: Representing a crucial step in validation and operational costs in a laboratory.
4. Analysis: In close relation with the instrument to deliver quantitative and qualitative information.
5. Data evaluation: Gaining insight and summarising the data collected (Karnes *et al*., 1991:421).

A.3.1.1 Analytical instrument

Ibuprofen permeation samples were analysed using an HP1100 series HPLC, equipped with a pump, auto sampler, UV detector and Chemstation Rev. A.06.02 data acquisition and analysis software, or equivalent. All reagents were of HPLC grade. The Luna C_{18}-2 column, 150 x 4.6 mm, 5 µm, 100 Å pores, 17.8% carbon load, endcapped, Phenomenex, Torrance, CA was used. Detection was at 240 nm. The mobile phase consisted of a filtered and degassed mixture of HPLC grade water/methanol (70/30). The mobile phase flow rate was 1.0 ml/min with an injection volume of 100 µl. The retention time of ibuprofen was 5 min and the stop time 7 min.

A.3.1.2 Preparation of standard solution

A mother solution was prepared by accurately weighing 50 mg of ibuprofen into a 100 ml volumetric flask. The flask was then filled to volume with 50/50 methanol/Milli-Q water and sonicated to ensure a homogenous solution. From this stock solution 2 , 5 , 6 , 8 and 10 ml were transferred into separate 100 ml volumetric flasks each and filled to volume with 50/50 methanol/Milli-Q water. HPLC vials were filled with samples from each volumetric flask and analysed in duplicate on the HPLC.

A.3.2 Franz cell diffusion experiments

A.3.2.1 Preparation of donor- and receptor phases

A.3.2.1.1 Donor phase

Saturated solutions of ibuprofen in the solvent vehicle were prepared according to the solubility data as described in Section A.3.2. The preparation of this solution occurred an hour before each diffusion experiment was initiated. The saturated solution was kept in a water bath at

32°C for 1 hour while stirring, to ensure that all of the ibuprofen had dissolved and to maintain the solution at skin temperature of 32°C. Depending on the mode of application, a certain volume of saturated solution was applied to the Carbosil® membrane, whilst the donor compartment was left open.

A.3.2.1.2 Receptor phase

The receptor phase was phosphate buffered solution (PBS) (pH 7.4) that consisted of 4.4 g of sodium chloride, 9.2 g of disodium orthophosphate dehydrate and 2.1 g of sodium dihydrogen dehydrate that were dissolved in 1,000 ml freshly prepared Milli-Q water.

A.3.2.2 Membrane preparation

Carbosil® membrane was cut into circles, big enough to cover the area available for diffusion (1.075 cm^2) of the Franz cell. With finite dose applications (< 150 µl), only a fraction of the available diffusion area of the Franz cell was covered by the delivery vehicle and as a result the diffusion area for each droplet had to be measured, as summarised in Table A.1, for use in calculations. Infinite dose applications (> 150 µl) covered the entirely available diffusion area of the Franz cell, hence. a surface area of 1.075 cm^2 was used in these calculations. Before conducting each experiment, all Carbosil® membrane circles were soaked for 12 hours at 32°C in the relevant donor phase vehicle used in the particular experiment, in order to saturate the membrane with the solvent vehicle. A membrane that is pre-saturated with the solvent vehicle allows the permeant to partition into the membrane at a faster rate than when unsaturated membranes are used. The membrane circles were removed from the soaking solution after 12 hours and dried with tissue paper to remove any excess donor solution on the surface of the membrane, before mounting it on the receptor compartment of the Franz cell.

A.3.2.3 Franz cell diffusion method

Twenty four glass vertical Franz diffusion cells with a diffusion area of 1.075 cm^2 and receptor capacity of approximately 2 ml were used during this study. Pre-soaked Carbosil® membrane was cut into circles big enough to cover the area available for diffusion of the Franz cell. The membranes were placed on the lower half of the vertical Franz diffusion cell, the donor compartments were placed on top, sealed with Dow-corning® vacuum grease and clamped with metal horseshoe clamps. A small stirrer bar was placed in each receptor compartment.

Table A.1: Diffusion area available for diffusion through the membrane measured for each finite dose droplet

Donor solvent	Volume (µl)	Diffusion area (cm2){ λr2}
80/20 (v/v) propylene glycol/water	2 µl	0.02376
100% propylene glycol		0.02924
80/20 (v/v) mineral oil/Miglyol®		0.05184
50/50 (v/v) mineral oil/Miglyol®		0.06023
20/80 (v/v) mineral oil/Miglyol®		0.04290
100% Miglyol®		0.03170
80/20 (v/v) propylene glycol/water	5 µl	0.04711
100% propylene glycol		0.03594
80/20 (v/v) mineral oil/Miglyol®		0.12062
50/50 (v/v) mineral oil/Miglyol®		0.16251
20/80 (v/v) mineral oil/Miglyol®		0.07350
100% Miglyol®		0.06690
80/20 (v/v) propylene glycol/water	10 µl	0.07888
100% propylene glycol		0.08968
80/20 (v/v) mineral oil/Miglyol®		0.15336
50/50 (v/v) mineral oil/Miglyol®		0.20258
20/80 (v/v) mineral oil/Miglyol®		0.14649
100% Miglyol®		0.10060
80/20 (v/v) propylene glycol/water	20 µl	0.13584
100% propylene glycol		0.19234
80/20 (v/v) mineral oil/Miglyol®		0.28543
50/50 (v/v) mineral oil/Miglyol®		0.29401
20/80 (v/v) mineral oil/Miglyol®		0.25954
100% Miglyol®		0.17860
50/50 (v/v) propylene glycol/water	50 µl	0.19546
80/20 (v/v) propylene glycol/water		0.19861
100% propylene glycol		0.35028
100% mineral oil		0.56183
80/20 (v/v) mineral oil/Miglyol®		0.40240
50/50 (v/v) mineral oil/Miglyol®		0.75545
20/80 (v/v) mineral oil/Miglyol®		0.48620
100% Miglyol®		0.40807
20/80 (v/v) propylene glycol/water	150 µl	0.47514
50/50 (v/v) propylene glycol/water		0.56849
80/20 (v/v) propylene glycol/water		0.72044
100% mineral oil		1.04902
80/20 (v/v) mineral oil/Miglyol®		0.89700
50/50 (v/v) mineral oil/Miglyol®		1.66872
20/80 (v/v) mineral oil/Miglyol®		1.03455
100% Miglyol®		1.14714

The receptor compartment was filled with PBS (pH 7.4 at 37°C), taking care not to allow bubbles in the receptor compartment. The diffusion cells were placed in a tray on a Variomag® stirrer plate in order to continuously stir the receptor phase inside a Grant® water bath at 32°C, simulating the temperature of human skin (Azarmi *et al.,* 2007:17).

Saturated solutions of ibuprofen in the different solvents or combination of solvent vehicles were prepared an hour before the experiment. The saturated solutions were kept at 32°C before application to the membrane. Depending on the experiment conducted a specific volume of saturated solution was applied to the membrane. The volumes that were tested are as follows: 2 µl, 5 µl, 10 µl, 20 µl, 50 µl, 150 µl, 250 µl, 500 µl and 1,000 µl. A separate experiment was done for each donor phase vehicle with a certain volume. Out of the twenty four Franz cells that were used per diffusion experiment, the receptor phase of the first four cells were extracted after 1 hour and were not refilled. After 2 hours the second 4 Franz cells were extracted. The aforementioned was done hourly until 6 hours have passed and all the receptor compartments were empty. The main reason for doing the experiment this way was to ensure that the diffusion area stays as consistent as possible; due to the fact that when the cell is turned upside down to refill the receptor compartment; the diffusion area will change for volumes that does not cover the entire diffusion area of the Franz cell. By leaving the cell unmoved until extraction ensures that the diffusion area stays the same for the duration of the experiment. Therefore, as a result of the design of this experiment it was not possible to use cumulative concentrations or flux. The Franz cells were left open and the donor solution was exposed to the effect of evaporation.

A.4 Results and discussion

A.4.1 Solubility study

Excess drug was added to each solvent vehicle and stirred with a magnetic stirrer for 24 hours (to attain equilibrium) in a water bath maintained at 32°C. A syringe (5 ml) and a filter were also pre-heated to 32°C. The supernatant was then filtered and diluted 1/10 (supernatant/methanol) with methanol. This dilute was then assayed on HPLC. Experiments were performed in triplicate and the solubility of the drug in the solvent was determined. For each experiment ibuprofen was added to the solvent vehicle in concentrations as determined by the solubility study in order to obtain saturated solutions of ibuprofen. This saturated ibuprofen/solvent solution was stirred for 12 hours at 32°C before each experiment. After assuring that no undissolved particles are present in the drug/solvent solution the vehicle was applied to the Carbosil® membrane as the donor phase.

The concentration of ibuprofen needed to make up a saturated solution of ibuprofen in solvent is illustrated in Tables A.2 and A.3.

Table A.2: Ibuprofen concentration (mg/ml) in different propylene glycol-water mixtures

Solvent (%)		Concentration (mg/ml)
Propylene glycol/Water	100/0	323.14
	80/20	26.65
	50/50	2.13
	20/80	1.02
	0/100	0.94

Table A.3: Ibuprofen concentration (mg/ml) in different mineral oil-miglyol® mixtures

Solvent (%)		Concentration (mg/ml)
Mineral oil/Miglyol®	100/0	10.69
	80/20	167.63
	50/50	1696.01
	20/80	53.61
	0/100	31.99

From the tables above it is clear that ibuprofen is highly soluble in propylene glygol and almost insoluble in water. The solubility in the binary phase solvents decrease as the amount of propylene glycol in the solvent decrease.

Ibuprofen is most soluble in the binary phase solvent containing 50/50 (v/v) mineral oil and Miglyol®. The 80/20 (v/v) mineral oil and Miglyol® had the second highest solubility followed by 20/80 (v/v) mineral oil and Miglyol®. The solubility of ibuprofen was the lowest in the sole solvent mineral oil and slightly more soluble in Miglyol®.

A.4.2 Diffusion and statistical analysis

Before analysing the samples of the diffusion studies, a standard solution was prepared and linearity was determined. In this study it was not possible to calculate cumulative amounts of ibuprofen per area (flux), because the Franz cells could not be refilled after a specific time interval. The concentration (µg/cm^2) of ibuprofen that diffused through the Carbosil® membrane for a certain time interval was calculated.

