Influence of selected formulation factors on the transdermal delivery of ibuprofen

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Dedicated to:

Mohammed Iqbal my dad, Yasmin my mum, Rizana my sister, Prof Awie Kotze, Dr Joe Viljoen and Dr Jan Steenekamp

“It’s hard to beat a person who **NEVER GIVES UP**”

-Baberuth-

“It’s important to remember that we cannot become what we need to be, by remaining what we are”

-Max DePree-

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ABSTRACT

A pharmaceutical dosage form is an entity that is administered to patients so that they receive an effective dose of an active pharmaceutical ingredient (API). The proper design and formulation of a transdermal dosage form require a thorough understanding of the physiological factors affecting percutaneous penetration and physicochemical characteristics of the API, as well as that of the pharmaceutical exipients that are used during formulation. The API and pharmaceutical excipients must be compatible with one another to produce a formulation that is stable, efficacious, attractive, easy to administer, and safe (Mahato, 2007:11). Amongst others, the physicochemical properties indicate the suitability of the type of dosage form, as well as any potential problems associated with instability, poor permeation and the target site to be reached (Wells & Aulton, 2002:337). Therefore, when developing new or improved dosage forms, it is of utmost importance to evaluate the factors influencing design and formulation to provide the best possible dosage form and formulation for the API in question.

Delivery of an API through the skin has long been a promising concept due to its large surface area, ease of access, vast exposure to the circulatory and lymphatic networks, and non-invasive nature of the therapy. This is true whether a local or systemic pharmacological effect is desired (Aukunuru *et al.,* 2007:856). However, most APIs are administered orally as this route is considered to be the simplest, most convenient and safest route of API administration. Since ibuprofen is highly metabolised in the liver and gastrointestinal tract, oral administration thereof results in decreased bioavailability. Furthermore, it also causes gastric mucosal damage, bleeding and ulceration. Another obstacle associated with oral API delivery is that some APIs require continuous delivery which is difficult to achieve (Bouwstra *et al.,* 2003:3). Therefore, there is significant interest to develop topical dosage forms for ibuprofen to avoid side effects associated with oral delivery and to provide relatively consistent API levels at the application site for prolonged periods (Rhee *et al.,* 2003:14).

The aim of this study was to determine the influence of selected formulation factors on the transdermal delivery of ibuprofen. In order to achieve this aim, the physicochemical properties of ibuprofen had to be evaluated. The aqueous solubility, pH-solubility profile, octanol-water partition coefficient (log P-value) and octanol-buffer distribution coefficient (log D-values, pH 5 and 7.4) of ibuprofen were determined. According to Naik *et al.,* (2000:319) the ideal aqueous solubility of APIs for transdermal delivery should be more than 1 $mg.ml^{-1}$. However, results showed that ibuprofen depicted an aqueous solubility of 0.096 $mg.ml^{-1}$ ± 25.483, which indicated poor water solubility and would therefore be rendered less favourable for transdermal delivery if only considering the aqueous solubility. The pH-solubility profile depicted that ibuprofen was less soluble at low

pH-values and more soluble at higher pH-values. Previous research indicated that the ideal log P-values for transdermal API permeation of non steroid anti-inflammatory drugs (NSAIDs) are between 2 and 3 (Swart *et al.,* 2005:72). Results obtained during this study indicated a log P-value of 4.238 for ibuprofen. This value was not included in the ideal range, which is an indication that the lipophilic/hydrophilic properties are not ideal, and this might therefore; contribute to poor ibuprofen penetration through the skin. Furthermore, the obtained log D-values at pH 5 and 7.4 were 3.105 and 0.386, respectively. Therefore, it would be expected that ibuprofen incorporated into a formulation prepared at a pH of 5 would more readily permeate the skin compared to ibuprofen incorporated into a formulation prepared at a pH of 7.4.

A gel, an emulgel and a Pheroid™ emulgel were formulated at pH 5 and 7.4, in order to examine which dosage form formulated at which pH would deliver enhanced transdermal delivery. Obtained diffusion results of the different semi-solid formulations were furthermore compared to a South African marketed commercial product (Nurofen® gel) in order to establish if a comparable formulation could be obtained.

An artificial membrane was used to conduct the membrane permeation studies over a period of 6 h, in order to determine whether ibuprofen was in fact released from the formulations through the membrane. Skin permeation studies were conducted using Franz diffusion cells over a period of 12 h where samples were withdrawn at specified time intervals.

All the formulations exhibited an increase in the average cumulative amount of ibuprofen released from the formulations and that permeated the membrane when compared to Nurofen® gel. This increase was statistically significant ($p<0.05$) for the gel, emulgel and Pheroid™ emulgel at pH 7.4. The gel at pH 7.4 exhibited the highest cumulative amount of ibuprofen that permeated the membrane. Preparations formulated at a pH of 5, did not differ significantly from Nurofen® when the average cumulative amount of ibuprofen that permeated the membrane were compared. The following rank order for the average cumulative amount released from the formulations could be established: Gel (pH 7.4) >>>> Pheroid™ emulgel (pH 7.4) > Emulgel (pH 7.4) >>> Gel (pH 5)> Pheroid™ emulgel (pH 5) ≈ Emulgel (pH 5) > Nurofen® gel.

On the other hand, all the formulations exhibited an increase in the average cumulative amount of ibuprofen that permeated the skin when compared to Nurofen® gel. This increase was statistically significant ($p < 0.05$) for the gel, emulgel and Pheroid™ emulgel at pH 5, as well as the emulgel and Pheroid™ emulgel at pH 7.4. The emulgel at pH 5 exhibited the highest cumulative amount of ibuprofen that permeated the skin. The following rank order for the average cumulative amount released from the formulations and that permeated the skin could be established: Emulgel (pH 5) >> Pheroid™ emulgel (pH 5) > Gel (pH 5) > Emulgel (pH 7.4)> Pheroid™ emulgel (pH 7.4) ≈ Emulgel (pH 7.4) >> Nurofen® gel > Gel (pH 7.4). From this rank order it was clear that a trend was followed where the pH of formulation also played a role in ibuprofen permeation.

All the formulations exhibited a higher release rate and flux when compared to Nurofen® gel. This was statistically significant for the emulgel, gel and Pheroid™ emulgel at pH 7.4. The gel at pH 7.4 exhibited the highest release rate and flux. This was observed for the membrane and skin permeation studies. All the formulations (including Nurofen® gel) presented a correlation coefficient (r^2) of 0.972 – 0.995 for membrane permeation studies, and 0.950 – 0.978 for skin permeation studies; indicating that the release of ibuprofen from each of the formulations could be described by the Higuchi model. Furthermore, all the formulations exhibited a prolonged lag time compared to Nurofen® gel which indicated that the ibuprofen was retained for a longer time by the base. This was statistically significant ($p < 0.05$) for the emulgel at pH 7.4, the gel and Pheroid™ emulgel at pH 5. The gel at pH 7.4 exhibited a lag time closest to that of Nurofen® gel and this difference could not be classified as statistically significant ($p > 0.286$). This was observed for the membrane and skin permeation studies.

Nurofen® gel exhibited the highest ibuprofen concentration in the stratum corneum as well as in the epidermis followed by the gel at pH 7.4. However, results obtained for all the formulations indicated that topical as well as transdermal delivery of ibuprofen was achieved.

The pH of a formulation plays an important role with respect to API permeation. Ibuprofen is reported to have a pK_a value 4.4 (Dollery, 1999:I1); and by application of the Henderson-Hasselbach equation, at pH 5, 20.08% of ibuprofen will be present in its unionised form and at pH 7.4, 0.1% ibuprofen will exist in its unionised form. Since the unionised form of APIs is more lipid soluble than the ionised form, unionised forms of APIs permeate more readily across the lipid membranes (Surber & Smith, 2000:27). Therefore, it would be expected that ibuprofen formulated at pH 5 would be more permeable than formulations at pH 7.4. However, this did not correspond to the results (membrane studies) obtained in this study. It may be attributed to the solubility of ibuprofen in the different formulations. According to the pH-solubility profile of ibuprofen obtained in this study, it was more soluble at pH 7.4 than at pH 5. This was due to the fact that ibuprofen is a weak acidic compound, and for every 3 units away from the pK_a*-value*, the solubility changes 10-fold (Mahato, 2007:14). However, with regard to the skin permeation studies, enhanced permeation was obtained with the formulations prepared at pH 5. This was in accordance with Corrigan *et al.,* (2003:148) who stated that NSAIDs are less soluble and more permeable at low pH values, and more soluble and less permeable at high pH values. This was most probably due to the fact that unionised species, although possessing a lower aqueous solubility than the ionised species, resulted in enhanced skin permeation due to being more lipid-soluble.

Finally, stability tests on the different semi-solid formulations for a period of three months at different temperature and humidity conditions were conducted to determine product stability. The formulations were stored at 25 °C/60% RH (relative humidity), 30 °C/60% RH and 40 °C/75% RH. Stability tests included: mass variation, pH, zeta potential, droplet size, visual appearance, assay, and viscosity.

No significant change was observed for mass variation, pH, zeta potential and droplet size over the three months for any of the different formulations stored at the different storage conditions. In addition, no significant change in colour was observed for the gel and emulgel formulations at pH 5 and 7.4 over the three months at all the storage conditions. However, it was observed that the formulations containing Pheroid™ showed a drastic change in colour at all the storage conditions. This might have been due to oxidation of certain components present in the Pheroid™ system. Consequently, further investigation is necessary to find the cause of the discolouration and a method to prevent it.

The gel formulated at pH 5 depicted the formation of crystals. This might have been due to the fact that the solubility of ibuprofen was exceeded, leading to it precipitating from the formulation. A possible contributing factor to the varying assay values obtained during the study might have been due to non-homogenous sample withdrawal. On the other hand, no significant change was observed for the emulgel and Pheroid™ emulgel formulated at pH 5 and 7.4. The emulgel and Pheroid™ emulgel formulated at pH 5 depicted relative instability (according to the International Conference on Harmonisation of Technical Requirements For Registration of Pharmaceuticals for Human Use, ICH) only at 40 °C/75% RH with a change in ibuprofen content of more than 5% (6.78 and 6.46%, respectively). The gel, emulgel and Pheroid™ emulgel at pH 7.4 exhibited the least variation in ibuprofen concentration at all of the storage conditions. This might indicate that the pH at which a semi-solid formulation is produced will have a direct influence on the stability of the product.

No significant changes in viscosity (%RSD < 5) was observed for the gel and emulgel formulated at pH 7.4 and stored at 25 °C/60% RH. The remaining formulations at all of the specified storage conditions exhibited a significant change in viscosity (%RSD > 5) with a decrease in viscosity being more pronounced at the higher temperature and humidity storage conditions. A possible contributing factor to the change in viscosity over three months at the specified storage conditions might have been due to the use of Pluronic® F-127 (viscosity enhancer). This viscosity enhancer possesses a melting point of approximately 56 °C (BAST Corporation. s.a). The problem with this might have been the temperature (70 °C) at which the formulations were prepared. The higher preparation temperature might have caused the Pluronic® F-127 to degrade, thereby losing its ability to function appropriately.

A balance must be maintained between optimum solubility and maximum stability (Pefile & Smith, 1997:148). Despite the lower skin permeation of the gel formulated at pH 7.4, this formulation performed the best, as it was considered stable (least variation during the 3 month stability test) and the obtained tape stripping results showed that this formulation depicted the highest ibuprofen concentrations in the stratum corneum and epidermis. Thus, topical as well as transdermal delivery were obtained.

Keywords: Ibuprofen, physicochemical properties, transdermal diffusion, pH, solubility, Higuchi model, stability

UITTREKSEL

‘n Farmaseutiese doseervorm word beskou as ‘n eenheid wat aan pasiënte toegedien kan word om ‘n effektiewe dosering van ‘n aktiewe farmaseutiese bestanddeel (AFB) af te lewer. Die deeglike ontwerp en formulering van ‘n transdermale doseervorm vereis dat nodige kennis van die fisiologiese faktore wat penetrasie deur die vel beïnvloed en fisiese-chemiese eienskappe van die verkose AFB, asook die hulpstowwe van die formulering, verkry moet word. Beide die AFB en die hulpstowwe moet met mekaar verenigbaar wees om ‘n stabiele, doeltreffende, aanvaarbare, maklik-toedienbare en veilige produk te produseer (Mahato, 2007:11). Die fisies-chemiese eienskappe van ‘n AFB beïnvloed die keuse van ‘n doseervorm en kan ook ‘n aanduiding gee van moontlike struikelblokke wat geassosieer word met onstabiliteit, swak deurlaatbaarheid en die teikenarea wat bereik wil word (Wells & Aulton, 2002:337). Dit is dus van die uiterste belang om die faktore wat die ontwerp en formulering van nuwe of verbeterde doseervorms beïnvloed, te evalueer. Dit sal die gevolg hê dat die beste moontlike doseervorm ontwerp word vir ‘n spesifieke AFB.