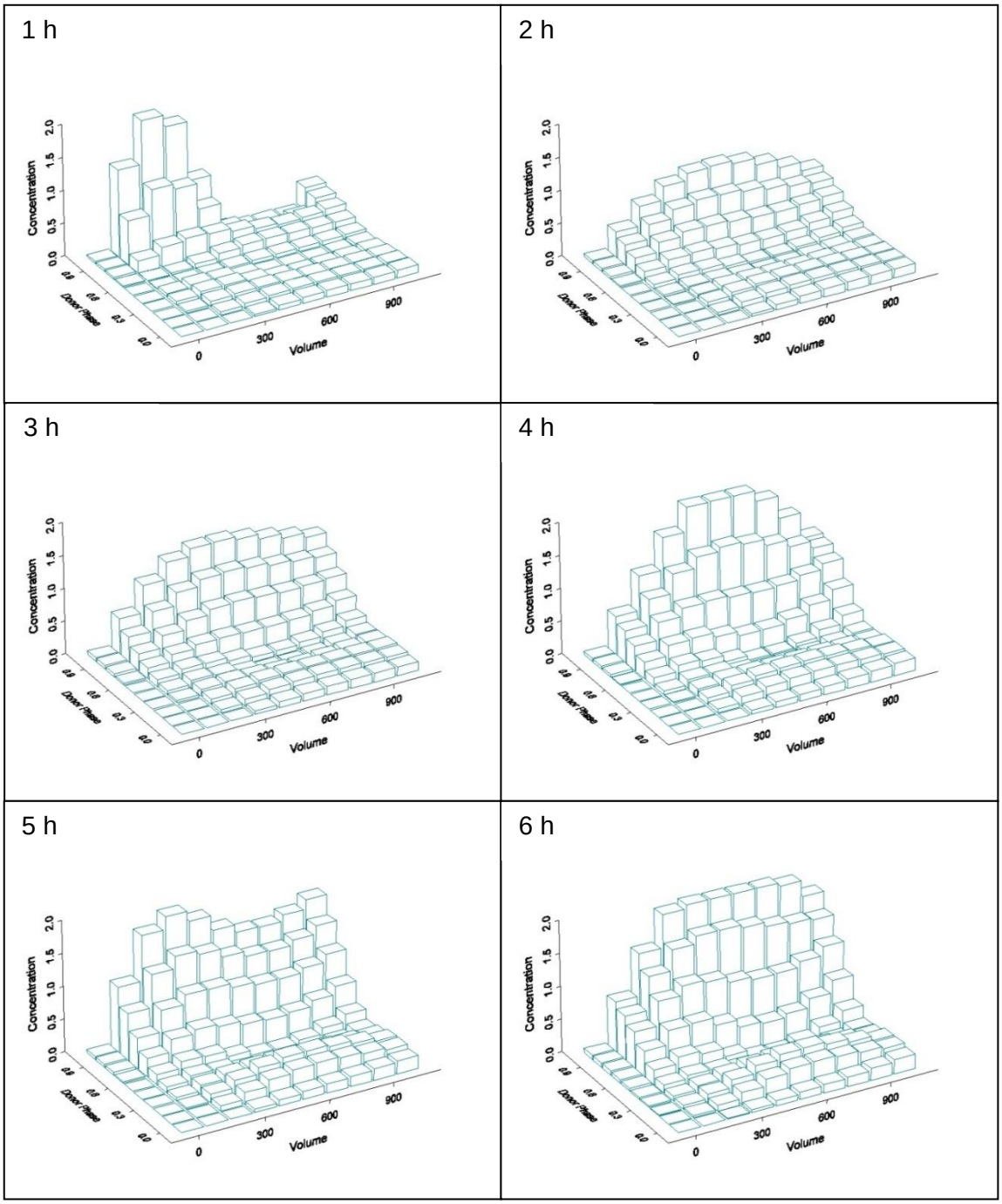

Figure A.1: Spline representation of the ibuprofen concentration (µg/ml) that diffused hourly over 6 hours when finite and infinite doses were applied to the Carbosil® membrane. Binary solvents of propylene glycol and water acted as the delivery vehicles.

Spline methodology was used to describe the statistical significance of the different doses for Miglyol®/mineral oil and propylene glycol/water. Smoothing spline is a method of smoothing (fitting a smooth curve to a set of noisy observations) using a spline function (Reinsch, 1967:177). It was decided to use this methodology because there was no pattern or order in

the way the data points were analysed. Histograms were also used to illustrate the difference in the penetration enhancement effect of the different delivery vehicles as well as the effect of the mode of application.

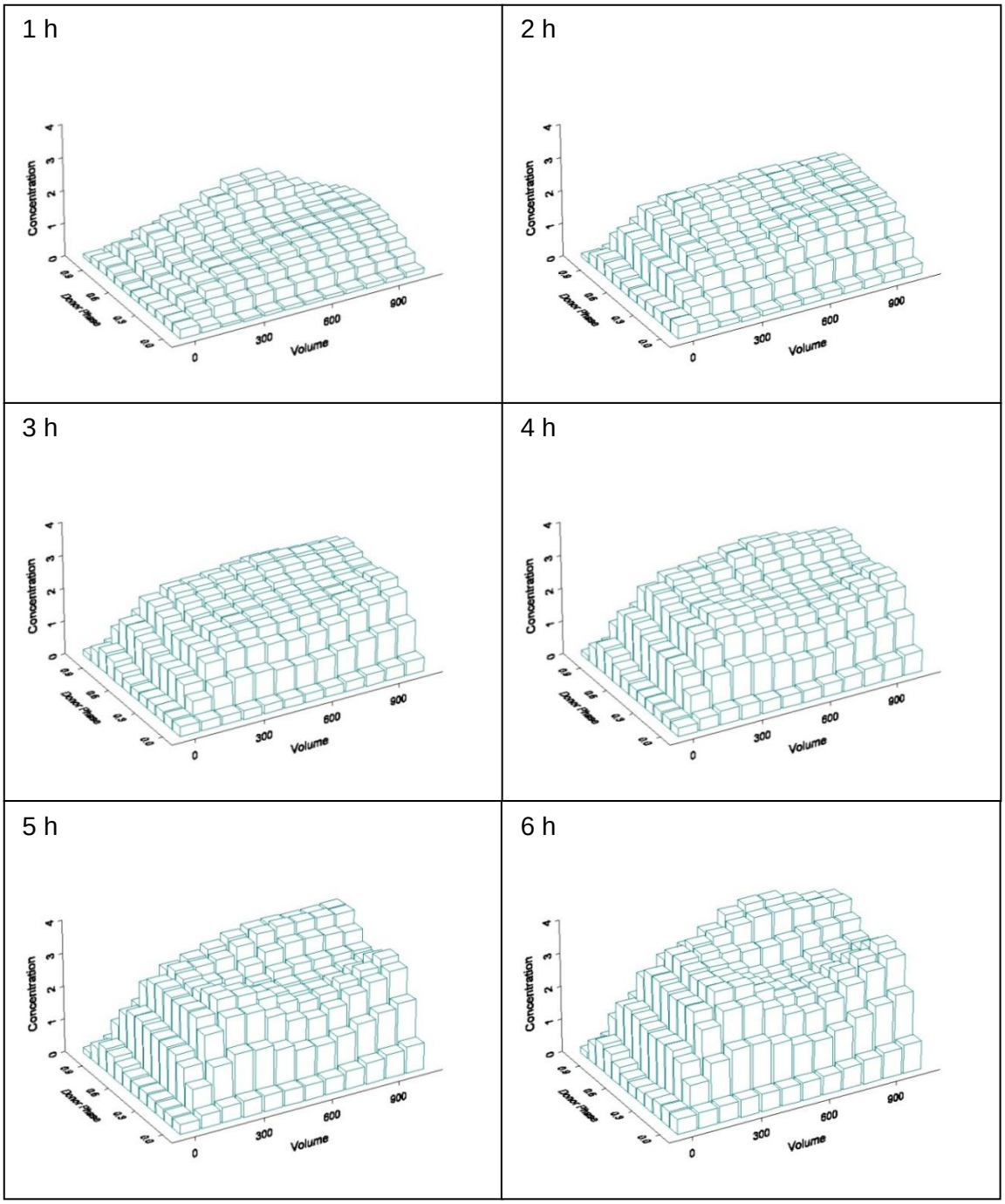

Figure A.2: Spline representation of ibuprofen concentration (µg/ml) that diffused hourly over 6 hours when finite and infinite doses were applied to the Carbosil® membrane. Binary solvents of Miglyol® and mineral oil solvents acted as the delivery vehicles.

A.4.3 Permeation of ibuprofen from propylene glycol and water vehicles

A.4.3.1 Finite dose application

Figures A.3 illustrates the concentration (µg/cm^2) of ibuprofen that permeated the membrane after each hour. In an attempt to keep the diffusion area consistent throughout the experiment the Franz cells were kept unmoved until the receptor phase was extracted. Twenty four Franz cells were used for each diffusion experiment and the receptor phase of 4 cells were extracted after each hour and indicated as one measurement on the histograms. Hence this unique experimental design 6 measurements for each volume are seen in the figures below (one measurement for each hour until 6 hours).

The concentration (µg/cm^2) of ibuprofen that permeated the Carbosil® membrane when propylene glycol and water were applied in finite doses is illustrated in Figure A.3.

The amount of ibuprofen that permeated the membrane when water was used as the delivery vehicle is shown in Figure A.3A and the concentration permeant measured was so low that it could not be measured. In general water will increase membrane hydration and increase the transdermal delivery of both hydrophilic and lipophilic permeants. The human statum corneum and Carbosil® membrane share both a heterogeneous nature and for this reason the water in the membrane is found in several 'states'. Some 25-35% of water present in the membrane can be seen as 'bound' or associated with structural elements in the membrane. The remaining of the water in the membrane is 'free' and available to act as a solvent within the membrane for polar permeants (Williams & Barry, 2004:606). Although extensive research has been done to understand the mechanism of action by which water increases transdermal delivery it is still unclear what the exact mechanism involves. The free water within the membrane could alter the solubility of the permeant in the membrane and modify the partitioning from the delivery vehicle into the membrane. Such a mechanism could explain increased transdermal delivery of hydrophilic permeants but would fail to explain hydration enhanced delivery of lipophilic drugs such as ibuprofen. (Williams & Barry, 2004:606). The low levels of ibuprofen measured from water as a delivery vehicle could be explained by this mechanism of hydration enhanced delivery as well as the low solubility of ibuprofen in water.

When the 20/80 (v/v) propylene glycol/water solvent vehicle was applied as the delivery vehicle, volumes lower than 150 µl could not be measured (Figure A.3B) due to the low water solubility of ibuprofen. With a log *P* of 3,6, it is clear that ibuprofen is a highly lipophilic drug and almost insoluble in a vehicle containing 80% water (Beetge *et al.,* 2000:262). Compared to water, permeation though the membrane with this solvent was measured when higher application

volumes (150 µl) were applied. This is due to the 20% propylene glycol addition to the delivery vehicle which increased the solubility of the permeant in the solvent.