Die toediening van ‘n AFB deur die vel dui op ‘n belowende konsep as gevolg van die oppervlakarea, gemak van toegang, wye blootstelling aan die sirkulasie- en limfatiese stelsels, en die nie-indringende aard van die terapie. Dit geld, ongeag of ‘n lokale of sistemiese farmakologiese effek vereis word (Aukunuru *et al.,* 2007:856). Die meeste AFBe word egter oraal toegedien omdat hierdie roete beskou word as die eenvoudigste, geriefliksfte en veiligste roete vir AFB aflewering. Aangesien ibuprofeen hoofsaaklik deur die lewer en gastro-intestinale stelsel gemetaboliseer word, word orale toediening van ibuprofeen geassosieer met verlaagde biobeskikbaarheid. Wat meer is, orale toediening van ibuprofeen veroorsaak skade aan die maagwand in die vorm van bloeding en ulserasie. ‘n Verdere struikelblok wat geassosieer word met die orale toediening van sommige AFBe is dat dit moeilik is om aanhoudende aflewering te verseker van sommige AFBe waarvan dit ‘n vereiste is (Bouwstra *et al.,* 2003:3). Dit is dus van betekenisvolle belang dat die ontwikkeling van topikale doseervorme vir ibuprofeen plaasvind, om so die newe-effekte geassosieer met orale toediening te vermy, asook om relatiewe konstante vlakke van die AFB op ‘n spesifieke area vir ‘n sekere periode te kan handhaaf (Rhee *et al.,* 2003:14).

Die doel van hierdie studie was om te bepaal wat die invloed van geselekteerde formuleringsfaktore op die transdermale aflewering van ibuprofeen sal wees. Om hierdie doel te bereik, is die fisies-chemiese eienskappe van ibuprofeen geëvalueer. Die wateroplosbaarheid, pH-oplosbaarheidsprofiel, oktanol-water-verdelingskoëffisiënt (log P-waarde) en oktanol-buffer-verdelingskoëffisiënt (log D-waardes, by pH 5 en 7.4) van ibuprofeen is bepaal. Volgens Naik *et al.,* (2000:319) is die ideale wateroplobaarheid vir 'n AFB in transdermale aflewering veronderstel om meer as 1 $mg.ml^{-1}$ te wees. Resultate het egter getoon dat ibuprofeen 'n wateroplosbaarheid van 0.096 $mg.ml^{-1}$ ± 25.483 gehad het. Hierdie waarde dui op swak wateroplosbaarheid, en dit is daarom ongeskik vir transdermale aflewering indien wateroplosbaarheid die enigste oorwegende faktor is. Die pH-oplosbaarheidsprofiel het daarop gedui dat ibuprofeen minder wateroplosbaar is by lae pH-waardes en meer oplosbaar is by hoër pH-waardes. Vorige navorsing, dui aan dat die ideale log P-waardes vir transdermale aflewering van nie-steroïed anti-inflammatoriese middels (NSAIMs) tussen 2 en 3 is (Swart *et al.,* 2005:72). Resultate verkry uit hierdie studie het egter getoon dat ibuprofeen 'n log P-waarde van 4.238 het. Hierdie waarde val nie binne die ideale grense nie, wat daarop dui dat die lipofiel-/hidrofiel-eienskappe nie ideal is nie en dus mag bydra tot swak deurlaatbaarheid deur die vel. Die verkrygde log D-waardes by pH 5 en 7.4 was 3.105 en 0.386, onderskeidelik. Dus kan daar verwag word dat ibuprofeen, wat geformuleer is by 'n pH van 5, meer geredelik deur die vel sal beweeg as 'n formulering wat by 'n pH van 7.4 geformuleer is.

'n Jel, emuljel en 'n Pheroid™-emuljel wat ibuprofeen as AFB bevat het, is geformuleer by pH's van beide 5 en 7, met die oog op evaluering van optimale transdermale aflewering van die AFB deur die doseervorm, asook die invloed van die pH daarop. Die diffusieresultate verkry vanaf die verskeie semi-soliede formulerings is verder ook vergelyk met 'n Suid-Afrikaans-bemarkte kommersiële produk (Nurofen® jel) om te bepaal of 'n vergelykbare of selfs verbeterde formulering verkry kon word.

Deurlaatbaarheidstudies deur kunsmatige membrane asook die vel is uitgevoer deur gebruik te maak van Franz-diffusieselle. 'n Kunsmatige membraan is gebruik om die membraan-deurlaatbaarheidstudies uit te voer oor 'n tydperk van 6 h, om te bepaal of ibuprofeen wel vanuit die formulerings vrygestel is en wel deur die membraan beweeg het. Veldeurlaatbaarheidstudies is oor 'n tydperk van 12 h uitgevoer.

Al die formulerings het 'n toename in die gemiddelde kumulatiewe hoeveelheid ibuprofeen, wat vanuit die formulerings vrygestel is, en gevolglik deur die membraan beweeg het, getoon, in

vergelyking met Nurofen® jel. Hierdie toename was statisties-beduidend ($p < 0.05$) vir die jel, emuljel en Pheroid™-emuljel by 'n pH van 7.4. Die jel by 'n pH van 7.4 het die hoogste kumulatiewe hoeveelheid ibuprofeen deur die membraan deurgelaat. Produkte wat by 'n pH van 5 geformuleer is, het nie 'n statisties-beduidende verskil getoon in die gemiddelde kumulatiewe hoeveelheid wat deur die membraan beweeg het nie, in vergelyking met Nurofen® jel. Die volgende rangorde vir die gemiddelde kumulatiewe hoeveelheid vrygestel vanuit die formulerings kon vasgestel word: Jel (pH 7.4) >>>> Pheroid™-emuljel (pH 7.4) > Emuljel (pH 7.4) >>> Jel (pH 5) > Pheroid™-emuljel (pH 5) ≈ Emuljel (pH 5) > Nurofen® jel.

Met betrekking tot die veldeurlaatbaarheidstudies was dit duidelik dat al die formulerings 'n toename in die gemiddelde kumulatiewe hoeveelheid ibuprofeen wat vrygestel is en deur die vel beweeg het, getoon het in vergelyking met Nurofen® jel. Hierdie toename was statisties-beduidend ($p < 0.05$) vir die jel, emuljel en Pheroid™-emuljel geformuleer by pH 5, asook die emuljel en Pheroid™-emuljel geformuleer by pH 7.4. Die emuljel geformuleer by pH 5 het die hoogste gemiddelde kumulatiewe hoeveelheid ibuprofeen deur die vel laat beweeg. Die volgende rangorde vir die gemiddelde kumulatiewe hoeveelheid vanuit die formulerings vrygestel, en wat deur die vel beweeg het, kon vasgestel word: Emuljel (pH 5) >> Pheroid™-emuljel (pH 5) > jel (pH 5) > Emuljel (pH 7.4) > Pheroid™-emuljel (pH 7.4) ≈ Emuljel (pH 7.4) >> Nurofen® jel > jel (pH 7.4). Uit hierdie rangorde is dit duidelik dat 'n neiging gevolg is waarin die pH van die formulerings ook 'n rol speel in die deurlaatbaarheid van ibuprofeen.

Vir beide die membraan- en veldeurlaatbaarheidstudies het al die formulerings 'n hoër vrystellingstempo en fluks getoon in vergelyking met Nurofen® jel. Dit was statisties-beduidend vir die emuljel, jel en Pheroid™-emuljel geformuleer by pH 7.4. Die jel geformuleer by pH 7.4 het die hoogste vrystellingstempo en fluks getoon. Al die formulerings (insluitend Nurofen® jel) het 'n korrelasiekoëffisiënt (r^2) van 0.972 – 0.995 getoon vir die membraandeurlaatbaarheid-studies en 0.950 – 0.978 vir die deurlaatbaarheidstudies deur die vel. Hierdie resultate is 'n aanduiding dat die vrystelling van ibuprofeen vanuit elk van die formulerings deur die Higuchi-model beskryf kon word. Al die formulerings het ook 'n verlengde vertragingstyd getoon in vergelyking met Nurofen® wat aangedui het dat ibuprofeen langer teruggehou is in die basis. Hierdie bevindinge was statisties-beduidend ($p < 0.05$) vir die emuljel geformuleer by pH 7.4, asook die jel en Pheroid™-emuljel geformuleer by pH 5. Die jel geformuleer by pH 7.4 het 'n vertragingstyd getoon wat die naaste aan dié van Nurofen® jel was ($p > 0.286$).

Deurlaatbaarheidstudies het getoon dat topikale sowel as transdermale aflewering met al die formulerings verkry is alhoewel Nurofen® jel die hoogste konsentrasie ibuprofeen in die stratum corneum getoon het, sowel as in die epidermis, gevolg deur die jel formulering by pH 7.4.

Die pH van 'n formulering speel 'n belangrike rol met betrekking tot AFB-deurlaatbaatheid. Daar is gerapporteer dat ibuprofeen 'n pK_a-waarde van 4.4 het (Dollery, 1999:I1); en deur die toepassing van die Henderson-Hasselbach-vergelyking, by pH 5 sal 20.08% van ibuprofeen voorkom in die ongeïoniseerde vorm en by pH 7.4 sal 0.1% van die ibuprofeen ongeïoniseerd wees. Aangesien die ongeïoniseerde vorm van AFBe meer lipied-oplosbaar is as die geïoniseerde vorm, sal ongeïoniseerde AFBe meer deurlaatbaar wees oor die lipiedmembrane (Surber & Smith, 2000:27). Dus kan daar verwag word dat wanneer ibuprofeen geformuleer word by pH 5, daar 'n verhoogde deurlaatbaarheid getoon sal word in vergelyking met ibuprofeen geformuleer by pH 7.4. Die teenoorgestelde is egter met die membraanstudies gesien. Dit kan toegeskryf word aan die oplosbaarheid van ibuprofeen in verskillende formulerings. Na aanleiding van die pH-oplosbaarheidsprofiel van ibuprofeen, bepaal in hierdie studie, is ibuprofeen meer oplosbaar by pH 7.4 as by pH 5. Dit kan toegeskryf word aan die feit dat ibuprofeen 'n swak suurverbinding is en vir elke 3 eenhede weg van die pK_a-waarde, verander die oplosbaarheid tienvoudig (Mahato, 2007:14). Met betrekking tot die veldeurlaatbaarheidstudies, het die formulerings by pH 5 beter ibuprofeendeurlaatbaarheid in vergelyking met die formulerings by pH 7.4 getoon. Dit stem ooreen met Corrigan *et al.,* (2003:148) wat verklaar het dat NSAIMs minder oplosbaar en meer deurlaatbaar is by lae pH-waardes, asook meer oplosbaar en minder deurlaatbaar by hoë pH-waardes sal wees. Dit kan moontlik toegeskryf word aan die feit dat alhoewel die ongeioniseerde spesie swakker wateroplosbaar is, dit beter deur die vel beweeg vanweë 'n hoër lipied-oplosbaarheid.

Laastens is stabiliteitstoetse op die verskillende semi-soliede formulerings uitgevoer oor 'n tydperk van drie maande, onder verskeie temperatuur- en humiditeitstoestande. Die formulerings is geberg by 25 °C/60% RH (relatiewe humiditeit), 30 °C/60% RH en 40 °C/75% RH. Stabiliteitstoetse sluit in: gehaltebepaling, pH, viskositeit, massaverandering, zeta-potensiaal, deeltjiegrootte en visuele voorkoms.

Geen beduidende veranderinge is waargeneem in massa, pH, zeta potensiaal en deeltjiegrootte oor die tydperk van drie maande by al die verskillende bergingstoestande nie.

Geen beduidende verskil in kleur is waargeneem vir die jel- en emuljel-formulering by pH 5 en 7.4 oor die drie maande by alle bergingstoestande nie. Die formulerings wat Pheroid™ bevat het, het egter ‘n drastiese verandering in kleur getoon by al die verskillende bergingstoestande. Dit kan wees as gevolg van oksidasie van sekere komponente teenwoordig in die Pheroid™-sisteem. Gevolglik is verdere ondersoek nodig om die oorsaak van verkleuring vas te stel, asook ‘n metode om dit te voorkom.

Die jel-formulering by pH 5 het die vorming van kristalle getoon. Dit mag toegeskryf word aan die feit dat die oplosbaarheid van ibuprofeen oorskrei is, wat kon lei tot presipitasie in die formulering. ‘n Moontlike bydraende faktor vir die wisselende gehaltebepalingswaardes (vir die jeformulering by pH 5) verkry in hierdie studie kon moontlik toegeskryf word aan nie-homogene monsteronttrekking, ‘n sekondêre gevolg van kristallisasie. Geen beduidende verandering is egter waargeneem in die emuljel en Pheroid™-emuljel geformuleer by pH 5 en 7.4 nie. Die jel, emuljel en Pheroid™-emuljel geformuleer by pH 7.4 het die minste variasie getoon in ibuprofeen-konsentrasie by alle bergingstoestande. Dit kan ‘n aanduiding wees dat die pH waarby ‘n semi-soliede formulering geproduseer is, ‘n groot invloed op die stabiliteit van die produk kan hê.

Geen beduidende verskille in viskositeit (%RSA < 5) is waargeneem by die jel en emuljel geformuleer by pH 7.4 en gestoor by 25 °C/60% RH nie. Die oorblywende formulerings by alle bergingstoestande het beduidende verskille getoon (%RSA > 5) met ‘n afname in viskositeit, wat meer merkbaar was by hoër temperatuur- en bergingstoestande. ‘n Moonlike bydraende faktor vir die verandering in viskositeit oor die drie maande by verskeie bergingstoestande mag die byvoeging van Pluronic® F-127 (viskositeitsverhoger). Hierdie viskositeitsverhoger het ‘n smeltpunt van ongeveer 56 °C (BAST Corporation. s.a). Die probleem in verband met die gebruik van Pluronic® F-127 kan moontlik wees dat die produkte geformuleer was by 70 °C, wat moontlik afbraak van hierdie viskositeitsverhoger kon veroorsaak het, met die verlies van sommige eienskappe.

‘n Balans moet gehandhaaf word tussen optimale oplosbaarheid en maksimum stabiliteit (Pefile & Smith, 1997:148). Ten spyte van minder deurlaatbaarheid van die jel geformuleer by pH 7.4, was die formulering meer stabiel by alle bergingstoestande (behalwe vir viskositeit by 30 °C/60% RH en 40 °C/75% RH). Dit was ‘n aanduiding dat die jel-formulering die beste presteer het, selfs in vergelyking met Nurofen® jel. Hierdie formulering het die hoogste konsentrasie ibuprofeen in die stratum corneum en epidermis getoon.

Sleutelwoorde: Ibuprofeen, fisies-chemiese eienskappe, transdermale diffusie, pH, oplosbaarheid, Higuchi-model, stabiliteit.

AIMS AND OBJECTIVES

To achieve an optimal response from a dosage form, an active pharmaceutical ingredient (API) should be delivered to its target site at a rate and concentration that minimises its side-effects and maximises its therapeutic effects (Mahato, 2007:29). Absorption of an API is possible only when it is present in solution. API absorption is dependent on its lipid and aqueous solubility, type of formulation and the route of administration (Mahato, 2007:11). Thus, prior to the development of new or improved dosage forms, a thorough understanding of the physiological factors affecting percutaneous penetration and the physicochemical properties of the API, as well as the compatibility of the formulation excipients is essential. This eliminates problems associated with stability and poor *in vivo* dissolution, leading to the formation of a stable, efficacious, easy to administer and safe pharmaceutical dosage form (Mahato, 2007:11, Wells & Aulton, 2002:337). Therefore, the aim of this study was to determine the influence of selected formulation factors on the transdermal delivery of ibuprofen.

In order to achieve the aim of this study, the following objectives were set:

- Validation of a high performance liquid chromatography (HPLC) method was conducted to determine the concentration of ibuprofen in the different semi-solid formulations and diffusion samples.
- The physicochemical properties of ibuprofen, e.g., aqueous solubility, log P (octanol-water distribution coefficient) and log D (octanol-buffer distribution coefficient), were determined.
- The effect of pH on ibuprofen solubility was determined by performing a pH solubility profile.
- Pre-formulation studies were performed in order to establish the appropriate components to be included in the different semi-solid formulations.
- Formulation of a gel, an emulgel and a Pheroid™ emulgel at pH 5.0 and 7.4, had to be performed in order to examine which preparation would deliver enhanced transdermal delivery.
- Membrane permeation studies had to be done to determine whether ibuprofen was in fact released from the different semi-solid formulations.
- Skin permeation studies had to be conducted to determine whether ibuprofen diffused through the skin after the application of the different semi-solid formulations.
- Tape stripping had to be done after completion of the skin permeation studies to determine whether topical or transdermal delivery of ibuprofen was achieved.

- Obtained diffusion results of the different semi-solid formulations had to be compared to a South African marketed commercial product (Nurofen® gel) in order to establish if a comparable formulation could be obtained.
- Stability tests on the different semi-solid formulations for a period of three months at different temperature and humidity conditions had to be conducted to determine product stability. The formulations were stored at 25 °C/60% RH (relative humidity), 30 °C/60% RH and 40 °C/75% RH. Stability tests included: assay, pH, viscosity, mass variation, zeta potential, droplet size and visual appearance.

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CHAPTER 1
FACTORS INFLUENCING TRANSDERMAL DRUG DELIVERY

1.1 INTRODUCTION

Controlled delivery of active pharmaceutical ingredients (APIs) into the body is one of the fundamental research topics in the pharmaceutical field. Most APIs are administered orally as this route is considered to be the simplest, most convenient and safest route of API administration (Bouwstra *et al.,* 2003:2, York, 2002:7). However, ibuprofen administered orally is highly metabolised in the liver (first pass metabolism) and in the gastrointestinal tract (Bouwstra *et al.,* 2003:2) resulting in decreased bioavailability. Furthermore, it also causes gastric mucosal damage, bleeding, and ulceration (Rhee *et al.,* 2008:14). Another obstacle associated with oral API delivery is that some APIs require continuous delivery which is difficult to achieve (Bouwstra *et al.,* 2003:3). Therefore, there is significant interest to develop topical dosage forms for ibuprofen to avoid oral side effects and to provide relatively consistent API levels at the application site for prolonged periods (Rhee *et al.,* 2003:14). Besides the need for transdermal administration of APIs as an alternative route, the target site is also considered to be an important factor (Bouwstra *et al.,* 2003:3).

There are 3 fundamental target sites for topical and transdermal API delivery, namely: (1) the skin surface, (2) the skin itself (epidermis or dermis) or (3) the systemic circulation. The surface of the skin may be a target when considering disinfectants, insect repellents or cosmetics. Targeting the various layers of the skin is termed topical API delivery and is relevant when the disease state presents within the organ itself. For example, treating neoplasias, inflammatory disorders, and microbial infections of the skin.However, when the systemic circulation is the principal target, transdermal API delivery is considered as an alternative to conventional systemic and oral routes of administration (Morrow *et al.,* 2007:37).

Skin is the heaviest and most versatile organ of the body (Sanders *et al.,* 1999:168). It is one of the key sites for non-invasive delivery of therapeutic agents into the body (Foldvari, 2000:417). Transdermal API delivery offers an advantageous route of API administration by eliminating first pass hepatic metabolism and providing sustained API release for prolonged time periods. It is painless when compared to needles, and therefore, offers superior patient compliance (Karande & Mitragotri, 2009:2362). However, the task of API delivery can be relatively challenging owing to the impermeability of the skin (Foldvari, 2000:417). As the interface between the body

and the environment, the skin inhibits the outward transport of water and the inward movement of topically contacting substances (Sanders *et al.,* 1999:168, Potts *et al.,* 1992:14). A unique hierarchical structure of lipid-rich matrix (15 µm) with embedded keratinocytes in the upper strata of skin, namely the stratum corneum (SC), is responsible for the barrier properties of the transport of hydrophilic substances (Karande & Mitragotri, 2009:2363, Saino *et al.,* 2010:444). The viable epidermis, situated below the stratum corneum is much more aqueous in nature and represents a significant barrier for highly lipophilic substances (Saino *et al.,* 2010:443).

At the surface of the skin, molecules interact with cellular debris; micro-organisms; sebum and other materials, which negligibly affect permeation (Barry, 2001:101). Prior to the uptake of these molecules by the blood vessels in the dermis, they dissolve in the stratum corneum and then diffuse through the remaining sub-layers of the epidermis and dermis (Ghafourian *et al.,* 2010:28). There are three potential pathways by which molecules can transverse the stratum corneum (Hadgraft, 2001:1). These pathways are: (1) through the hair follicles with associated sebaceous glands, (2) via sweat ducts or (3) across the continuous stratum corneum between these appendages (Barry, 2001:101). However, these pathways are not restricted and it is likely that most molecules will permeate by a combination of these routes (Williams, 2003:31).The next section deals with these three pathways.

1.2 API PENETRATION PATHWAYS

As previously stated, there are basically three ways in which API molecules can transverse the intact stratum corneum. These include the transcellular route (over the cells), the intercellular route (between cells) and the transappendageal route (shunt route). In order for an API to permeate the skin, a combination of these routes may be used. These pathways contribute to the gross flux controlled by the physicochemical properties of the molecule (Morrow *et al.,* 2007:38).

1.2.1 TRANSCELLULAR ROUTE

APIs entering the skin via the transcellular route pass through the corneocytes. The corneocytes contain highly hydrated keratin which provides an aqueous environment for the transport of hydrophilic APIs (Morrow *et al.,* 2007:38). This accounts for the rapid diffusion of hydrophilic molecules through the keratinocytes. The keratin-filled cells are not in isolation (Williams, 2003:33). They are embedded by a lipid envelope which connects the cells to the interstitial lipids. Multiple lipid bilayers separate the keratinised cells. There are approximately twenty such lamella between each corneocyte. Hence, a molecule crossing the intact stratum

corneum via the transcellular route requires several partitioning and diffusion steps (Morrow *et al.,* 2007:38).

Subsequent to partitioning into and diffusing through the relatively aqueous corneocytes, the permeant must partition into the surrounding lipid envelope and then partition in and out of the multiple bilayers separating the corneocytes (Morrow *et al.,* 2007:38). The multiple bilayered lipids that the molecules must transverse between the keratinocytes remain the rate-limiting barrier for penetration via this route (Williams, 2003:33). Therefore, the physicochemical properties of the permeant will have an important influence on whether the transcellular route is the predominant route taken by the API to diffuse the skin (Morrow *et al.,* 2007:38).

1.2.2 INTERCELLULAR ROUTE

The intercellular pathway involves API diffusion through the continuous lipid matrix. This route is a significant obstacle, mainly for two reasons. Firstly, as depicted by the "brick and mortar" model of the stratum corneum, the intercellular route provides a more complex diffusional pathway than that of the relatively direct path of the transcellular route. Previous research has estimated that water travels approximately 50 times further via this route, compared to the transcellular route. Secondly, the intercellular domain is a region of alternating structured bilayers, causing an API to partition into, and diffuse through repeated aqueous and lipid domains. Small uncharged molecules penetrate the skin via this pathway (Morrow *et al.,* 2007:38).

1.2.3 TRANSAPPENDAGEAL ROUTE (SHUNT ROUTE TRANSPORT)

The continuity of the stratum corneum is interrupted by skin appendages and their associated paths (Gunther, 1982:30). Skin appendages provide a continuous channel directly across the stratum corneum barrier (Morrow *et al.,* 2007:38). Initially, skin appendages were not acknowledged to be a significant transdermal penetration route; as evidence suggested that it occupied approximately 0.1% of the skin surface area (Knorr *et al.,* 2009:173), thereby limiting the area available for penetration (Morrow *et al.,* 2007:38). However, the hair follicles represent invaginations which extend deep into the dermis. These invaginations increase the actual surface area available for penetration. With a rich perifollicular vascularisation and changes in the differentiation pattern along the follicular duct, the hair follicles possess distinct characteristics which favour penetration. Multiple studies suggest that the follicular route may be especially appropriate for hydrophilic and high molecular weight molecules; as well as particle-based API delivery systems (Knorr *et al.,* 2009:173).