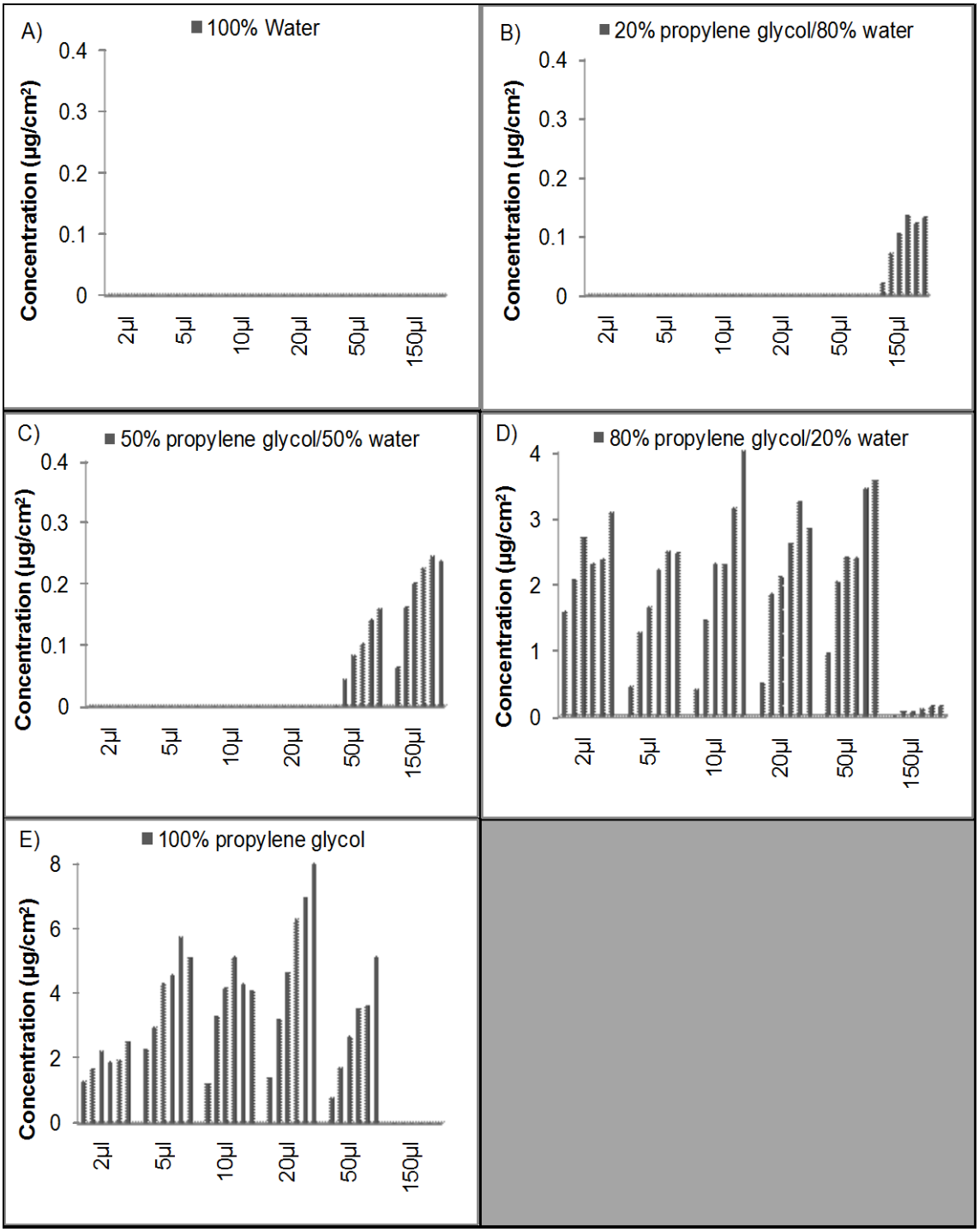

Figure A.3: Ibuprofen concentration (µg/cm^2) that diffused over 6 hours through the Carbosil® membrane when finite dose volumes were applied using propylene glycol and water as delivery vehicles. Standard deviation was in all instances less than 1.0.

In Figure A.3C the binary solvent of propylene glycol and water 50/50 (v/v) was applied in finite doses. The only two finite dose volumes that showed diffusion of the active from the delivery

vehicle through the membrane were 50 µl and 150 µl. The concentration that permeated from the 150 µl application was higher than the permeated concentration from the 50 µl application. This can be due to the higher application concentration with the 150 µl application compare to the 50 µl application. All volumes lower that 50 µl could not be measured due to the increased but still low solubility of the active ingredient in the solvent vehicle.

The results obtained when the binary solvent of propylene glycol and water 80/20 (v/v) was used in finite dose applications, is shown in Figure A.3D.

From the above results it is clear that the concentration (µg/cm^2) of ibuprofen that permeated the membrane from all the finite dose applications lower than 150 µl showed good permeation through the membrane. The 10 µl application shows the highest level of permeation after 6 hours. When 150 µl was applied the amount of permeated ibuprofen was significantly lower compared to the other finite dose applications used. One explanation that could be considered is the effect of evaporation. Then finite dose applications are used the amount of propylene glycol in the solvent vehicle depletes due to evaporation and consequently the drug crystallisation occur on and in the membrane (Santos *et al*., 2011:77). The effect of evaporation might not have been so prominent with the smaller volumes because less solvent was available for evaporation.

The concentration of ibuprofen that penetrated the membrane when 100% propylene glycol was applied in finite dose volumes is illustrated in Figure A.3E. The amount of ibuprofen that permeated the membrane from propylene glycol as a delivery vehicle is very similar to what was seen when 80/20 (v/v) propylene glycol and water was used to deliver the active. The amount of permeant that diffused through the membrane from volumes lower than 150 µl was higher than when 150 µl was applied. The same explanation can be given as for 80/20 (v/v) propylene glycol and water.

A.4.3.2 Infinite dose application

The concentration (µg/cm^2) of ibuprofen that permeated the Carbosil® membrane when propylene glycol and water were applied in infinite doses is illustrated in Figure A.4. The dose applied (250 µl, 500 µl and 1,000 µl) covered the entire area available for diffusion of the Franz cell and stayed constant at 1.075 cm^2.

In Figure A.4A the single phase solvent of 100% water was used to deliver ibuprofen through the Carbosil® membrane. The amount of ibuprofen that permeated the membrane from this delivery vehicle increase as the dose applied increase. The concentration permeant measured from 250 µl was the lowest and from 1,000 µl the highest with 500 µl between the other two. As mentioned when finite dose application the water solubility of ibuprofen is very low. This

explains the low permeation of ibuprofen through the membrane but as the dose applied increase the concentration of ibuprofen that permeates the membrane increase.

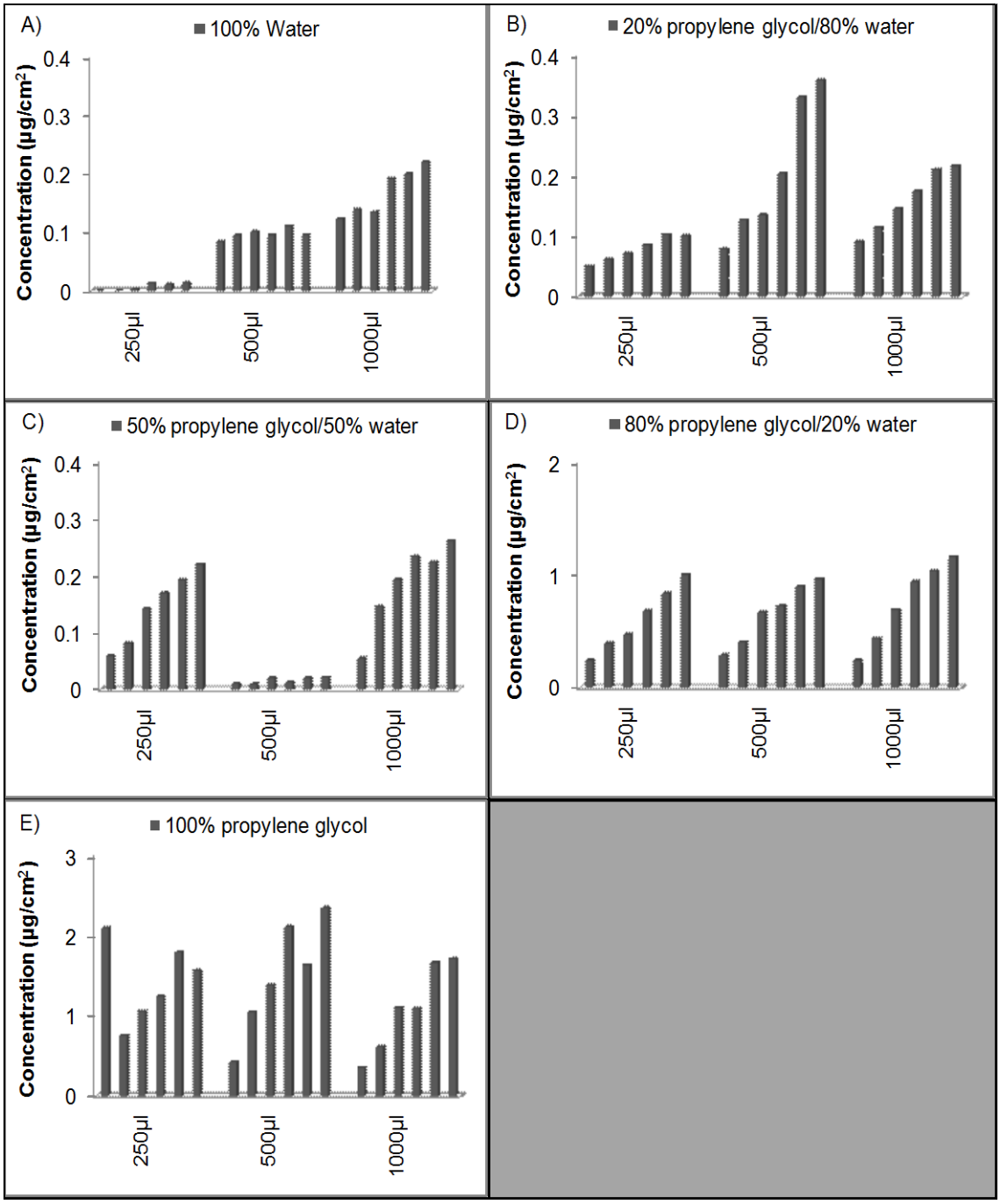

Figure A.4: Ibuprofen concentration (µg/cm^2) that diffused over 6 hours through the Carbosil® membrane when infinite dose volumes were applied using propylene glycol and water as delivery vehicles. Standard deviation was in all instances less than 0.2.

The amount of ibuprofen that permeates through the membrane when infinite doses of the binary solvent propylene glycol and water 20/80 (v/v) was used as the delivery vehicle is shown in Figure A.4B. The highest concentration (µg/cm^2) of permeated ibuprofen was measured from

the 500 µl application. The 1,000 µl application showed the second highest permeation through the membrane and the concentration permeated from the 250 µl application showed the lowest concentration of ibuprofen that diffused through the membrane. The lower permeation of ibuprofen from a higher applied dose (1,000 µl) might be due to the effect of evaporation and less solvent was available to carry the permeant through the membrane.

Figure A.4C illustrates the permeation results measured then the binary solvent of propylene glycol and water 50/50 (v/v) was used in infinite doses. The amount of ibuprofen that permeated the membrane from the 1,000 µl and 250 µl application were almost the same. But the concentration permeated ibuprofen measured from the 500 µl application was much less compared to the other two volumes. The lower measurement from the 500 µl application might be due an experimental error.

The results obtained when the binary solvent of propylene glycol and water 80/20 (v/v) was used in infinite dose applications, is shown in Figure A.4D. The amount of ibuprofen that permeated the membrane from all three volumes applied was the same. The concentration of permeant that was measured using this delivery vehicle is much higher compared to the previous delivery vehicles mentioned for infinite dose applications. This is due to the high solubility of ibuprofen in propylene glycol and the increased levels of propylene glycol in the solvent.

The concentration of ibuprofen that penetrated the membrane when 100% propylene glycol was applied in infinite dose volumes is illustrated in Figure A.4E.

The amount of ibuprofen that permeated the membrane from the 500 µl application was slightly higher after 6 hours compared to the 250 and 1.000 µl applications. Results from the 1,000 µl application showed to be slightly lower and this might be due to the effect of evaporation and depletion of propylene glycol from the donor compartment (Santos *et al*., 2011:77).

Diffusion from finite and infinite dose applications through the Carbosil® membrane after 6 hours is illustrated in Figure A.5.

When propylene glycol was employed as the delivery vehicle solvent the concentration ibuprofen that permeated the Carbosil® membrane was at its highest. Water showed the lowest permeation of the active through the membrane.