Sweat ducts are either empty or actively secreting an aqueous salt solution. Although many APIs favour an aqueous pathway across the skin, permeation may be limited as sweat is travelling against the diffusion pathway of the permeant. Furthermore, sebaceous glands are filled with a lipid rich sebum, which may present a barrier to hydrophilic APIs (Morrow *et al.,* 2007:38).

Permeation pathways are followed according to some of the physicochemical characteristics of the API (table 1.1) (Williams, 2003:36).

Table 1.1: *Selection of permeation pathways according to physicochemical properties of API (Williams, 2003:36)*

MOLECULE	Log P	Preferred penetration route
Lipophilic	1-3	Intercellular
Highly lipophilic	>3	Intercellular
Hydrophilic	<1	Transcellular
Highly hydrophilic & charged	<1	Appendageal

Ibuprofen possesses a Log P value of 4 (Saino *et al.,* 2010:443). From table 1.1 it is therefore clear that this value indicates that ibuprofen would mainly penetrate the skin via the intercellular route.

1.3 FACTORS THAT AFFECT PERCUTANEOUS PENETRATION

For successful transdermal API delivery, candidates have to adhere to specific physicochemical constraints. Ideally, an API must possess both lipophilic and hydrophilic properties in order to effectively permeate the skin (Naik *et al.,* 2000:319). The main factors that affect the extent of API absorption from a formulation and the rate at which it is absorbed are broadly classified into two categories, i.e., physiological- and physicochemical factors (Potts *et al.,* 1992:23, Surber & Davis, 2002:432). The next sections will deal with these two categories.

1.3.1 PHYSIOLOGICAL FACTORS

1.3.1.1 SKIN AGE

Although ageing effects of normal skin on API delivery are negligible, there are important morphological, and hence, permeability differences between normal (mature) skin and that of a neonate (pre-term) (Williams, 2003:15). In contrast to a full term baby who has a stratum

corneumas effective as that of a mature adult, a pre-term baby can be born without a stratum corneum, or the stratum corneum may still be incompletely formed (Potts *et al.,*1992:23, Barry, 2002:510). The immature epidermal barrier results in a highly permeably skin, thereby increasing the susceptibility to the toxic effects of applied APIs and exogenous substances (Darlenski *et al.,* 2009:295). However, it may be advantageous in treating breathing difficulties with caffeine, or pain with buprenorphine through topical application instead of intravenous administration (Barry, 2002:510).

As skin ages, it is submitted to structural and functional variations. The quantity of topically applied APIs entering the systemic circulation is hindered by age-related alterations that occur beyond the skin membrane. Ageing skin results in a decreased blood flow which could, *in vivo*, reduce transdermal flux (Williams, 2003:14).

1.3.1.2 SKIN CONDITION

The defence mechanism of the body begins with intact, healthy skin (Barry 2002:566, Barry, 1983:130). Maintenance of the stratum corneum protects the body against both chemical and microbiologic attacks (Wickett & Visscher, 2006:S98). Chemical invasion damages the tissue by injuring the barrier cells, thereby increasing penetration (Barry, 1983:130). Severe injuries resulting in extensive tissue damage necessitate immediate coverage to aid repair and regeneration, in order to restore normal skin function (Balasubramani *et al.,* 2001:534). Within 3 days of injury, the skin constructs a temporary barrier that remains until the regenerating epidermis can form normal keratinising cells, resulting in reduced penetration (Barry, 2002:509). Conditions resulting in a thickened stratum corneum (with corns, calluses, warts), may also reduce permeability (Barry, 2002:510).

1.3.1.3 BODY SITE

Variations in coetaneous permeability around the body depend on the thickness and the nature of the stratum corneum, as well as the density of the skin appendages (Barry, 2002:510). An inverse relationship exists between the diffusion path length (h) and the partition coefficient (K) of a penetrant across the stratum corneum. This results in decreased permeability at anatomical sites where the stratum corneum is the thickest (Potts *et al.,* 1992:23). Equation 1.1 describes this relationship (Smith, 1990:7).

$$P = \frac{DK}{h} \quad \textbf{[1.1]}$$

Where:

- P is the permeability coefficient,
- D is the diffusion coefficient,
- K is the partition coefficient, and
- h is the membrane thickness.

Although site-to-site variation in permeability is complex, a generalised rank order of site permeabilities exists: genitals > head and neck > trunk > arm > leg. Thus, there is a clear scientific hypothesis for selecting the application site based on permeability. However, important to note is that the absorption rate for a specific substance varies significantly passing through identical skin sites in different individuals. It is therefore valuable to consider the regional variations in transdermal API absorption (Williams, 2003:16).

1.3.1.4 SKIN METABOLISM

Far from being a passive membrane for the permeation of APIs, skin possesses the capability of metabolising permeants (Surber & Davis, 2002:435, Potts *et al.,* 1992:24). It serves as a source of extra-hepatic metabolism for many xenobiotics and topically applied APIs through a wide range of oxidative, reductive, hydrolytic and conjugative reactions (Surber & Davis, 2002:435). However, the physicochemical properties of certain APIs themselves are not always optimal for transdermal API delivery, thereby rendering topical API administration unsuitable. This problem can be overcome by creating a prodrug of the API, which yields the desired physicochemical properties. Once the prodrug has overcome the skin barrier, it is metabolised to the parent compound of established safety and efficacy (Surber & Davis, 2002:435).

The driving force for absorption or transport of any penetrant is proportional to the concentration gradient of that penetrant within the skin. Any degradation of the penetrant to another species reduces the concentration of the penetrant species in the skin, and therefore, reduces the flux (Smith, 1990:27).

1.3.1.5 CIRCULATORY EFFECTS

In principle, variations in blood flow through the dermis can affect percutaneous absorption. Blood flow rate can only control the systemic appearance of the penetrant, if transport across the stratum corneum is highly rapid (Potts *et al.,* 1992:24). Thus, the length of time in which a penetrant remains in the dermis is reduced by an increase in blood flow, thereby raising the concentration gradient across the skin (Barry, 1983:137).

1.3.1.6 SPECIES DIFFERENCES

Mammalian skin obtained from different species displays significant differences in skin thickness, density of appendages (hair follicles and glands), vascularity and metabolic enzymes. Furthermore, different pharmacokinetics are expected at different skin regions in the same individual (Barry, 1983:138, Mills & Cross, 2006:219). In frequently-utilised laboratory rodents, the skin is significantly thinner at a specific site and possesses increased hair density compared to humans, resulting in increased and rapid penetration in mice, rats and rabbits. Furthermore, absorption through skin of hairless rodent species is often significantly higher than that seen when using human skin (Riviere, 1993:117).

1.3.1.7 SKIN HYDRATION

According to Riviere (1993:117), skin hydration is one of the most important factors affecting the rate and extent of percutaneous absorption. Adequate hydration of the stratum corneum is required for the important metabolic processes occurring in this layer. These processes include conversion of probarrier lipids to barrier lipids that maintain barrier function and the hydrolysis of desmosomes necessary for normal desquamation (Wickett & Visscher, 2006:S105). Upon hydration, the tissue softens, swells and wrinkles, resulting in an increase in transdermal API delivery (Williams, 2003:17). Significant skin hydration may occur as a result of (1) environments with a high relative humidity (>80%); (2) water diffusing from underlying epidermal layers; or (3) perspiration that accumulates after the application of an occlusive vehicle or dressing (Riviere, 1993:117, Barry, 2002:511).

1.3.1.8 API-SKIN BINDING

Percutaneous absorption may be retarded by a diffusing molecule binding to various components of the skin. However, if an API intended for transdermal delivery exhibits a strong tendency to bond to components of the stratum corneum, uptake into the circulation will be hindered. This may require the use of a higher applied dose, thereby enabling the level of mobile, freely diffusing species, to be increased. Since binding is generally reversible, the bound API acts as a local reservoir releasing API to replace the free species as the circulation clears it. This may be advantageous for topically applied APIs used in dermatology (Potts *et al.,* 1992:24).

1.3.1.9 TEMPERATURE

The stratum corneum is a unique and thin barrier to the permeation of molecules through the skin, as penetration depends on the translational movement of such molecules. However, the tissue is a poor heat insulator as heat can flow by contact between molecules (Barry, 1983:158). Thus, the

human body maintains a temperature gradient across the skin from approximately 37 °C inside to approximately 32 °C at the outer surface (Williams, 2003:18). Cutaneous blood flow increases when the environmental temperature exceeds body temperature, resulting in heat loss through the skin. In contrast, blood flow decreases or is totally shunted in cold temperatures to prevent surface heat loss (Riviere, 1993:118).

The stratum corneum is able to resist heat damage, enduring temperatures as high as 60 °C for several hours without serious alteration to its barrier properties. However, when heated above 65 °C, the horny layer suffers irreversible structural alterations (Barry, 1983:158).

1.3.2 PHYSICOCHEMICAL FACTORS

1.3.2.1 MOLECULAR STRUCTURE

Ibuprofen (IB) is a chiral Nonsteroid Anti-inflammatory Drug (NSAID) that exists in two enantiomeric forms, namely, the (R)(–) enantiomer anddexibuprofen(S)(+) enantiomer. The chemical structures are illustrated in figure 1.1 (Cilurzo *et al.,* 2010:71, Leising *et al.,* 1996:3S). Racemic ibuprofen contains both enantiomers in equal amounts, whereas dexibuprofen is the pure S (+) enantiomer (Leising *et al.,* 1996:3S).

(R)(-)-Ibuprofen **(S)(+)-Ibuprofen**

Figure 1.1: *The chemical structure of the R(-) and S(+) enantiomers of ibuprofen (Cilurzo et al., 2010:71, Leising et al., 1996:3S)*

Despite the fact that the pharmacological activity of ibuprofen resides in the S (+) enantiomer, it is usually administered as the racemate. The reason for this is that on oral administration of the racemate, extensive bioconversion of the pharmacologically inactive R(-) enantiomer to the active S(+) enantiomer occurs (Cilurzo *et al.,* 2010:71). Figure 1.2 represents the process of bioconversion (Williams, 1995:121).

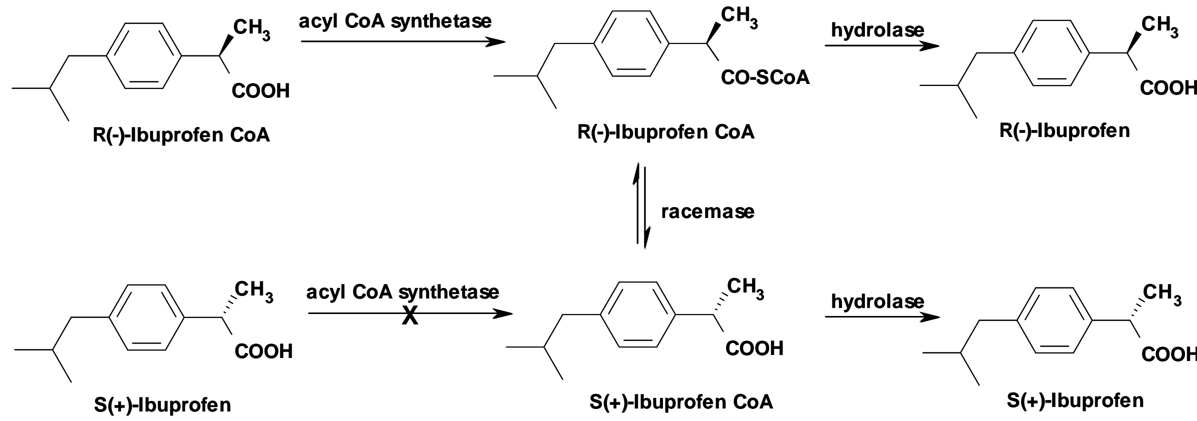


Figure 1.2: *Bioconversion of R(-) to S(+)-ibuprofen (Williams, 1995:121)*

The NSAID acyl CoA thioester is the main intermediate for the bioconversion of ibuprofen and the formation of thisthioester is stereospecific for the R(-) enantiomer. Racemic ibuprofen is subjected to*in vivo* metabolic inversion to the S(+) enantiomer via the formation, epimerisation, and hydrolysis of their respective acyl CoA thioesters. Stereoselective thioester formation (not stereoselectivity of the epimerisation or hydrolysis steps is involved) of (R)-ibuprofen CoA is responsible for the unidirectional inversion of R(-) to S(+) ibuprofen. However, S(+)-ibuprofen does not form its CoA thioester *in vivo* (Williams, 1995:120).