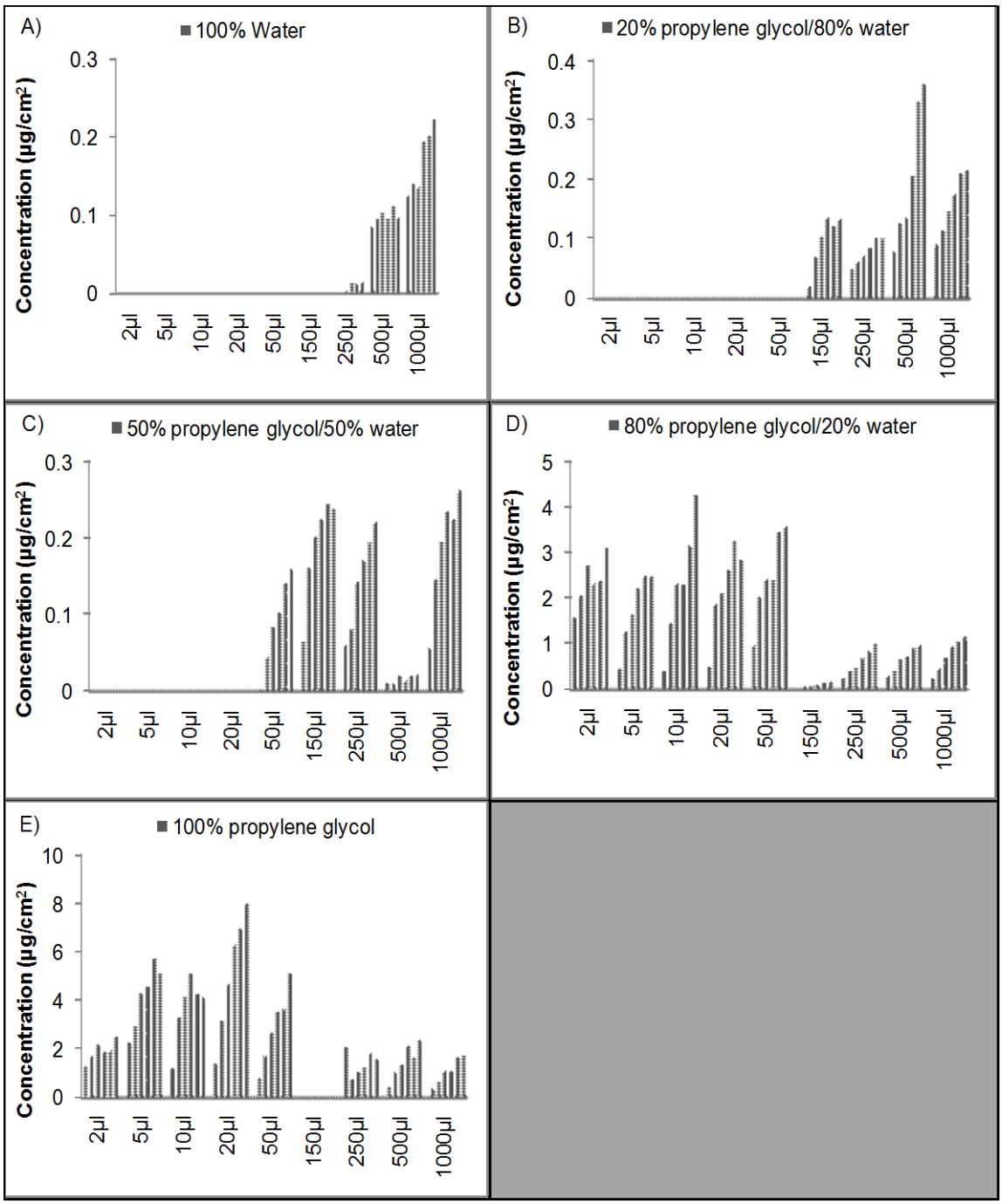

Figure A.5: Ibuprofen concentration (µg/ml) that diffused over 6 hours when finite and infinite doses were applied to Carbosil® membrane by using binary solvents of propylene glycol and water as delivery vehicles. The standard deviation for finite dose applications was in all instances less than 1.0 and for infinite dose applications less than 0.2.

Because of the relationship between the flux of both propylene glycol and the permeant (Trottet *et al.,* 2004:214) further hypothesised that the permeant flux would depend on the dose of propylene glycol under conditions of finite dose of propylene glycol. Smith & Maibach (1995:481) suggest in a review of penetration enhancers that the effect of penetration

enhancers could be dependent on the dose applied. It is clear from the results above that the dose applied will have an influence on the penetration enhancement effect of a solvent. The concentration permeant that penetrated the membrane from the finite dose applications is significantly higher compared to the infinite dose applications. Chen *et al*. (2011:231) found that with an infinite dose application the donor compartment will be filled with a thick liquid formulation layer covering on the surface of the membrane with a height of 1.6 mm, while the finite dose application will form a thin layer on the surface of the membrane of 0.1 mm. As a result the hydration levels of the membrane will increase from infinite dose applications. This increase in the hydration levels of the membrane will facilitate higher permeability of the membrane (Chen *et al*., 2011:231). Increased membrane hydration appears to increase the transdermal delivery of both hydrophilic and low lipophilic compounds due to the partitioning of the active into the membrane (Williams & Barry, 2004:603). This hydration effect on the membrane will make penetration of hydrophilic compounds through the membrane easier but it will be more difficult for high lipophilic compounds (log P > 2) to partition into the hydrated membrane (Zhang *et al.,* 2010:894). Ibuprofen is a high lipophilic drug with a log P of 3.6 (Beetge *et al.,* 2000:262). This drug will find it very difficult to partition into a hydrated membrane and this explains the low concentration of ibuprofen that permeated through the membrane from the infinite dose applications. The high levels of permeation through the membrane from finite dose volumes can be explained by the effect of evaporation and the penetration enhancement effect of propylene glycol through the membrane.

A.4.4 Permeation of ibuprofen from Miglyol® and mineral oil vehicles

A.4.4.1 Finite dose application

The concentration (µg/cm^2) of ibuprofen that permeated the Carbosil® membrane when the mineral oil and Miglyol® was applied in finite doses is illustrated in Figure A.6.

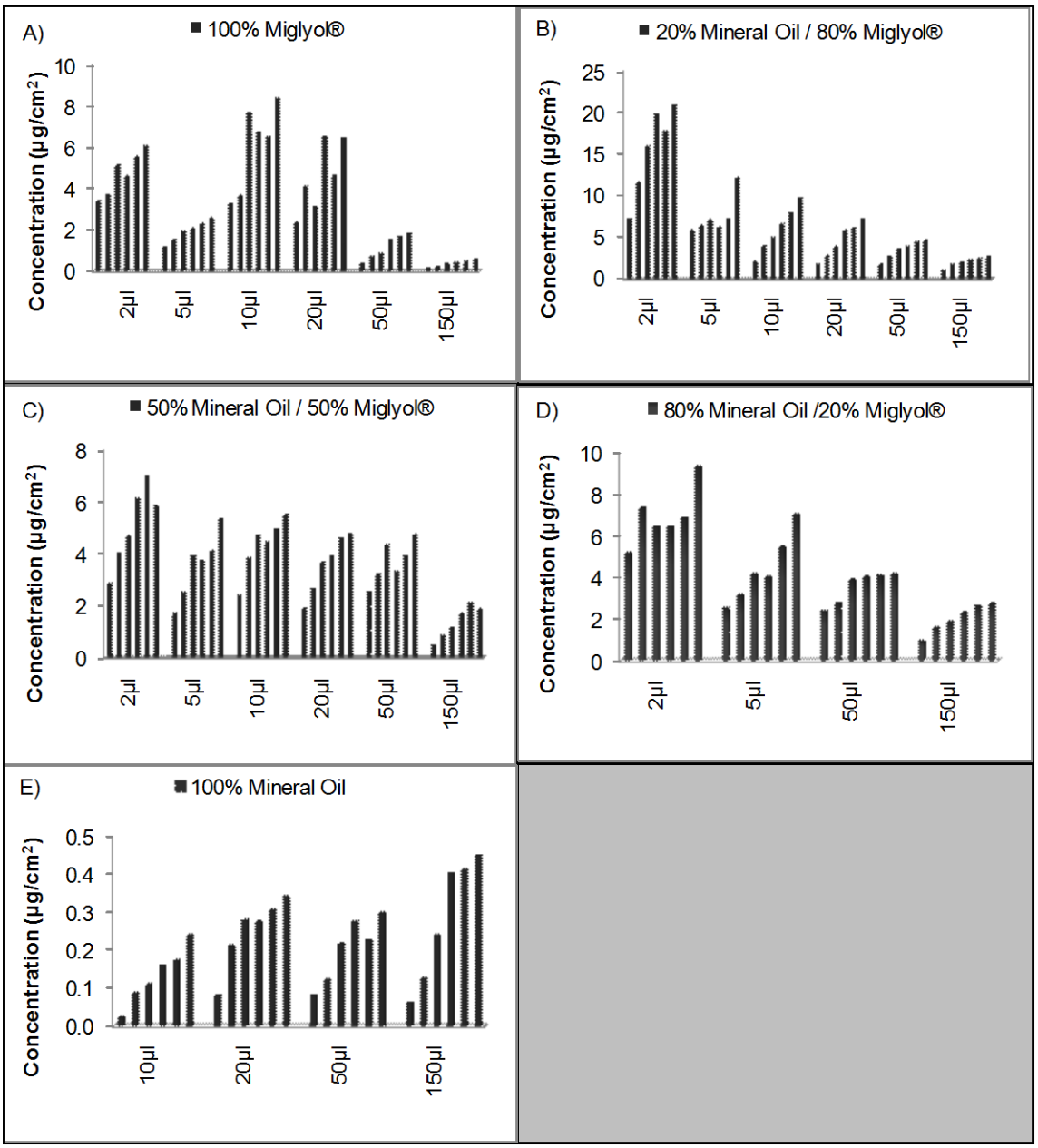

Figure A.6: Ibuprofen concentration (µg/cm^2) that diffused over 6 hours through the Carbosil® membrane when finite dose volumes were applied using mineral oil and Miglyol® as delivery vehicle. Standard deviation was in all instances less than 1.0.

In Figure A.6A the permeation of ibuprofen from finite dose applications is shown when Miglyol® was used to deliver the active ingredient. The smaller volumes showed higher levels of penetration through the membrane. The lowest level of permeation is seen when 50 and 150 µl was applied. Chen *et al*. (2011:231) found that with an infinite dose application the donor compartment will be filled with a thick liquid formulation layer covering on the surface of the membrane with a height of 1.6 mm, while the finite dose application will form a thin layer on the surface of the membrane of 0.1mm. As a result the hydration levels of the membrane will increase from infinite dose applications. This increase in the hydration levels of the membrane

will facilitate higher permeability of the membrane (Chen *et al*., 2011:231). Increased membrane hydration appears to increase the transdermal delivery of both hydrophilic and low lipophilic compounds due to the partitioning of the active into the membrane (William & Barry, 2004:603). This hydration effect on the membrane will make penetration of hydrophilic compounds through the membrane easier but it will be more difficult for high lipophilic compounds (log $P_{o/w}$ >2) to partition into the hydrated membrane (Zhang *et al*., 2010:894). Ibuprofen is a high lipophilic drug with a log $P_{o/w}$ of 3.6 (Beetge *et al*., 2000:262). Thus the lower permeation results from higher finite dose volume are due to higher levels of membrane hydration which makes it more difficult for a lipophilic drug like ibuprofen to penetrate through the membrane and for this reason we see lower levels of penetration of the active through the membrane when bigger finite dose volumes were applied (Chen *et al*., 2011:231).

Figure A.6B shows the permeation from finite dose applications with 20/80 (v/v) mineral oil/Miglyol® as the delivery vehicle. When the 20/80 (v/v) mineral oil/Miglyol® vehicle was used as the delivery vehicle the concentration ibuprofen that permeated the membrane increased as the volume applied decreased. The 2 µl application showed the highest concentration of permeated ibuprofen and this concentration decrease from 5 µl to 150 µl which showed the lowest concentration of permeated ibuprofen. These high levels of penetration after application of finite doses might be due to a synergistic effect of penetration enhancement between the two solvents in the right combination.

Figure A.6C illustrates the permeation from the binary solvent of Miglyol® and mineral oil 50/50 (v/v) when finite doses were applied. The concentration of ibuprofen that permeated the membrane is almost the same for all finite dose volumes applied. The 2 µl application shows a slightly higher level of permeated ibuprofen and 150 µl shows the lowest concentration of permeated ibuprofen.