Administration of ibuprofen as the individual enantiomers causes the main metabolites to be isolated. These are the (+)-isomers irrespective of the configuration of the administered enantiomer. However, the R(-) enantiomer is inverted to the S(+) enantiomer *in vivo*, considering the observation that the two enantiomers are bioequivalent *in vivo* (Borne, 1995:561). The metabolism of ibuprofen is illustrated in figure 1.3 (Borne, 1995:562).

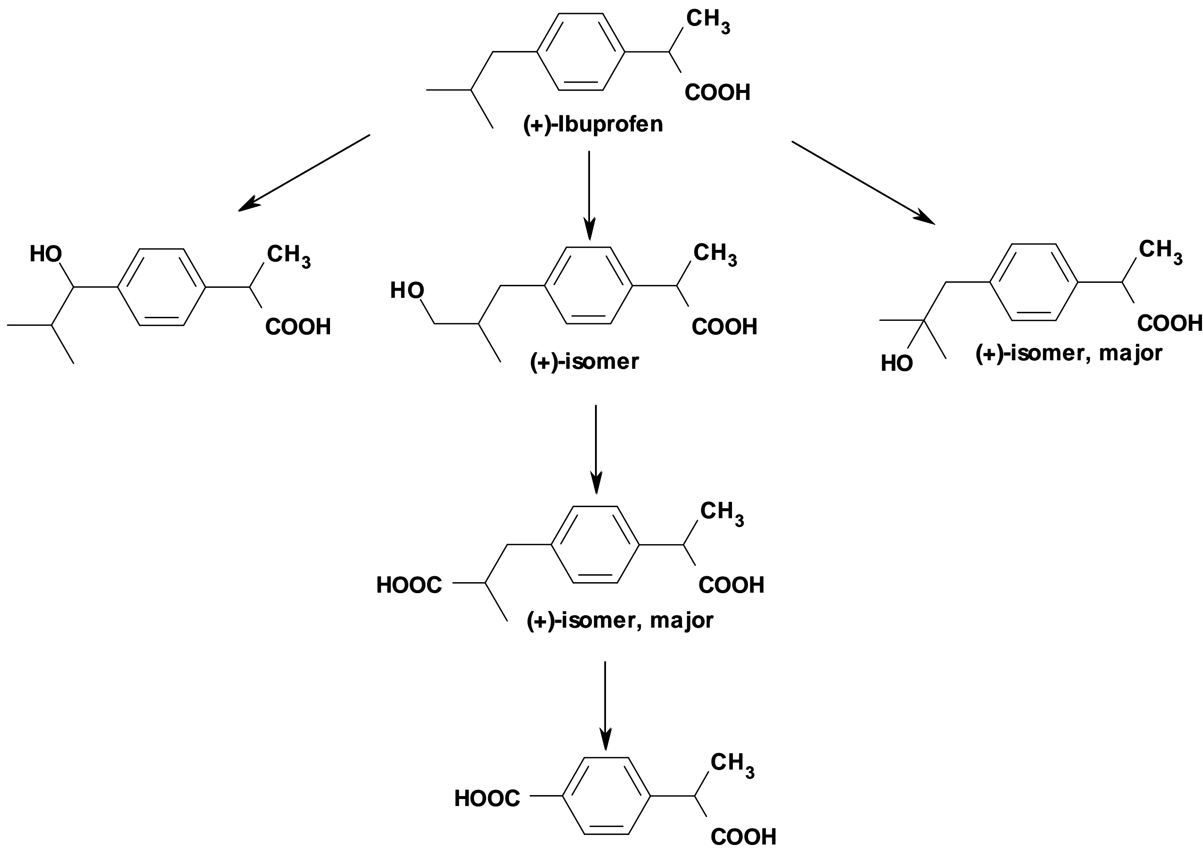


Figure 1.3: *Metabolism of ibuprofen (Borne, 1995:562)*

Topical administration of ibuprofen provides faster relief in comparison with the oral route as a result of low plasma concentrations and a lower incidence of systemic side effects. However, skin metabolism does not result in the chiral conversion of enantiomers. Therefore, the anti-inflammatory effect of topical ibuprofen is due to half the administered dose (Cilurzo *et al.,* 2010:71).

Selection of enantiomers is not only related to the intrinsic pharmacological activity of the API, but also to its physicochemical properties. The lower the melting point of the stereoisomers of a chiral compound, the higher the solubility in the vehicle and, consequently, the higher the flux through the skin (Cilurzo *et al.,* 2010:71). Dexibuprofen shows significantly higher solubility, higher dissolution rates, a lower melting temperature, and a different crystal structure when compared to the racemate (Leising *et al.,* 1996:3S). The melting point of dexibuprofen is approximately one third lower than the racemate and thus, it is expected that its flux through human skin would be higher than that of the racemate (Cilurzo *et al.,* 2010:71).

In this study, racemic ibuprofen(as indicated by the supplier, Shasun Pharmaceuticals, Puducherry, India) was incorporated into selected “topical” formulations. These formulations thus

contained equal amounts or concentrations of the two enantiomers; and any anti-inflammatory effect that these formulations would demonstrate, would be due to half the administered dose.

1.3.2.2 MELTING POINT

APIs with high melting points and high enthalpies of melting have relatively low aqueous solubilities at normal temperatures and pressures (Williams, 2003:37). Therefore, optimal transdermal penetration requires an API to possess a low melting point, which correlates to ideal solubility. An API with a melting point of less than 200 °C is a suitable candidate for transdermal delivery because of the higher aqueous solubility and dissolution rate it will possess(Barry, 2002:513). If the only consideration for ideal transdermal delivery was the melting point of the API, ibuprofen could have been rendered a suitable candidate, as it exhibits a melting point of 75 – 77 °C (Drug bank, 2012).

1.3.2.3 SOLUBILITY

As the concentration of the API substance in the vehicle increases, the quantity of API percutaneously absorbed per unit surface area per time interval also increases (Ansel & Popovich, 1990:309). However, for an API to be percutaneously absorbed, it must be in solution.Aqueous solubility determines the concentration of an API that is presented to the absorption site. The rate across the absorption site is strongly influenced by the partition coefficient (Ansel & Popovich, 1990:309). Steady-state flux of an API transversing the tissue is the product of the permeability coefficient and the applied concentration. Since lipophilic molecules permeate the skin faster than more hydrophilic molecules, solubility within the intercellular lipids can be correlated with the permeability coefficient for a homologous series of compounds. Although lipophilicity is a prerequisite for percutaneous penetration, some degree of solubility of the molecule in both oil and water is also considered essential for effective percutaneous absorption. Therefore, solutes with adequate solubility in mineral oil and water can effectively permeate the skin (Williams, 2003:37, Ansel & Popovich, 1990:309).

According to Naik *et al.,* (2000:319) the ideal aqueous solubility of APIs for transdermal delivery should be more than 1 $mg.ml^{-1}$. However, according to Mura *et al.,* (1998:190), the aqueous solubility of ibuprofen at 25 °C is 0.05 $mg.ml^{-1}$. Thus, ibuprofen is considered practically insoluble in water and would therefore be rendered unsuitable for transdermal delivery (British Pharmacopoeia, 2013).

Solubility of an API in a formulation is furthermore influenced by the presence of a co-solvent that alters the dissolution medium from a single solvent to a mixture of two or more chemicals that then

contain a more complex solvent environment. The majority of topical formulations are mixtures of several chemicals that would each influence the solubility of the API to be delivered. The influence of co-solvency is, therefore, an important basis in the formulation of topical delivery vehicles. A simple example of a co-solvent system is the addition of a relatively small fraction of propylene glycol to water. Propylene glycol has a dramatic effect on the solubilising potential of the aqueous system for lipophilic APIs. Lipophilic APIs would present a higher saturation solubility in the co-solvent system than in pure water and one would therefore expect API permeation rates to be proportionally higher from the co-solvent system. This concept assumes that the applied solvent interacts negligibly with the membrane with which it is in contact with (Surber & Smith, 2000: 27).

During this study, 20% propylene glycol was used as co-solvent in ibuprofen gel formulations. Based on the above conclusion of Surber and Smith (2000:27), ibuprofen would have a higher saturation solubility in the co-solvent system than in pure water and one would therefore expect ibuprofen permeation rates to be proportionally higher from the co-solvent system.

Once an API is in solution, it is able to diffuse through the skin. Diffusion is a passive kinetic process that occurs down a concentration gradient from a region of high diffusant concentration to one of lower concentration. The difference in concentration across the barrier membrane is considered as the driving force for the net movement of the APImolecules between the donor vehicle and the receptor environment. This concentration difference is estimated by subtracting the receptor-phase concentration (C_R) from the donor-phase concentration (C_D), if partitioning at either surface of the membrane is ignored (Surber & Smith, 2000:25).

1.3.2.4 DIFFUSION APPARATUS

In vitro permeation studies which determine the diffusion of an API through the skin may be performed using various types of diffusion apparatus in order to determine diffusant solubility. These range in complexity from a single two-compartment "static" diffusion cell to multijacketed "flow-through" cells. Static diffusion cells are usually of the upright ("Franz") or side-by-side type, with receptor chamber volumes of approximately 2 - 10 ml and surface areas of approximately 0.2 – 2 cm^2(Watkinson & Brain, 2002:199).

Side-by-side cells can be used for the measurement of permeation from one stirred solution, through a membrane, into another stirred solution. This is of particular advantage when examining flux from saturated solutions in the presence of an excess of API, if accumulation of the API on the membrane surface must be prevented. Upright cells are particularly useful for studying absorption from semi-solid formulations spread on the membrane surface; and are optimal for simulating *in vivo* performance. The donor compartments may be capped to provide occlusive conditions, or

left open, according to the objectives of the particular study. Receptor medium is manually removed at predetermined intervals (Watkinson & Brain, 2002:199).

Flow-through cells, on the other hand, can be useful when the permeant has a very low solubility in the receptor medium. Sink conditions are maximised as the receptor medium is continually replaced using a suitable pump (at a rate of approximately 1.5 $ml.h^{-1}$). These cells consist of an automated flow-through system which allows unattended sampling (Brain *et al.,* 2002:200).

In a biological system *(in vivo)* and in a flow-through diffusion cell system (*in vitro)*, the concentration of the diffusant on the lateral side of the barrier membrane always tends to be zero, as the molecules are instantly swept away by the solvent environment. This is termed "sink" - diffusion conditions, as the diffusant is not allowed to accumulate at the target location. Therefore, under sink-diffusion conditions, $C_R \rightarrow 0$ and $(C_D - C_R) \rightarrow C_D$. However, in a system using Franz cells, the diffusant concentration often increases considerably on the receptor side of the membrane and true sink-diffusion conditions may be destroyed. As the diffusant concentration in the receptor medium increases, the concentration difference across the membrane decreases and, theoretically, so does the permeant flux (Surber & Smith, 2000:25).

During this study, Franz diffusion cells were used in order to determine thetransdermal diffusion of ibuprofen.

1.3.2.5 DIFFUSION COEFFICIENT

Diffusion of molecular species through the stratum corneum is expressed as the diffusion coefficient, which is expressed in units of area/time, usually cm^2h^{-1} or cm^2s^{-1} (Williams, 2003:27). The ease with which a molecule is able to diffuse through a solvent system is described by the magnitude of its diffusion coefficient (*D*) in that medium. Physicochemical properties of the API; the respective diffusion medium; and the interaction between these two factors, determine the diffusion coefficient of an API present in the topical donor vehicle or in the membrane (Smith & Surber, 2000:28).

1.3.2.6 MOLECULAR SIZE

Considering that the stratum corneum is a compact membrane and that diffusing molecules follow a tortuous path through it, an inverse relationship exists between the diffusion coefficient and the molecular weight. This phenomenon may be described by equation 1.2 (Zatz, 1993:28).

$$D_m = D_m^0 MW^{-n} \quad \text{#} \qquad \textbf{[1.2]}$$

Where:

- D_m is the diffusion coefficient of the compound,
- D_m^0 and *n* are constants that depend on the nature of the membrane,
- *m* is a constant that depends on the membrane, and
- *MW* is the molecular weight.

Smaller molecules permeate faster than larger molecules (Barry, 2002:513). However, the specific effect of the size of the permeating molecule on the flux can only be determined when the effect of the size is withdrawn from the consequent change in solubility characteristics. Since the partition coefficient is so dominant, this process is difficult. It is even more complex to determine the effect of molecular shape on permeating molecules. Thus, nothing is known about this factor in skin permeability (Barry, 2002:513).