The amount of ibuprofen that permeates through the membrane when finite doses of the binary solvent Miglyol® and mineral oil 20/80 (v/v) was used as the delivery vehicle is shown in Figure A.6D. The concentration (µg/cm^2) of diffused ibuprofen from the 20/80 (v/v) Miglyol®/mineral oil vehicles was the highest from the 2 µl application. The 5 µl application also showed high levels of diffused ibuprofen. Volumes 10 and 20 µl could not be measured due to the viscosity of this solvent, which was of such a nature that the diffusion area could not be kept consistent for these two volumes. The droplets of the 10 µl and 20 µl applications did not cover the entire diffusion area of the Franz cell but adhered to the walls of the cell and a constant diffusion area could not be measured and for this reason the results from these two experiments could not be used. The 50 µl and 150 µl applications covered the entire diffusion area of the Franz cell and could be measured but showed lower concentrations of permeated ibuprofen

compared to 2 µl and 5 µl. The 50 µl results are higher than the 150 µl application. This can also be explained by the effect of membrane hydration as stated by Chen *et al*. (2011:231).

In Figure A.6E the permeation of ibuprofen from finite dose applications is shown when mineral oil was used to deliver the active ingredient. Compared to Miglyol® the solubility of ibuprofen in mineral oil is low and for this reason the concentration ibuprofen applied from the 2 µl and 5 µl applications were very low and because of this we see no permeation through the membrane. The application of 10 µl of the delivery solvent showed permeation through the membrane but in comparison with the other volumes the concentration permeated from this volume was the lowest. The permeation concentration increased when 20 µl and 50 µl were applied and 150 µl showed the highest level of permeated ibuprofen. As the dose applied increased because of the bigger volumes the more ibuprofen is available to permeate the membrane and hence the increase in permeation as the volumes applied increase.

A.4.4.2 Infinite dose application

The concentration (µg/cm^2) of ibuprofen that permeated the Carbosil® membrane when mineral oil and Miglyol® was applied in infinite doses is illustrated in Figure A.7.

In Figure A.7A, Miglyol® was used as the sole delivery vehicle and applied in infinite doses the amount of ibuprofen that permeated the membrane was low. The permeation measured from the 1,000 µl application was slightly lower compared to 500 and 250 µl that was very similar to one another. The low permeation concentrations measured might be due to poor penetration enhancement of the sole solvent or a second possible can be due to the low solubility of ibuprofen in the solvent.

The results obtained when the binary solvent of Miglyol® and mineral oil 80/20 (v/v) was used in infinite dose applications, is shown in Figure A.7B. The permeation from 500 and 1,000 µl applications is almost the same. Whereas, 250 µl showed a slightly lower permeation of ibuprofen compared to 500 and 1,000 µl but all three volumes applied showed very similar results. The concentration of ibuprofen that permeated is higher compared to the results where Miglyol® was used as the sole solvent. This might be due to an increase in the solubility of the active in the solvent combination or secondly due to a synergistic effect between mineral oil and Miglyol®.

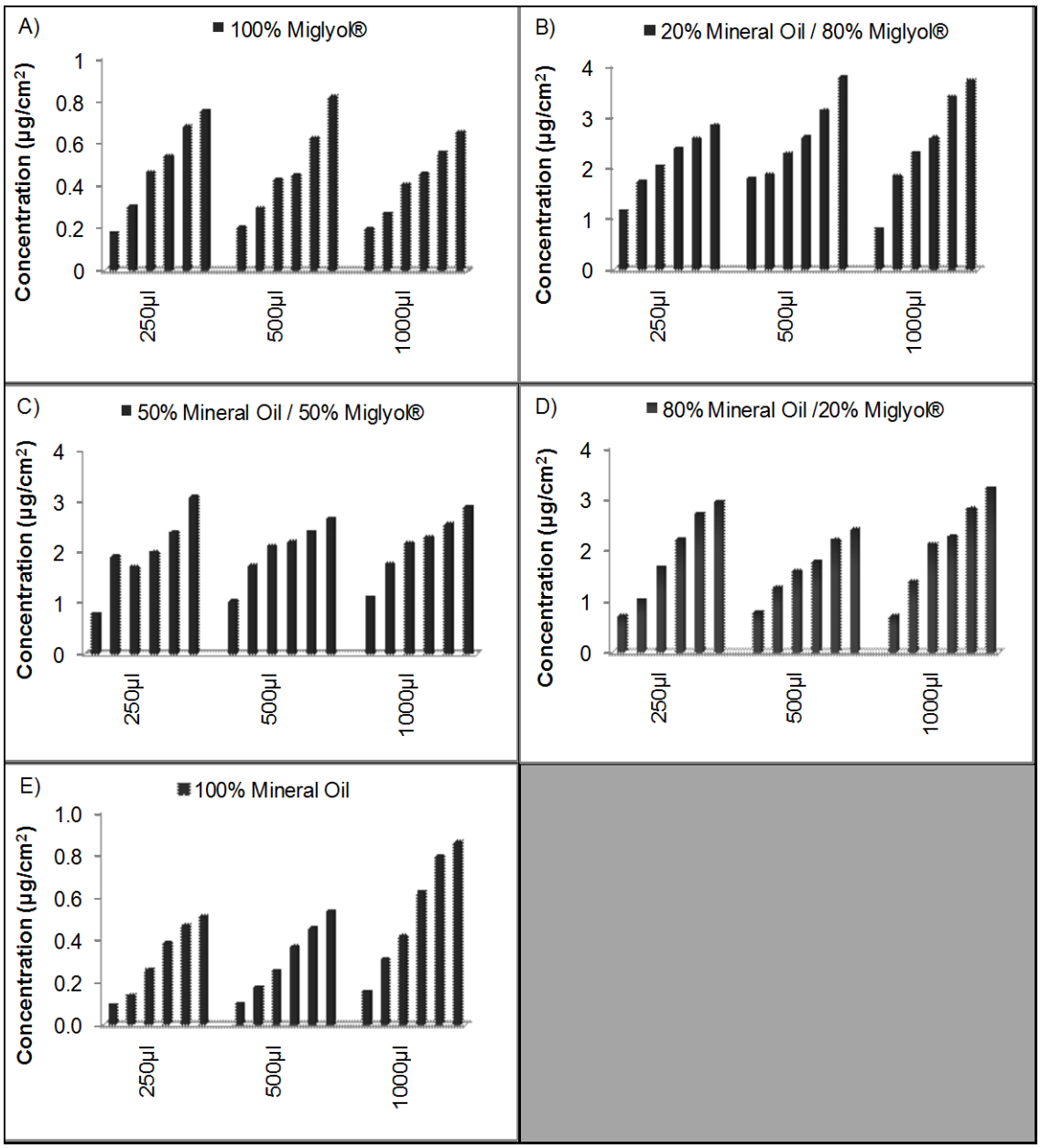

Figure A.7: Ibuprofen concentration (µg/cm^2) that diffused over 6 hours through the Carbosil® membrane when infinite dose volumes were applied using mineral oil and Miglyol® delivery vehicles. Standard deviation was in all instances less than 0.2.

Figure A.7C shows the permeation results of the binary solvent containing Miglyol® and mineral oil 50/50 (v/v) in infinite doses. When the solvent of 50/50 (v/v) Miglyol® and mineral oil was employed as the delivery vehicle, good permeation concentrations of ibuprofen was measured with small difference between the results measured from the different infinite volumes. Again this can be due to the synergistic action of using the two penetration enhancer solvents in combination with one another.

The amount of ibuprofen that permeates through the membrane when infinite doses of the binary solvent Miglyol® and mineral oil 20/80 (v/v) was used as the delivery vehicle is shown in

Figure A.7D. The application of 500 µl of the delivery solvent showed a slightly lower concentration of permeated ibuprofen compared to 250 µl and 1,000 µl, which showed higher permeation levels of permeation. The difference between the results obtained from the three different volumes was very small. The overall concentration of ibuprofen that penetrated the membrane from this binary solvent was also higher compared to the single solvent Miglyol® These results almost confirm that there is a synergistic action between the two solvents used in combination and as the permeations levels measured from the 80/20 (v/v) combination showed the highest level of penetration it can be assumed that this is the best combination to use.

Figure A.7E illustrated the permeation of ibuprofen from the sole penetration enhancer solvent, mineral oil. The amount of ibuprofen that permeated through the membrane increased as the volume applied increased. The 1,000 µl application showed the highest concentration of permeated ibuprofen and 250 µl and 500 µl the lowest with a slight difference between the two. The over all concentration ibuprofen that penetrated the membrane from this single penetration enhancer solvent is much lower compared to the binary solvent results and more similar to the results obtained from the single Miglyol® solvent, Where mineral oil is used as the sole solvent there is no synergistic effect between two solvents and the end results is smaller.

Diffusion from finite and infinite dose applications through the Carbosil® membrane after 6 hours is illustrated in Figure A.8.

When 100% Miglyol® was used to deliver ibuprofen through the membrane the differences between the infinite dose results were very small and the concentration measured was much lower compare to finite dose measurements. Absorption of the active from finite dose conditions was higher compared to infinite dose conditions. This is due to the effect of membrane hydration with infinite dose conditions which decrease permeation of lipophilic compounds through the membrane. With finite dose conditions the effect of hydration of the membrane is less and it is easier for a lipophilic drug like ibuprofen to penetrate through the membrane and as a result we see higher levels of penetration of the active with finite dose applications (Chen *et al*., 2011:231).

The 20/80 (v/v) mineral oil/Miglyol® showed the highest level of diffusion through the membrane compared to all other solvents used. This can be due to a synergistic effect of penetration enhancement between the two solvents in the right combination. From the results obtained using this solvent the finite dose measurements were also higher than the infinite dose measurements.