Permeability through human skin is predicted by equation 1.3, derived by Potts and Guy (1992:664) and described by Hadgraft (2001:11).

$$\log k_p = -2.7 + 0.71 \log K_{oct} - 0.0061 MW \qquad \textbf{[1.3]}$$

Where:

- K_p is the permeability coefficient (cm.h^{-1}),
- K_{oct} is the octanol/water partition coefficient, and
- *MW* is the molecular weight.

It is evident from equation 1.3 that as the lipophilicity of the API increases, its permeability increases due to improved partitioning into the skin (Hadgraft, 2001:11). APIs with a molecular weight ranging between 100 - 500 Dalton (Da) are suitable candidates for optimum transdermal API delivery (Williams, 2003:37). Ibuprofen possesses a molecular weight of 206 Da which lies within the optimum range and would therefore prefer the intercellular route of absorption (Saino *et al.,* 2010:443). Preferred routes of API absorption based on molecular weight are presented in table 1.2 (Ashford, 2002:243).

Table 1.2: Preferred routes of API absorption based on molecular weight (Ashford, 2002:243)

MOLECULAR WEIGHT	PREFERRED ROUTE OF ABSORPTION
< 200 Da and upto 400 Da	Intercellular
< 500 Da	Transcellular passive diffusion
>500 Da	Transcellular passive diffusion (absorption less efficient)

1.3.2.7 PARTITION COEFFICIENT

The partition coefficient (log P) is an indication of the lipophilicity of a molecule which can be used as a prediction of the ability to cross a biological membrane (Ashford, 2002:307). It is determined by its ability to partition between a suitable lipid-like solvent and water or an aqueous buffer at a constant temperature. Octanol is usually selected as the organic solvent to mimic the biological membrane, because of its similarities (Ashford, 2002:243).

Optimal transport through the skin requires an API to possess lipophilic, as well as hydrophilic properties. If an API is too hydrophilic, the molecule will be unable to transverse into the stratum corneum and if it is too lipophilic, the API will tend to remain in the stratum corneum layers (Naik *et al.,* 2000:319). Previous research indicated the ideal log P-values for transdermal API permeation of NSAIDs to be between 2 and 3 (Swart *et al.,* 2005:72). As previously mentioned, ibuprofen, possesses a log P value of 4 (Saino *et al.,* 2010:443). This value is not included in the ideal range, which is an indication that the lipophilic/hydrophilic properties are not ideal and this will contribute to poor ibuprofen penetration through the skin. The partition coefficient remains the most significant parameter affecting API absorption and permeation (Zatz, 1993:31). NSAIDs are less soluble and more permeable at low pH values, and more soluble and less permeable at high pH - values (Corrigan *et al.,* 2003:148).

The steady-state of molecules traversing biological membranes is defined as a solubility-diffusion process (Potts *et al.,* 1992:663). The product of the partition coefficient and the diffusion coefficient, divided by the diffusion path length yields the permeability coefficient (K_p) (Bosman, 1998:76). K_p relates solute flux to the concentration gradient across the membrane and is mathematically expressed by equation 1.4 (Potts *et al.,* 1992:663).

$$K_p = K_m . D_m . \partial^{-1} \quad \textbf{[1.4]}$$

Where:

- K_m is the membrane/water partition coefficient of the permeant,
- D_m is the permeant diffusivity within the membrane, and
- ∂ is the diffusion path length.

The membrane/water partition coefficient (K_m) is frequently substituted with the more readily accessibleoctanol/water partition coefficient (K_{oct}). Substitution occurs as a result of difficulty in measuring K_m (Potts *et al.,* 1992:663).

1.3.2.8 IONISATION

One of the most important factors influencing the formulation process is pH. The stratum corneum is exceptionally resistant to variations in pH, enduring a pH-range of 3 - 9 (Barry, 2002:511). Most APIs (but not all) are either weak acids or weak bases and their solubility in the aqueous phase of a given vehicle is determined by the state of ionisation (determined by the pH and pK_a) in that medium (Surber & Smith, 2000:27).

Unionised species of APIs are more lipid-soluble than ionised (hydrophilic) species, therefore, unionised molecules pass more readily across lipid membranes (Surber & Smith, 2000:27). However, ionised molecules do penetrate the stratum corneum, but to a limited extent. In saturated or nearly saturated solutions, these ionised molecules have a much higher aqueous solubility than neutral species, resulting in a significant contribution to the total flux (Barry, 2002:511). The total solubility, S_T, is the function of intrinsic solubility, S_0; and the difference between the molecule's pK_a and the pH of the solution. This is described by equation 1.5 and 1.6 for weak acids and weak bases, respectively (Mahato, 2007:14).

For a weak acid:

$$S_T = S_0\left(1 + 10^{pH - pKa}\right) \quad \textbf{[1.5]}$$

For a weak base:

$$S_T = S_0\left(1 + 10^{pka - pH}\right) \quad \textbf{[1.6]}$$

Weak acidic compounds may be solubilised at pH-values below their acidic dissociation constant (pK_a), whereas weak basic compounds may be solubilised at pH-values above their basic dissociation constant (pK_a). For every pH unit away from the pK_a, the solubility of the weak acid/base changes 10-fold. Therefore, solubility may be achieved as long as the pH of the formulation is at least 3 units away from the pK_a in the direction where the API becomes more ionised (Mahato, 2007:14). This means that for weak basic APIs, the pK_a value should decrease, where as for acidic APIs, the pK_a value should increase, in order to obtain increased solubility.Ibuprofen is a weak acidic compound with a pK_a value of 4.4 (Dollery, 1999:I1). Thus, at a pH of 7.4, it is expected that ibuprofen would be more soluble than at a pH of 5.This is in

accordance to Corrigan *et al.,* (2003:148) who stated that NSAIDs are less soluble and more permeable at low pH values, and more soluble and less permeable at high pH-values.

The percentage ionised weak acidic API present at a given pH is obtained from the Henderson-Hasselbalch equation (Shargel *et al.,* 2005:137) as follows:

$$pH = pKa + \log\left[\frac{ionised}{unionised}\right] \quad \textbf{[1.7]}$$

From equation 1.7, at pH 5, 20.08% of ibuprofen will be present in the unionised form and at pH 7.4, 0.1% ibuprofen will be present in the unionised form. If only percentage ionisation is taken into consideration for optimum skin permeability, then ibuprofen formulations at pH 5 would be most suitable. However, ibuprofen will be less soluble at pH 5 compared to at pH 7.4. This indicates that formulations at pH 5 may encounter instabilities such as crystallisation.Altering the pH of the formulation will influence the solubility and stability of weakly acidic or basic compounds (Mahato, 2007:14). Therefore, the pH for optimum solubility is not always the pH of maximum stability of the compound. A balance must be maintained between optimum solubility and maximum stability (Pefile & Smith, 1997:148).

1.3.2.9 HYDROGEN BONDING

Hydrogen bonds arise from electrostatic attraction between one hydrogen atom and one electron negative atom, such as oxygen (Buckton, 2002:110). An excess of hydrogen bonds within a molecule may hinder API absorption. API absorption in general is considered thorough when the molecule has no more than 5 hydrogen bond donors and no more than 10 hydrogen bond acceptors. The sum of the nitrogen and oxygen atoms in a molecule provides a rough indication of hydrogen bond acceptors (Ashford, 2002:295). Ibuprofen possesses only 2 hydrogen bond acceptors and 1 hydrogen bond donor, which would therefore indicate that it should be able to penetrate the stratum corneum with relative ease (Drug bank, 2012)

1.4 PENETRATION ENHANCERS

In an attempt to overcome the problems arising from skin permeability and biological variability, as well as to increase the number of API candidates suitable for transdermal API delivery, the use of penetration enhancers has been introduced to reversibly decrease the barrier resistance of the stratum corneum. Penetration enhancers are chemical compounds which are themselves pharmacologically inactive, but can partition into, and interact with stratum corneum constituents

when incorporated into a transdermal formulation. This results in reduced resistance to skin API diffusion. A penetration enhancer may also increase the thermodynamic activity of an API, thus initiating enhanced API flux (Suhonen *et al.,* 1999:149). A new approach to enhance the permeation of an API through the skin is known as Pheroid™ technology (Grobler *et al.,* 2008:283).

The Pheroid™ delivery system is a colloidal system that constitutes unique and stable lipid-based submicron- and micron-sized structures, called Pheroids. Pheroids are uniformly distributed in a dispersion medium that may be adapted to the indication it is needed for. The dispersed structures (dispersed phase) can be manipulated in terms of morphology, structure, size and function. Colloidal systems are used as carriers of APIs in order to enhance the efficacy of the administered compounds and to reduce unwanted side effects (Grobler *et al.,* 2008:284).

A Pheroid™ system consists mainly of modified essential fatty acids which are also inherent components of the skin. These fatty acids assist in the normalisation of the physiological micro-environment, resulting in added anti-inflammatory action, suppression of epidermal hyper-proliferation, normalisation of the water barrier of the skin, and fast and efficient API delivery (Grobler *et al.,* 2008:309).

During this study, the Pheroid™ system was incorporated into two emulgel formulations to determine whether it enhanced the delivery of ibuprofen though the skin.

1.5 MATHEMATICS CONCERNING SKIN PERMEATION

APIs are transported into and through the skin by a solution-diffusion process. The penetrant must dissolve in the skin, diffuse across it, and partition into the body fluids or tissues beneath the skin. Due to the extraordinary barrier that skin represents to most penetrants, diffusion across it is typically the slowest, and is therefore the rate-limiting step in the process (Smith, 1990:24). The mathematical description of the diffusion processes through the membranes is generally described by Fick's laws of diffusion (Rieger, 1993:38).

1.5.1 FICK'S FIRST LAW OF DIFFUSION

Flux (J) is defined as the total quantity of permeant that will diffuse through the membrane. This parameter is dependent on the surface area of the membrane to which the delivery vehicle containing the API is applied, as well as the total contact time of the delivery vehicle with the membrane (Surber & Smith, 2000:25). Equation 1.8 describes the flux (Martin, 1993:325).

$$J = \frac{dM}{S.dt}$$ **[1.8]**

Where:

- *J* is the flux, and
- *M* is the amount of material flowing through a unit surface area, *S*, of a barrier in unit time, *t*.

The flux in turn, yields Fick's first law of diffusion (equation 1.9) which states that the rate of transfer of diffusing substance through a unit area of a section is proportional to the concentration gradient $\frac{\partial c}{\partial x}$ (Martin, 1993:325).

$$J = -D\frac{\partial c}{\partial x}$$ **[1.9]**

Where:

- *J* is the flux ($cm^2.sec^{-1}$),
- *D* is the diffusion coefficient of the permeant ($cm^2.sec^{-1}$),
- *C* is the concentration ($g.cm^{-3}$),
- x is the distance in *cm* of movement perpendicular to the surface of the barrier, and
- $\frac{\partial c}{\partial x}$ is the concentration gradient.

The negative sign indicates that the diffusion occurs in a direction (the positive *x* direction) opposite to that of increasing concentration; i.e., this indicates that diffusion occurs in the direction of decreasing concentration of the permeant. Therefore, the flux is always a positive quantity (Martin, 1993:325).

1.5.2 FICK'S SECOND LAW OF DIFFUSION

It is normally assumed that diffusion is unidirectional when a topically applied permeant enters the skin, resulting in a concentration gradient from the outer surface into the tissue (Williams, 2003:41) This reduces the number of variables by one (Barry, 2002:506). Unidirectional diffusion is expressed mathematically by Fick's second law of diffusion (equation 1.10) which is derived from equation 1.9. (Williams, 2003:42).

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}$$ **[1.10]**

Where:

- *t* is time.

Thus, Fick's second law of diffusion states that the rate of change in concentration with time, *t*, at a point within a diffusional field is proportional to the rate of change in the concentration gradient at that point (Williams, 2003:42).

Fick's laws are more relevant if certain parameters are specified (Watkinson & Brain, 2002:65). The system imposed must be that of a well-designed diffusion experiment. The permeant must be at a high, fixed activity on one side of an inert homogenous membrane through which it diffuses into a sink on the other side. Prior to the start of the experiment, the membrane must be completely devoid of permeant (Watkinson & Brain, 2002:66). This indicates that the diffusive flow commences at the donor side which has a high concentration of permeant, where $C = 0$ and $x = h$ (h is the thickness of the membrane) at all time intervals *(t)*. Prior to the ingress of permeant, there is no diffusing material indicating that at $t = 0$, $C = 0$ for all values of x. Diffusion occurs in the direction of decreasing, x, towards the opposite side of the membrane where $x = 0$ and $C = 0$ (sink receptor phase) for all time intervals *(t)*. The cumulative mass *(m)* of permeant that diffuses through a unit area of a membrane in a time, *t*, is shown in equation 1.11 (Watkinson & Brain, 2002:65, Barry, 2002:572).

$$\frac{dm}{dt} = \frac{DC_0K}{h} \qquad \textbf{[1.11]}$$

Fick's second law has been expanded to equation 1.12 as time approaches infinity (Watkinson & Brain, 2002:69).

$$Q = C_o h\left[\frac{Dt}{h^2} - \frac{1}{6}\right] = \frac{DC_0}{h}\left[t - \frac{h^2}{6D}\right] \qquad \textbf{[1.12]}$$

From equation 1.12, time *(t*) can be solved if, $Q = 0$; yielding the value of the time axis intercept known as the lag time (figure 1.4). The lag time relates inversely to the diffusion coefficient and directly to the diffusional path length, as indicated by equation 1.13 (Watkinson & Brain, 2002:69).

$$t_{lag} = \frac{h^2}{6D} \qquad \textbf{[1.13]}$$

A constant-activity dosage form may not exhibit a steady-state process from the initial time of release. Figure 1.4 depicts steady state and non-steady-state regions. It is observed that the curve of this figure is convex to the time axis in the early stage and then becomes linear. The early stage is the non-steady-state condition. At a later stage, the rate of diffusion is constant, the curve is essentially linear, and the system is at steady state (Martin, 1993:328).

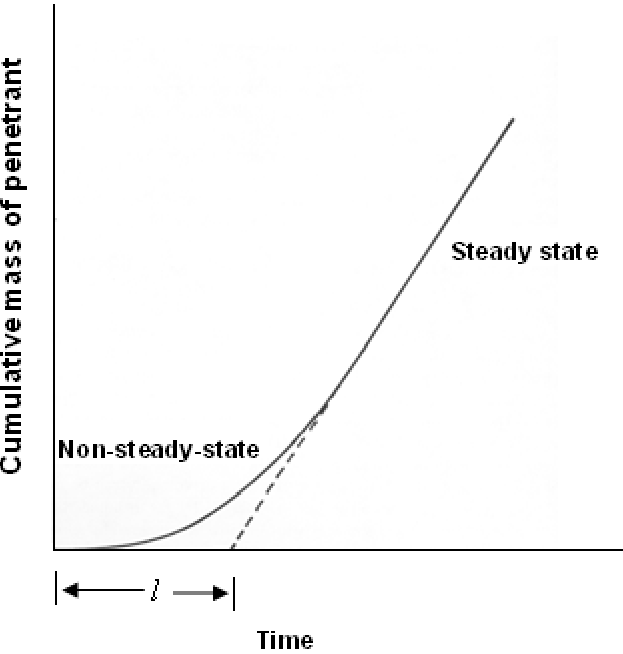


Figure 1.4: *Cumulative mass of penetrant diffusing across skin, as a function of time, showing an estimation of lag time (Martin, 1993:328)*

When equation 1.12 is distinguished relative to time, equation 1.14 is obtained which is possibly the most well-known form of Fick's law of diffusion that describes the flux, *J*, at steady state (Watkinson & Brain, 2002:69).

$$\frac{dQ}{dt} = J = \frac{DC_0}{h} \qquad \textbf{[1.14]}$$

It is often impractical to use the forms of equations 1.12 and 1.14 due to the fact that they include C_0 (the concentration of permeant in the outer layer of the membrane), that is extremely difficult to measure. The value C_0 is replaced with a term that links to the concentration in the vehicle $C_v$through the partition coefficient *K*. Equation 1.14 then rearranges to render equation 1.15 (Watkinson & Brain, 2002:70).

$$\frac{dQ}{dt} = J = \frac{DKC_v}{h} \qquad \textbf{[1.15]}$$

Frequently, particularly in biological membranes, there is a practical difficulty in measuring the diffusional pathlength. Due to this difficulty and the fact that information regarding the individual effects of changes in *K* and *D* values are often not required, a composite parameter (permeability coefficient) is normally used in equation 1.12 in order to obtain equation 1.16. The permeability coefficient *P*, is thus defined as (Watkinson & Brain, 2002:72):

$$J = PC_v \quad [1.16]$$

Equation 1.16 is probably the most basic and frequently used expression in the routine evaluation of membrane permeability. However, it should always be noted that the basis of this equation stipulates that the donor concentration is constant and that the diffusion has reached steady state. It is often difficult to assess when this period has been reached; and by only using this steady-state data, data collected at time points before this region can be lost. This obstacle is overcome by making use of, and fitting the entire set of diffusion data, using a non-linear curve-fitting software package (Watkinson & Brain, 2002:72).

1.5.3 HIGUCHI'S MODEL

Higuchi considered the release of an API from a thin ointment film into the skin under the following conditions (Siepmann & Peppas, 2011:7):

- API transport through the ointment base was rate-limiting, whereas API transport within the skin was rapid.
- The skin acted as a "perfect sink": the API concentration in this compartment was considered negligible.
- Initial API concentration in the film was significantly higher than the solubility of the API in the ointment base.
- The API was finely dispersed within the ointment base (the size of the API particles was significantly smaller than the thickness of the film).
- The API was initially homogeneously distributed throughout the film.
- Dissolution of API particles within the ointment base was rapid compared to the diffusion of dissolved API molecules within the ointment base.
- The diffusion coefficient of the API within the ointment base was constant and did not depend on time or the position within the film.
- Edge effects were negligible: the surface of the ointment film exposed to the skin was large compared to its thickness. A mathematical description of API diffusion could be restricted to one dimension.
- The medium (ointment base) did not swell or dissolve during API release.

The basic ideas of the above conditions resulted in the derivation of Higuchi's simple equation which is detailed in the following:

Exposure to perfect sink conditions allowed dissolved API molecules from the ointment base to diffuse into the skin. Initially, this occurred only close to the surface of the ointment film. API dissolution was rapid and a large excess of API was provided. Molecules that leached out of the

system were rapidly replaced by the (partial) dissolution of non-dissolved API particles located in this region. Thus, the concentration of dissolved API molecules within the ointment base remained constant, as long as non-dissolved API excess was provided (saturated solution). Only when all API particles located in the region next to the surface were finally dissolved, the concentration of dissolved API molecules in this region fell below saturation concentration. Due to concentration gradients subsequently, also dissolved API molecules located further away from the film's surface diffused through the ointment base into the skin. It is important to note that the concentration of dissolved API molecules in this newly concerned region remained constant (saturated concentration) as long as non-dissolved API excess was provided in that region (Siepmann & Peppas, 2011:7).

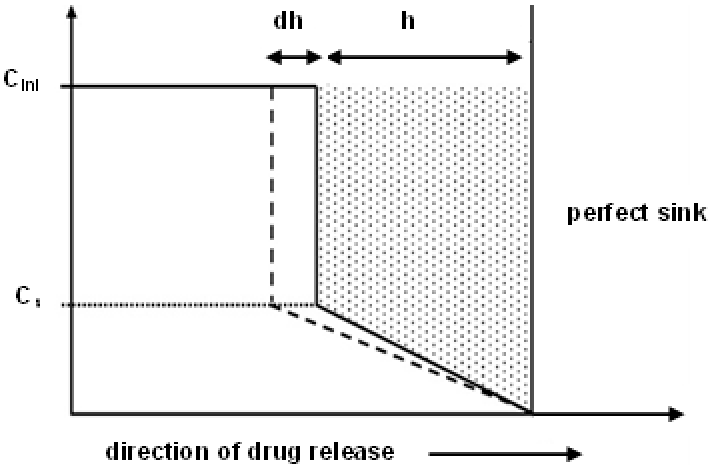


Figure 1.5: *Illustration of the API concentration-distance-profile within the ointment base after exposure to perfect sink conditions at time, t (solid line) and at time, t + dt (dashed line).* C_{ini} *and* C_s *represent the initial API concentration and API solubility, respectively; h represents the distance of the front which separates ointment free of non-dissolved API excess from ointment still containing API excess from the "ointment-skin" interface at time, t; dh represents the distance this front moves inwards during the time interval, dt (Siepmann & Peppas, 2011:7)*

After a given time t, the API concentration-distance-profile was obtained in the ointment film. This is represented by the solid line in figure 1.5. The y-axis represents the API concentration and the x-axis represents the distance. Figure 1.5 can be seen as a cross-section through the ointment film and the skin (located on the right hand side and providing perfect sink conditions). It is important to note that the figure illustrates API solubility, C_s, as relatively high when compared to the initial API concentration C_{ini}. Ideally, C_{ini}, should be significantly larger than C_s(by a factor of 10 or more). The dotted area in figure1.5 illustrates that parts of the ointment have been depleted of API at that time point. At a certain distance from the surface, API concentration steeply increased from saturation concentration to "initial concentration". This is illustrated by a sharp front on figure 1.5. Part of the ointment which still contained non-dissolved API particles (left hand side) and part of

the ointment which was free of non-dissolved API excess (right hand side) were separated by this front. It was located at the distance from the film's surface and is termed "diffusion front". To calculate the amount of API released from the ointment film at this time point t, the API concentration-distance-profile within the part of the ointment depleted of API excess must be known (Siepmann & Peppas, 2011:7).

Higuchi used a pseudo-steady-state approach to describe the API concentration gradient in the ointment zone located between the "diffusion front" and the skin. This is usually valid for systems containing initially a large excess of API (API loading >>API solubility). The idea was the following: If the initial API concentration was significantly higher than API solubility in the ointment base (ideally by factor 10 or more), it took a longer time to dissolve all the API excess at the distance,*h*, from the film's surface. Therefore, the concentration at this position could be considered constant during a certain time period. In addition, perfect sink conditions were provided at the film's surface. The ointment base did not swell or dissolve, resulting in pseudo-steady-state conditions for API diffusion. These conditions included a saturated API solution on the one side, perfect sink conditions on the other side and a constant distance in-between. Fick's second law of diffusion shows that under these conditions, the API concentration-distance-profile between the surface of the film and the "diffusion front" is linear. This is represented by the solid line in figure 1.5. The dotted trapezoid in figure 1.5 represents the amount of API released from the ointment film at time,*t.* It is important to note that under non-pseudo-steady-state conditions, the API concentration gradient in the ointment zone free of drug excess is non-linear, resulting in more complicated geometries (Siepmann & Peppas 2011:7).

The dotted trapezoid representing the amount of API released from the film at time,*t,* is focused on in figure 1.6. Figure 1.6 represents a cross section of the ointment film. Thus, the surface of the dotted trapezoid corresponded to the cumulative amount of API released divided by the surface area of the film exposed to the skin (Siepmann & Peppas 2011:7).