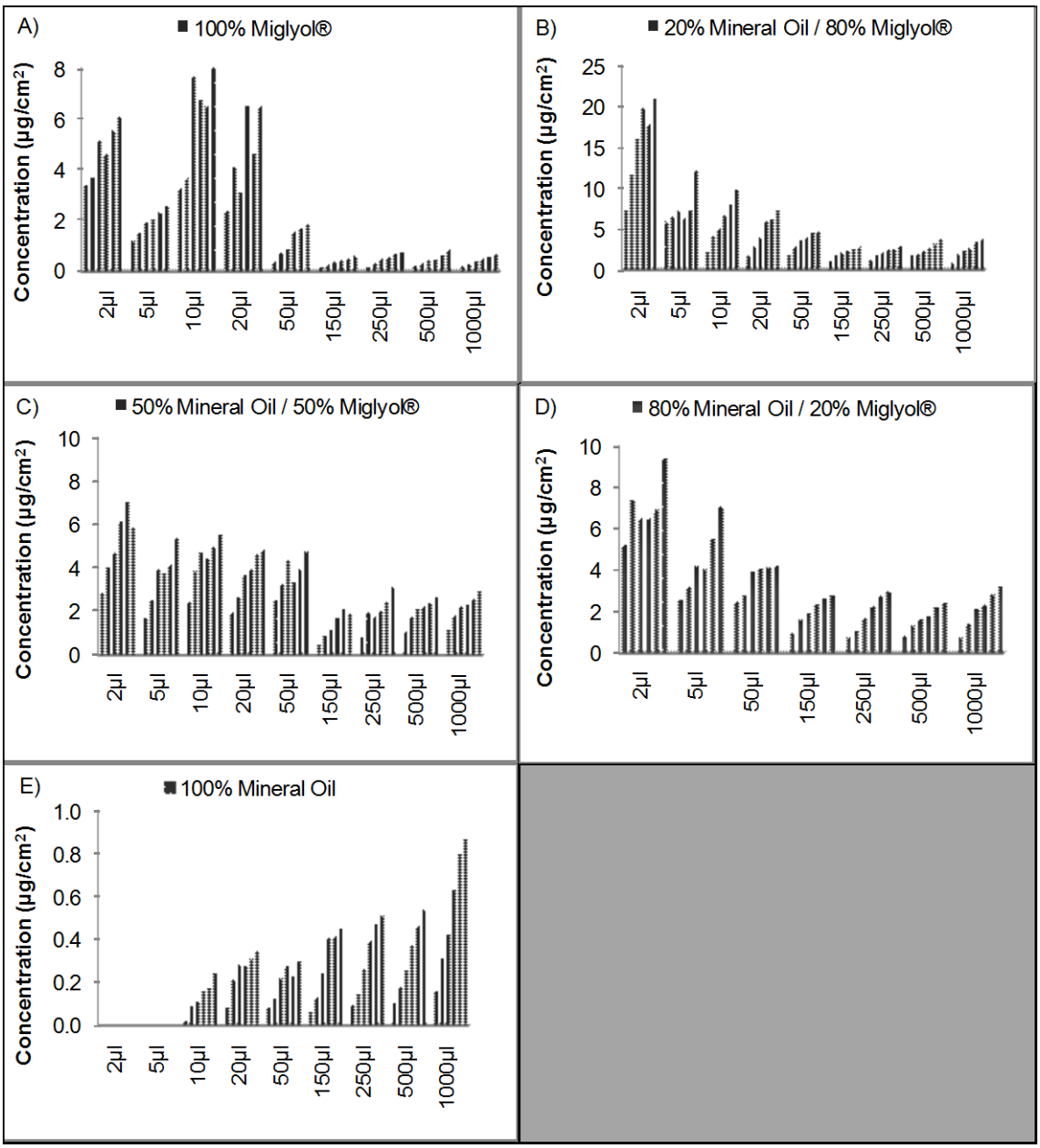

Figure A.8: Ibuprofen concentration (µg/ml) that diffused over 6 hours when finite and infinite doses were applied to Carbosil® membrane by using binary solvents of mineral oil and Miglyol® as delivery vehicles. The standard deviation for all finite dose applications was in all instances less than 1.0 and for infinite dose applications less than 0.2.

When the delivery solvent 50/50 mineral oil/Miglyol® the absorption from finite dose condition was higher compare to infinite dose conditions.

Using 80/20 (v/v) mineral oil/Miglyol® solvent as the delivery vehicle the permeant absorption from finite dose applications was much higher compared to infinite dose applications. Again this can be due to the effect of membrane hydration in the case of infinite dose conditions which decrease permeation of lipophilic compounds through the membrane. With finite dose

conditions the effect of hydration of the membrane is less and it is easier for a lipophilic drug like ibuprofen to penetrate the membrane and as a result we see higher levels of penetration of the active with finite dose applications (Chen *et al*., 2011:231).

When 100% mineral oil was applied as the sole solvent vehicle, it was found that the absorption concentration is much lower compared to the binary solvents used. The solubility of ibuprofen in mineral oil is very low and for this reason is the concentration active diffused through the membrane measured not to be more than 1µg/cm^2. The concentration of permeant that diffused through the membrane increase as the dose applied increase. Mineral oil is the only solvent used that show this permeation profile for all others the permeation levels were higher for finite dose applications. With solvent type permeant preparations applied to a membrane, three types of penetration-influencing parameters should be taken into account: (a) thermodynamic effects resulting from different permeant solubility's in the different vehicles; (b) penetration enhancing effect between the vehicle and the membrane; c) permeant depletion in the vehicle in the case of finite dose conditions. The extent of permeant depletion in the vehicle depends on the thickness of the applied solvent layer on the surface of the membrane (Leopold, 1998:166). Mineral oil does not show good penetration enhancement effect with finite dose applications and the reason for the decrease in ibuprofen absorption through the membrane with finite dose conditions can be due to the thin application layer and as a result permeant depletion in the solvent.

A.4.5 Permeation enhancement effect of the single and binary phase solvents

A.4.5.1 Propylene glycol and water

Figure A.9 illustrates the concentration of ibuprofen (µg/cm^2) that diffused from the propylene glycol/water vehicles through the Carbosil® membrane after 6 hours when different modes of application were used.

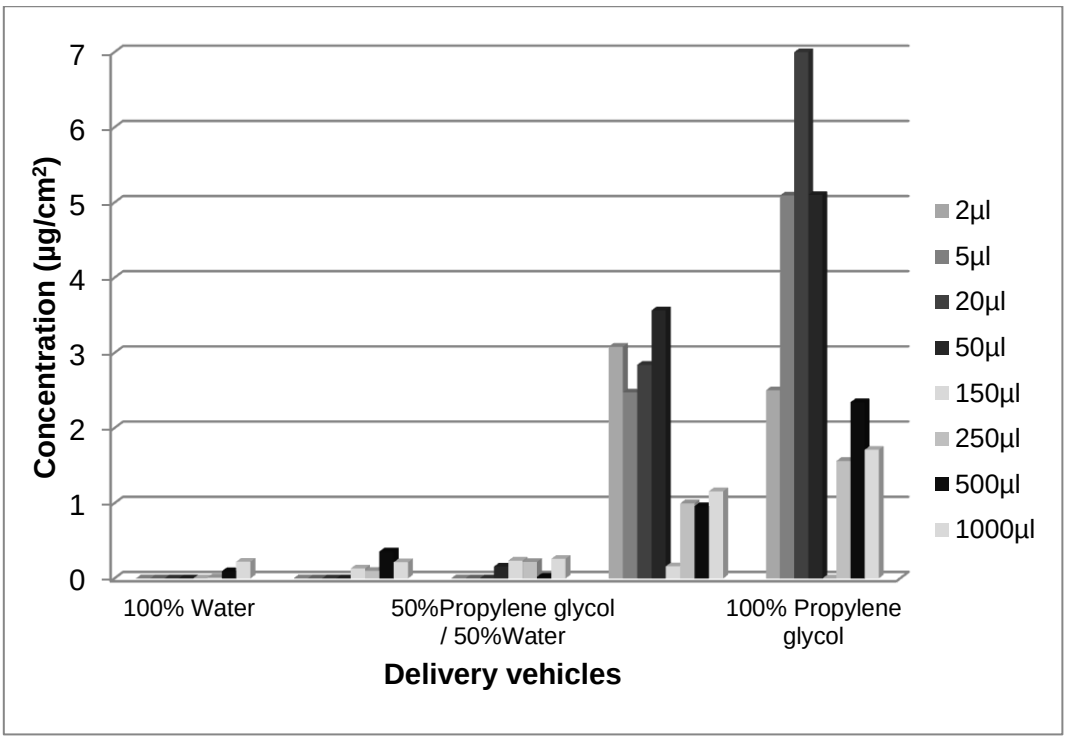

Figure A.9: Comparison of ibuprofen concentration (µg/cm^2) that diffused through the Carbosil® membrane from single and binary phase solvents of propylene glycol/water after application of different volumes after 6 hours.

It is clear from Figure A.9 that the concentration ibuprofen diffused increase as the amount of propylene glycol in the delivery vehicle increased. This is due to the mechanism of action of propylene glycol to partition into the membrane and increase permeant solubility in and diffusion through the membrane (Squillante *et al.,* 1998:266). It is also clear from the obtained permeation results of propylene glycol/water vehicles that the higher levels of diffusion of the active through the membrane was measured when finite dose volumes was applied in 100% propylene glycol as the carrier vehicle. This is the opposite of what Trottet *et al.* (2004:215) found. Trottet *et al.* (2004:215) showed a correlation between the amount of propylene glycol dosed on the skin and the amount of drug permeated. From these results Trottet *et al.* (2004:215) suggest that when finite dose applications are used propylene glycol will show substantial permeation through the membrane and the active showed relatively small permeation, which strongly suggests that the low permeation levels of the active is due to depletion of propylene glycol at the surface of the membrane. Results from this study showed that the finite dose applications with high levels of propylene glycol in the delivery vehicle showed the highest levels of permeation through the membrane. Because of the lipophilic nature of ibuprofen it is difficult to penetrate highly hydrated membranes (Chen *et al.,* 2011:231). When infinite doses are applied to the membrane a larger surface area is covered by the vehicle and the layer of vehicle solvent on the surface of the membrane will be thinker which results in higher hydration of the membrane. Also water is the most natural penetration

enhancer (Roberts & Walker, 1993:15). By soaking the membrane in water the humidity within the membrane will increase.

With finite dose applications the hydration of the membrane is less because of a smaller area of the membrane that is covered by the solvent solution and the layer on the surface of the membrane is thinner as this result in lower levels of hydration which makes it easier for lipophilic drugs to penetrate the membrane.

A.4.5.2 Miglyol® and mineral oil

In Figure A.10 the concentration (µg/cm^2) ibuprofen that diffused through the membrane from mineral oil and Miglyol® vehicles is illustrated.

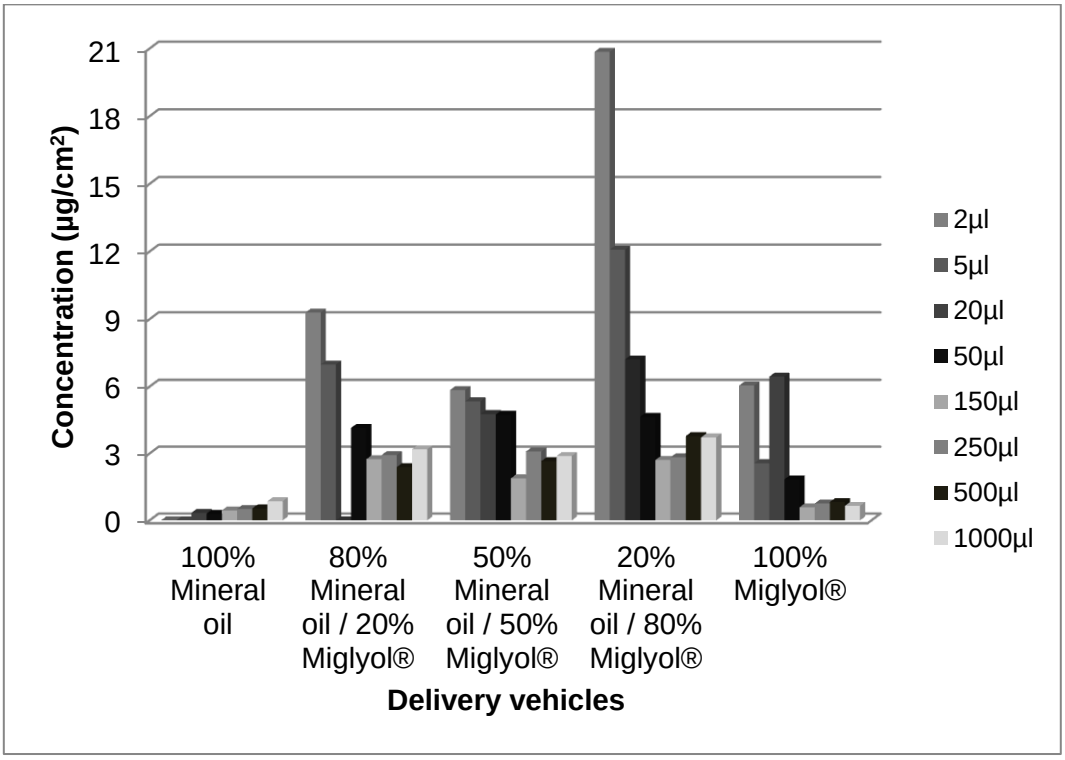

Figure A.10: Comparison of ibuprofen concentration (µg/cm^2) that diffused through the Carbosil® membrane from single and binary phase solvents of Miglyol®/mineral oil after application of different volumes after 6 hours.

From Figure A.10 it is clear that 100% mineral oil showed the lowest penetration enhancement effect compared to all other enhancer vehicles. The solubility of ibuprofen in mineral oil is the lowest. This could be the reason for the low levels of diffused ibuprofen from this vehicle. The binary solvent of 20/80 (v/v) mineral oil/Miglyol® showed the highest penetration enhancement effect compare to other solvents. This 20/80 (v/v) combination of the two penetration enhancer solvents could have a synergistic effect if used in combination with one another.

Miglyol® is known to modify the intercellular lipids of the stratum corneum in order to disrupt the barrier of the stratum corneum and increase the diffusivity through the membrane (Moser *et al*., 2001:105), while mineral oil carry active compound through the membrane (Hori *et al.,* 1991:33). Similar to the propylene glycol permeation profile the permeation of ibuprofen through the membrane is found to be higher from finite dose applications compare to infinite dose application and can also be explained due to the effect of hydration of the membrane and the lipophilic nature of the drug.

A.4.6 Conclusion

From the finite and infinite dose results it is clear that with increased levels of a penetration enhancer solvent like propylene glycol in the delivery vehicle that not only will the penetration of the active through the membrane increase but it will also enhance penetration of the active through the membrane from finite dose applications. It is also clear that in the presence of a penetration enhancer, absorption through the membrane increase with finite dose applications compared to infinite dose applications.

Mineral oil was the only solvent that showed a different permeation profile with increased permeation through the membrane as the volumes applied increased.

The potential of chemicals to cross the skin is an issue of increasing interest, especially in risk assessment studies. From the results above it is important to take the following characteristics into account when a person is exposed to a toxic substance: The first characteristic to consider is the concentration of the active (saturated or supersaturated), it is clear from the results above that the higher the concentration applied is the higher is the levels of permeant that penetrated the membrane. With infinite dose application the effect of evaporation causes the solvent to become supersaturated and the result is higher levels of diffusion through the membrane. Secondly, you have to take into account the vehicles used (penetration enhancer present), it is also clear from the results obtained that in the presence of a penetration enhancer the permeation of the active through the membrane is more significant even when small volumes are applied to the membrane. This fact is very important to take into account in risk assessment studies as well as in product development. Exposure to a small amount of a chemical in the presence of a penetration enhancer will have a significant effect on the permeation of the chemical through the membrane (skin). A third characteristic that should be looked at is the amount applied (membrane hydration), the amount of solvent applied to the membrane influence the degree of hydration of the membrane. Increased hydration will enhance the permeation of hydrophilic substances but it will decrease permeation of lipophilic substances like ibuprofen. This can have a positive and negative effect on permeation of toxic substances depending on the lipophilicity or hydrophilicity of the permeant. The two last characteristic is the

diffusion area and the time of exposure (Sartorelli *et al*., 2000:138). From the data obtained in this study it is clear that higher levels of the active compound permeated the membrane through a small diffusion area compared to the bigger diffusion area of infinite conditions. The results also showed that in some cases after one hour with finite dose applications higher concentrations of ibuprofen penetrated the membrane compared to concentrations after 6 hours from an infinite dose application. This illustrated that very short exposure time might have a significant effect on penetration of a compound through the membrane (skin).

The effect of finite dose conditions compared to infinite dose conditions on all the above characteristics should be explored further on human skin.

If the penetration enhancement effect of the different solvents was studies it is clear that the binary vehicle of 20/80 (v/v) mineral oil and Miglyol® showed the best penetration enhancement effect for finite dose and infinite dose conditions. Because of the lipophilic nature of these solvents it might be that when it was applied to the membrane it increased the hydration levels of the membrane and decreased the barrier function of the membrane. For this reason higher levels of the lipophilic drug (ibuprofen) penetrated the membrane (Chen *et al.,* 2011:231). The results can also be explained by the synergistic action of the combination of the two enhancers.

The two solvents work in synergy with each other to enhance penetration through the membrane. Miglyol®'s main mechanism of action is to modify the intercellular lipids of the stratum corneum in order to disrupt the barrier of the stratum corneum and increase the diffusivity through the membrane (Moser *et al*., 2001:105). While mineral oil which is part of the hydrocarbon group will enhance penetration through the membrane by partitioning into the membrane and carrying the active through the membrane (Hori *et al.,* 1991:33).

Propylene glycol is widely used as a penetration enhancers or a vehicle for penetration enhancers. When used with other penetration enhancers it shows synergistic action. Previous data reports on the efficacy of propylene glycol as a penetration enhancer showed mixed results, evidence suggest that propylene glycol has very mild enhancement effect on molecules such as estradiol and 5 fluorouracil (Williams & Barry, 2004:611). Propylene glycol and water delivery vehicles used in this study showed lower penetration enhancement action compared to the mineral oil and Miglyol® vehicles. Results from this study showed that the penetration enhancement effect of propylene glycol increase as the level of propylene glycol increase in the solvent vehicle. The mechanism of action is to partition into the stratum corneum and increase solubility of the permeant in the membrane, which increase the flux of both the propylene glycol and the permeant (Trottet *et al.,* 2004:215).

It would be interesting to conduct further studies using human skin as the membrane and investigate more penetration enhancer vehicles used in combination with one another like ternary solvent vehicles.

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APPENDIX B

GUIDELINES FOR AUTHORS

INTERNATIONAL JOURNAL OF PHARMACEUTICS GUIDE FOR AUTHORS

B.1 The arrangement of full length papers should accord with the following:

B.1.1 Title

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

B.1.2 List of authors

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

B.1.3 Affiliation(s)

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

B.1.4 Abstract

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

B.1.5 Keywords

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

B.1.6 Corresponding author

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

B.1.7 Text

The text should be divided into main sections, such as the following: 1. Introduction; 2. Materials and methods; 3. Results, 4. Discussion; Acknowledgements; References; Figure legends; Tables and Figures. These sections must be numbered consecutively as indicated. Subdivisions of a section should also be numbered within that section, for example,

2.1. Materials; 2.2. Relative humidity measurement; 2.3. Sample preparation, etc.

B.1.8 Nomenclature

Standard nomenclature should be used throughout; unfamiliar or new terms and arbitrary abbreviations should be defined when first used. Unnecessary or ambiguous abbreviations and symbols are to be avoided. Data should be expressed in SI-units.

B.1.9 Figure legends, Table legends, Footnotes

Figure legends, tables and footnotes should be typed on separate sheets, lines double spaced. Footnotes, to be numbered consecutively in superscript throughout the text, should be used as little as possible.

B.2 References

B.2.1 Text citation

The Harvard system of citation must be used. References should be cited in the text within parentheses: where several citations are given within a single set of parentheses, they should be arranged in ascending order of year of publication; where more than one reference with the same year of publication is cited, they should be arranged in alphabetical order of the first authors' names. When referring to a work of more than two authors, the name of the first author should be given, followed by et al.

B.2.1.1 Examples of text citations

(Gesztes et al., 1988; Chestnut et al., 1989; Legros et al., 1990; Mhando and Li Wan Po, 1990; Korsten et al., 1991; Langerman et al., 1991, 1992a,b; Masters et al., 1991; Bonhomme et al., 1992; Kolli et al., 1992).

(Shaw et al., 1978; Nakano and Arita 1990b; Nakano et al., 1990a,b; Bone et al., 1992).

B.2.2 Reference list

All references cited in the text should be listed at the end of the paper (typed with double spacing) and assembled alphabetically. More than one paper from the same author(s) in the same year must be identified by the letters a b c, etc. placed after the year of publication.

References must consist of names and initials of all authors, year, title of paper, abbreviated title of periodical, and volume and first and last page numbers. 'Personal communication' and 'unpublished data' should be cited in the text only. Papers referred to as 'submitted for publication' must include the name of the journal to which submission has been made. Journal titles should be abbreviated according to the 'List of Serial Title Word Abbreviations' (available from International Serials Data System, 20, rue Bachaumont, 75002 Paris, France. ISBN 2-904939-02-8).

B.2.2.1 Example of arrangement in the reference list

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B.2.2.2 Examples of presentation for various types of publications

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Du Plessis, J., 1992. Topical liposomal delivery of biologically active peptides. Ph.D Thesis, Potchefstroom University for CHE, South Africa.

B.2.3 Use of digital object identifier (DOI)

The digital object identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document particularly "Articles in press" because they have not yet received their full bibliographic information.

The correct format for citing a DOI is shown as follows: doi:10.1016/j.ijpharm.2005.01.041

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

B.3 Figures and Tables

B.3.1 Figures

Line drawings (including graphs) should be drawn in black ink on white paper or on tracing paper with blue or faint grey rulings; graduation will not be reproduced. Lettering should be large enough to permit photographic reduction. If figures are not to be reduced, their format should not exceed 16 x 20 cm. Photographs (or half-tone illustrations) must be of good quality, submitted as black and white prints on glossy paper, and have as much contrast as possible. The magnification of micrographs should be indicated by a scale bar in the figure. Figures should be clearly marked on the reverse side with the number, orientation (top) and author's name; a soft pencil or a felt-tipped pen should be used for marking photographs. The illustrations should be numbered with Arabic numerals. The legends should be typed separately with double spacing.

Color illustrations should be submitted as original photographs, high-quality computer prints or transparencies, close to the size expected in publication, or as 35 mm slides. Polaroid color prints are not suitable. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge that these figures will appear in color on the web (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. For color reproduction in print, you will receive information regarding the total cost from Elsevier after receipt of your accepted article. The 2008 price for color figures is EUR 285 for the first page and EUR 191 for subsequent pages. For further information on the preparation of electronic artwork, please see http://www.elsevier.com/artworkinstructions.

Please note: Because of technical complications which can arise by converting color figures to 'grey scale' (for the printed version should you not opt for color in print) please submit in addition usable black and white prints corresponding to all the color illustrations.

B.3.2 Tables

All tables must be numbered consecutively (with Arabic numerals) and be cited in the text. Titles should be short but descriptive. Tables should be compiled on separate sheets, together with a legend and/or footnotes identified by superscripts a, b, c, etc. Do not use vertical lines and keep horizontal rules to a minimum.

APPENDIX C

GUIDELINES FOR AUTHORS
JOURNAL OF DRUG DELIVERY

C.1 About the Journal

C.1.1 Aims and Scope

Drug Delivery serves the academic and industrial communities with peer reviewed coverage of basic research, development, and application principles of drug delivery and targeting at molecular, cellular, and higher levels. Topics covered include all delivery systems and modes of entry, such as controlled release systems; microcapsules, liposomes, vesicles, and macromolecular conjugates; antibody targeting; protein/peptide delivery. Papers on drug dosage forms and their optimization will not be considered unless they directly relate to the original drug delivery issues. Published articles present original research and critical reviews.

C.1.2 Editors-in-chief

Alfred Stracher, The State University of New York, Brooklyn, NY, USA Vladimir Torchilin, Northeastern University, Boston, MA, USA.

C.2 Manuscript submission

All submissions should be made online at Drug Delivery's ScholarOne Manuscripts site. New users should first create an account. Once a user is logged onto the site, submissions should be made via the Author Centre. If you experience any problems with your submission or with the site, please contact ScholarOne support through the "get help now" link.

All submissions to the journal must include full disclosure of all relationships that could be viewed as presenting a potential conflict of interest. If there are no conflicts of interest, authors should state that there are none. This must be stated at the point of submission (within the manuscript, after the main text under a subheading "Declaration of interest", and, where available within the appropriate field on the journal's ScholarOne Manuscripts site).

Please see our full Declaration of Interest Policy for further information.

C.3 Manuscript preparation

C.3.1 File preparation and types

Manuscripts are preferred in Microsoft Word format (.doc files). Documents must be double-spaced, with margins of one inch on all sides. Tables and figures should not appear in the main text, but should be submitted as separate digital files and designated with the appropriate file type on ScholarOne Manuscripts. References should be given in Harvard style (see References section for example).

Manuscripts should be compiled in the following order: title page; abstract; main text; acknowledgments; Declaration of Interest statement; appendices (as appropriate); references; tables with captions (on separate pages); figures; figure captions (as a list).

Drug Delivery publishes the following manuscript types: Original papers, Reviews, Book, reviews.

C.3.2 Title page

A title page should be provided comprising the manuscript title plus the full names and affiliations of all authors involved in the preparation of the manuscript. One author should be clearly designated as the corresponding author and full contact information, including phone number and email address, provided for this person. Five key terms that are not in the title should also be included on the title page. The keywords will assist indexers in cross indexing your article. The title page should be uploaded separately to the main manuscript and designated as “title page” – not for review on ScholarOne Manuscripts.

C.3.3 Abstract

All original articles and reviews should start with an abstract of 250 or fewer words, summarizing the central core of knowledge that is the focus of the paper. The recommended format is as a structured abstract, with the following headings for an original article: context, objective, materials and methods, results, discussion and conclusion. For a review article, it should be structured as follows: context, objective, methods (including data sources, study selection and data extraction), results and conclusion. It should be written in an informative style permitting its use, without revision, by abstracting services, give essential details of research findings without further reference to the text, and avoid generalizations and nonessential information.

C.3.4 Main text

C.3.4.1 Original articles

The body of the article should include the following sections: introduction; methods; results; discussion; conclusions.

Introduction: This section should state the relevance and background to the study, and its rationale and purpose.

Methods: This section should include only information that was available at the time the plan or protocol for the study was being written. You should describe your selection of the observational or experimental participants, identify the methods, apparatus and procedures in sufficient detail to allow others to reproduce the results, and describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. Drug Delivery requires that studies involving humans, both volunteers and patients, or animals be approved by an institutional review board, in accordance with approved published guidelines, prior to actually performing the research and publishing the data. Details including clinical trial registration number must be provided in the methods section if research includes studies conducted on human volunteers.

Results: Present your results in logical sequence in the text, tables, and illustrations.

Discussion: This should include implications of the findings and their limitations, with reference to all other relevant studies and the possibilities these suggest for future research.

Conclusions: This must summarize the main paper. Ensure that extrapolations are reasonable and that conclusions are justified by the data presented, and indicate if the study design can be generalized to a broader study population.

C.3.4.2 Reviews

The body of a review article should be a comprehensive, scholarly evidence-based review of the literature, accompanied by critical analysis and leading to reasonable conclusions. Wherever appropriate details of the literature search methodology should be provided, i.e. the databases searched (normally Medline and at least one or two other databases), the search terms and inclusive dates, and any selectivity criteria imposed.

Wherever possible, use primary resources, avoiding “Data on File”, “Poster” or other unpublished references.

C.3.4.3 Acknowledgments and Declaration of interest

Acknowledgments and Declaration of interest sections are different, and each has a specific purpose. The Acknowledgments section details special thanks, personal assistance, and dedications. Contributions from individuals who do not qualify for authorship should also be acknowledged here.

Declarations of interest, however, refer to statements of financial support and/or statements of potential conflict of interest. Within this section also belongs disclosure of scientific writing assistance (use of an agency or agency/ freelance writer), grant support and numbers, and statements of employment, if applicable. For a more detailed list of points to include, please see “Declaration of Interest section” below.

C.3.4.4 Acknowledgments

Any acknowledgments authors wish to make should be included in a separate headed section at the end of the manuscript preceding any appendices, and before the references section. Please do not incorporate acknowledgments into notes or biographical notes.

Declaration of Interest section: All declarations of interest must be outlined under the subheading “Declaration of interest”. If authors have no declarations of interest to report, this must be explicitly stated. The suggested, but not mandatory, wording in such an instance is: The authors report no declarations of interest. When submitting a paper via ScholarOne Manuscripts, the ”Declaration of interest” field is compulsory (authors must either state the disclosures or report that there are none). If this section is left empty authors will not be able to progress with the submission.

Please see our full Declaration of Interest Policy for further information.

Please note: for NIH/Wellcome-funded papers, the grant number(s) must be included in the Declaration of Interest statement.

C.3.4.5 References

References should be given in the Harvard style. Citation in the text is by author and date (Smith, 2001). The list of references appears alphabetically by primary author’s last name.

Examples

Journal: Iyengar BS, Dorr RT, Remers WA. (2004). Chemical basis for the biological activity of Imexon and related Cyanaziridines. *J Med Chem*, 47, 218-23.

Book: Vyas SP, Khar RK. (2001). Targeted and controlled drug delivery. New Delhi, India: CBS **Publisher and Distributor.**

Contribution to a Book: Chandrasekaran SK, Benson H, Urquhart J. (1978). Methods to achieve controlled drug delivery: The biomedical engineering approach. In: Robinson JR, ed. Sustained and Controlled Release Drug Delivery Systems. New York: Marcel Dekker, 557-93

Electronic Resources: Lin A-S, Shibano M, Nakagawa-Goto K, Tokuda H, Itokawa H, Morris-Natschke, SL, Lee K-H, (2007). Cancer Preventive Agents. 7. Antitumor-Promoting Effects of Seven Active Flavonolignans from Milk Thistle (*Silybum marianum*) on Epstein-Barr Virus Activation. *Pharm Biol* [Online] Available at: http://www.informapharmascience.com/doi/abs/10.1080/13880200701585592. Accessed on 12 April 2009.

Periodical abbreviations should follow the style given by Index Medicus.

C.3.4.6 Tables

Tables should be used only when they can present information more efficiently than running text. Care should be taken to avoid any arrangement that unduly increases the depth of a table, and the column heads should be made as brief as possible, using abbreviations liberally. Lines of data should not be numbered nor run numbers given unless those numbers are needed for reference in the text. Columns should not contain only one or two entries, nor should the same entry be repeated numerous times consecutively. Tables should be grouped at the end of the manuscript on separate pages.

C.3.4.7 Illustrations

Illustrations (line drawings, halftones, photos, photomicrographs, etc.) should be submitted as digital files for highest quality reproduction and should follow these guidelines:

300 dpi or higher Sized to fit on journal page EPS, JPG, TIFF, or PSD format only Submitted as separate files, not embedded in the text Legends or captions for figures should be listed on a separate page, double spaced.

For information on submitting animations, movie files and sound files or any additional information including indexes and calendars.

For information on color figures and charges.

C.3.5 Notes on style

C.3.5.1 General style

Authors are asked to take into account the diverse audience of the journal. Please avoid the use of terms that might be meaningful only to a local or national audience, or provide a clear explanation where this is unavoidable. However, papers that reflect the particularities of a social and cultural system are acceptable. Some specific points on style follow:

1. Authors should write in clear, concise US English. Language and grammar should be consistent with Fowler's English Usage; spelling and meaning of words should conform to *Webster's Dictionary*. If English is not your native language please ensure the manuscript has been reviewed by a native speaker. Please note: extensive rewriting of the text will not be undertaken by the editorial staff.
2. Latin terminology, including microbiological and species nomenclature, should be italicized.
3. Use standard convention for human and animal genes and proteins: italics for genes and regular font for proteins, and upper case for human products and lower case for animal products.
4. “US” is preferred to “American”, “USA” to “United States”, and “UK” to “United Kingdom”.
5. Double quotation marks rather than single are used unless the “quotation is ”within another”.
6. Punctuation of common abbreviations should adhere to the following conventions: “e.g.”; “i.e.”; “cf.”. Note that such abbreviations should not generally be followed by a comma or a (double) point/period.
7. Upper case characters in headings and references should be used sparingly, e.g. only the first word of paper titles, subheadings and any proper nouns begin upper case; similarly for the titles of papers from journals in the references and elsewhere.
8. Apostrophes should be used sparingly. Thus, decades should be referred to as follows: “The 1980s [not the 1980’s] saw …”. Possessives associated with acronyms (e.g. APU), should be written as follows: “The APU’s findings that …” but note that the plural is “APUs”.
9. All acronyms for national agencies, examinations, etc., should be spelled out the first time they are introduced in text or references. Thereafter the acronym can be used if appropriate, e.g. “The work of the Assessment of Performance Unit (APU) in the early 1980s …” and subsequently, “The APU studies of achievement …”, in a reference “(Department of Education and Science [DES] 1989a)”.

10. Brief biographical details of significant national figures should be outlined in the text unless it is quite clear that the person concerned would be known internationally. Some suggested editorial comments in a typical text are indicated in the following with square brackets: “From the time of H.E. Armstrong [in the 19th century] to the curriculum development work associated with the Nuffield Foundation [in the 1960s], there has been a shift from constructivism to heurism in the design of [British] science courses”.

11. The preferred local (national) usage for ethnic and other minorities should be used in all papers. For the USA, “African-American”, “Hispanic” and “Native American” are used, e.g. “The African-American presidential candidate, Jesse Jackson …”; for the UK, “Afro-Caribbean” (not “West Indian”), etc.

12. Material to be emphasized by italicization in the printed version should be italicized in the typescript rather than underlined. Please use such emphasis sparingly.

13. Numbers in text should take the following forms: 300, 3000, 30 000 (not 30,000). Spell out numbers under 10 unless used with a unit of measure, e.g. nine pupils but 9 mm (do not use full stops (periods) within units). For decimals, use the form 0.05 (not 05, × 05 or 0 × 05). “%” (not “per cent”) should be used in typescripts.

14. Appendices should appear before the references section and after any acknowledgments section. The style of the title is shown by the following example:

“Appendix C: The random network generator”. Figures and tables within appendices should continue the sequence of numbering from the main body of the text. Sections within appendices should be numbered, for example, C.1, C.2. Equations in appendices should be numbered, for example, (C 1), (C 2). If there is only one appendix, it is referred to as “the appendix” and not called “Appendix A”.

C.3.5.2 Abbreviations and nomenclature

For abbreviations and nomenclature, authors should consult the latest edition of the *CSE Style Manual* available from the Council of Science Editors, 60 Revue Drive, Suite 500 Northbrook, IL, 60062, USA.

C.3.5.3 Mathematics

Please click here for more information on the presentation of mathematical text.

C.3.5.4 Footnotes

Footnotes are not to be used except for designation of the corresponding author of the paper or current address information for an author (if different from that shown in the affiliation). Information concerning grant support of research should appear in a separate Declaration of interest section at the end of the paper. Acknowledgements of the assistance of colleagues or similar notes of appreciation belong in a separate Acknowledgements section.

Footnotes to tables should be typed directly below the table and are indicated by the following symbols: * (asterisk or star), † (dagger), ‡ (double dagger), ¶ (paragraph mark), § (section mark), || (parallels), # (number sign). Reinitialize symbol sequence within tables.

C.4 Editorial policies

C.4.1 Authorship

According to the International Committee on Medical Journal Ethics (ICMJE), an author is defined as one who has made substantial contributions to the conception and development of a manuscript. Informa Pharmaceutical Science adheres to the ICMJE guidelines (http://www.icmje.org/#author), which state that "authorship credit should be based on all of the following: 1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or advising it critically for important intellectual content; and 3) final approval of the version to be published"[1]. All other contributors should be listed as acknowledgements.

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C.4.2 Submission

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C.4.3 Peer review

Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication. Available at: http://www.icmje.org/ All manuscripts will be subjected to confidential peer review by experts in the field and, on the basis of reviewers' feedback papers, will be accepted unconditionally, accepted subject to revision or rejected.

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C.4.6 Declaration of interest

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If there are no declarations, authors should explicitly state that there are none. This must be stated at the point of submission (within the manuscript, after the main text, under a subheading "Declaration of interest", and within the appropriate field on the journal's ScholarOne Manuscripts site). Manuscript submission cannot be completed unless a declaration of interest statement (either stating the disclosures or reporting that there are none) is included.

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C.5 Additional information

C.5.1 Proofs

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