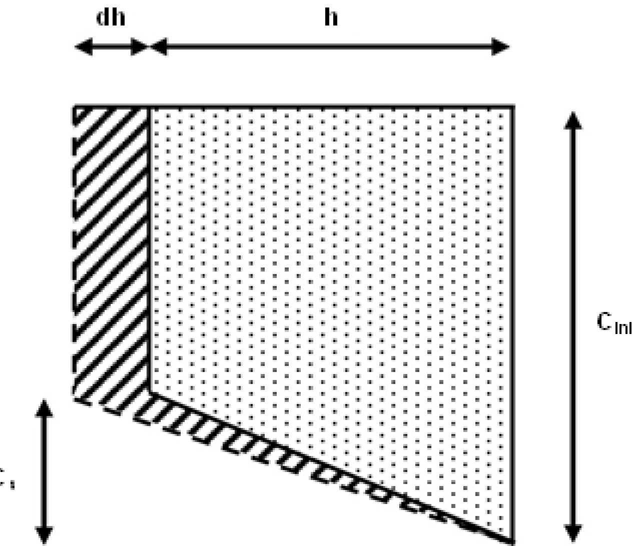


Figure 1.6: *Surfaces representing the amounts of API released from the ointment base at time, t (dotted trapezoid) and at time, t +dt (dashed trapezoid + dotted trapezoid) (Siepmann & Peppas 2011:7)*

The cumulative amount of API released from the ointment film*Mt* at time, *t*, can be calculated using equation 1. 17.

$$\frac{Mt}{A} = h\left(C_{ini} - \frac{C_s}{2}\right)$$ **[1.17]**

However, for the purpose of this equation, *h* must be known. Higuchi considered the API concentration-distance-profile within the ointment film at a certain time period *(dt)* later: *t* + *dt*, thereby resulting in the expression,*h,* as a function of other variables. This is represented by the dashed line in figure 1.6. The “diffusion front” separating ointment free of API excess; and ointment still containing API excess, moved the distance,*dh,* away from the surface. Fundamentally, the API concentration gradient between the new front position,*h* + *dh,* and the skin could be considered linear. This linear relation could be obtained due to the high excess of API (compared to the APIs solubility) and the above described pseudo-steady-state approach. The dashed trapezoid in figure 1.6 illustrates the cumulative amount of API released per unit surface area (*dM/A)* in the time interval, *dt*. This is shown in equation 1.18 (Siepmann & Peppas, 2011:7).

$$\frac{dM}{A} = C_{ini}dh - \frac{Cs}{2}dh$$ **[1.18]**

To quantify the amount of API released from the ointment film in time interval, *dt* (considering a saturated API solution at distance,*h,* from the surface and perfect sink conditions) can be

expressed using Fick's first law of diffusion as seen in equation 1.19 (Siepmann & Peppas, 2011:7).

$$\frac{dM}{dt} = AD\frac{C_s}{h} \quad \textbf{[1.19]}$$

Importantly, combining equations 1.18 and 1.19, allows,*h*, to be expressed as follows in equation 1.20 (Siepmann & Peppas, 2011:7):

$$h = 2\sqrt{\frac{DtC_s}{2C_{ini} - C_s}} \quad \textbf{[1.20]}$$

Substituting equation 1.20 into equation 1.17 and simplifying the substitution, leads to (Siepmann & Peppas, 2011:7):

$$\frac{M_t}{A} = \sqrt{(2C_{ini} - C_s)DtC_s} \quad \textbf{[1.21]}$$

For a high initial excess of API ($C_{ini} >> C_s$), equation 1.21 can be further simplified to equation 1.22, which represents the classical Higuchi equation (Siepmann & Peppas, 2011:7).

$$\frac{Mt}{A} = \sqrt{2C_{ini}DC_st} \quad \textbf{[1.22]}$$

However, equation 1.22 can be further simplified to equation 1.23, which is expressed in the more general form (Siepmann & Peppas, 2011:7).

$$Mt = k\sqrt{t} \quad \textbf{[1.23]}$$

Where:

$$k = A\sqrt{2C_{ini}DC_s} \quad \textbf{[1.24]}$$

Thus, the classical Higuchi equation describes a "square root of time" release kinetics (Siepmann & Peppas, 2011:7). This relation is used to describe API dissolution from several types of modified released pharmaceutical dosage forms, such as transdermal systems and matrix tablets with water soluble APIs (Costa & Lobo, 2001:126). Equations 1.25 and 1.26 describe API release according to the Higuchi model (Higuchi, 1962:803).

$$Q = 2C_0\left(\frac{Dt}{\pi}\right)^{\frac{1}{2}}$$ **[1.25]**

Where:

- Q is the amount of API release per unit area,
- C_o is the initial API concentration in the ointment,
- D is the diffusion coefficient of the API, and
- t is the time after application.

$$R = 200\left(\frac{Dt}{\pi h^2}\right)^{\frac{1}{2}}$$ **[1.26]**

Where:

- R is the percent of API released, and
- h is the thickness of the layer.

If the rate of API release obeys this law, the amount of API release is a linear function of the square root of time($t^{1/2}$). Thus, D can be calculated from the slope. Higuchi (1962:802) summarised the main assumptions as:

- The API is the only component diffusing out of the vehicle,
- Sink conditions are maintained in the acceptor phase, and
- D is constant with respect to time and position in the vehicle (Higuchi, 1962:802).

1.6 SUMMARY

The quantity of an API that can be transported into or through the skin (dermal or transdermal delivery) depends on the ability of an API to permeate the skin at a rate, and in a quantity, sufficient to attain an effective concentration in the biological tissue. Several factors are involved in this process, for example, skin permeability, physicochemical properties of the API and characteristics of the vehicle. Penetration of an API through the skin is hindered by the stratum corneum (SC), the cutaneous outermost external layer, consisting of keratin-rich dead cells embedded in a complex lipid matrix base of ceramides, cholesterol, and free fatty acids. The SC is a significant barrier to the transport of hydrophilic substances whereas the viable epidermis, situated below the SC is much more aqueous in nature and represents a significant barrier for highly lipophilic substances.

Inadequate skin permeability of ibuprofen, procures a significant interest for cutaneous dosage forms that could provide appropriate API levels at the application site, avoiding adverse effects, e.g., gastric mucosal damage and bleeding associated with oral delivery. However, high doses of API have to be applied on the skin to obtain a therapeutic amount of API at the target site. The total quantity of delivered API to the target site might be improved by influencing the physicochemical properties of APIs without changing the pharmacodynamics. These include lipophilicity, solubility, molecular weight or size, melting point, ionisation, molecular structure and hydrogen bonding ability, and/or without the incorporation of a penetration enhancer in the formulation. Penetration enhancers can increase API diffusion through the skin by modifying the intercellular lipid packing of the skin. However it can also increase the partition coefficient of the API between skin and vehicles, by solubilising into the skin. The incorporation of Pheroid™ technology as a penetration enhancer may also assist in overcoming the barrier function of the skin.

CHAPTER 2
MATERIALS AND METHODS

2.1 INTRODUCTION

A pharmaceutical dosage form is an entity that is administered to patients so that they receive an effective dose of an active pharmaceutical ingredient (API). The proper design and formulation of a dosage form requires a thorough understanding of the physiological factors that affect percutaneous penetration and the physicochemical characteristics (as discussed in section 1.3.1 and 1.3.2, respectively) of the API, as well as that of the pharmaceutical excipients that are used during formulation. The API and pharmaceutical excipients must be compatible with one another to produce a formulation that is stable, efficacious, attractive, easy to administer, and safe (Mahato, 2007:11). Therefore, when developing new or improved dosage forms, it is of utmost importance to evaluate the above-mentioned factors influencing design and formulation to provide the best possible formulation. This chapter describes the pharmaceutical excipients (materials) used in the various semi-solid formulations, as well as the experimental procedures of this study.

2.2 MATERIALS

The pharmaceutical excipients (materials) used in the study are presented in table 2.1.

Table 2.1: *Materials, suppliers and batch numbers used in the different formulations*

Materials	Supplier	Batch number
Cremophor® RH 40	BASF Aktiengesellschaft, Ludwigshafen, Germany	04517856PO
dl-α-Tocopherol	BASF Aktiengesellschaft, Ludwigshafen, Germany	UT11070044
Ethanol	Merck Chemicals®, Midrand, South Africa	230811EX
Ibuprofen	DB Fine Chemicals®, Johannesburg, South Africa	IB11060208
Isopropyl Myristate	Merck Chemicals®, Midrand, South Africa	54990302826
Octan-1-ol	ACE (Associated Chemical Enterprises), Johannesburg, South Africa	25341
Pluronic® F-127	Sigma Aldrich, Johannesburg, South Africa	BCBC6633V
Polyethylene Glycol 400	Merck Chemicals®, Midrand, South Africa	1027497
Potassium Chloride	Merck Chemicals®, Midrand, South Africa	1019710
Potassium Dihydrogen Orthophosphate	Labchem, Johannesburg, South Africa	19082011
Propylene Glycol	Merck Chemicals®, Midrand, South Africa	1033058
Sodium Hydroxide	Merck Chemicals®, Midrand, South Africa	1035057
Vitamin F Ethylester	BASF Aktiengesellschaft, Ludwigshafen, Germany	3102976

2.3 PREPARATION OF BUFFER SOLUTIONS

2.3.1 PREPARATION OF PHOSPHATE BUFFER SOLUTION (PH 7.4)

Sodium hydroxide pearls (1.571 g) were dissolved in 393 ml Milli-Q® water and added to a solution prepared by dissolving potassium dihydrogen orthophosphate (6.82 g) in 250 ml Milli-Q® water. The pH was measured, and if necessary, it was adjusted to 7.4 with phosphoric acid (British Pharmacopoeia, 2013).

2.3.2 PREPARATION OF PHOSPHATE BUFFER SOLUTION (pH 5.0)

Potassium dihydrogen phosphate (2.72 g) was dissolved in 800 ml Milli-Q® water. The pH was measured, and if necessary, it was adjusted to pH 5.0 with sodium hydroxide. Milli-Q® water was used to fill the beaker to volume (1 000 ml) (British Pharmacopoeia, 2013).

2.4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD VALIDATION

The basis of high quality data is a reliable analytical method which is obtained through a validation process. This process objectively demonstrates the inherent quality of an analytical method by fulfilment of minimum acceptance criteria (Peters & Musshoff, 2007:216). It ensures the reliability and sensitivity in the determination and recovery of active pharmaceutical ingredients (APIs), for example, in formulated products. Important to note is that only the analytical method is validated and not the results (Araujo, 2009:2225; Ermer, 2005:3-4).

It was important to accurately determine the amount of ibuprofen released by the various semi-solid formulations, as well as the amount of ibuprofen that diffused into, and through skin during *in vitro* skin diffusion studies. A high performance liquid chromatographic (HPLC) method for the analysis of ibuprofen was developed and validated at the Analytical Technology Laboratory (ATL) at the North-West University, Potchefstroom Campus. Method validation was done according to the guidelines of the International Conference on Harmonisation (ICH, 1995:7; ICH 1996:8).

2.4.1 CHROMATOGRAPHIC APPARATUS AND CONDITIONS

The following chromatographic apparatus and conditions were employed for the analysis of ibuprofen:

Analytical instrument: HPLC analysis of Ibuprofen was performed using an Agilent® 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA) in a controlled laboratory environment at 25 °C (figure 2.1). This instrument was equipped with a G1322A degasser, G1311A quaternary pump, G1313A autosampler injection mechanism, G1316A column oven and a G1315A multi wavelength diode array detector. Chromatograms were processed using Chemstation Rev. A.10.03 software. All validation calculations were done with Microsoft® Excel™ 2007, Office Package.

Figure 2.1: *A photograph illustrating an Agilent® 1100 Series HPLC*

Column: High performance silica based, reversed phase C18-2 column with a 5 µm particle size, endcapped, 150 x 4.60 mm (Venusil XBP Agela Technologies, Newark, DE) was used.

Mobile phase A mixture of 1% acetic acid, 70% acetonitrile and 30% HPLC grade (Milli-Q®) water (1:70:30) was filtered and degassed using an ultrasonic bath prior to use.

Solvent: Phosphate buffer solution (pH 7.4) was used as solvent for the validation of ibuprofen. Methanol was used as solvent to analyse the ibuprofen concentration for the assay during stability testing.

Flow rate: 1.0 $ml.min^{-1}$

Injection volume: The injection volume was set to 25 µl for the validation of ibuprofen and 20 µl for the assay determination during stability testing.

Detection: The UV-absorbance of ibuprofen was measured using a UV-detector set at 265 nm.

Retention time: The retention time was approximately 5.2 min for the validation of ibuprofen, whereas it was approximately 5.5 min for the assay determination during stability testing.

Run time: The run time was approximately 10.0 min for the validation of ibuprofen and approximately 7 min for the assay during stability testing.

2.4.2 PREPARATION OF STOCK SOLUTION

A stock solution was prepared as follows:

1. Ibuprofen (50.4 mg) was accurately weighed in a 100 ml volumetric flask.
2. The ibuprofen was dissolved in a small quantity of methanol. To ensure that it was completely dissolved, the solution was sonicated in an ultrasonic bath for approximately 5 min.
3. The volumetric flask was filled to 100 ml with phosphate buffer solution (pH of 7.4).

A concentration of 504.0 $\mu g.ml^{-1}$ was thus prepared as stock solution.

2.5 VALIDATION PARAMETERS

2.5.1 LINEARITY AND RANGE

The linearity of an analytical method is an indication of (1) how well a calibration plot of peak area *versus* concentration produces an approximate straight line, or (2) how well the data fit to the linear equation (equation 2.1), i.e., the residual standard deviation (RSD). The RSD is also understood as the standard error compared to the calculated y-value at a certain target concentration level (Snyder *et al.,* 1997:644).

$$y = mx + c \qquad \textbf{[2.1]}$$

Where:

- y is the peak area of the analyte,
- m is the slope,
- x is the concentration of the analyte ($\mu g.ml^{-1}$), and
- c is the y-intercept.

A concentration range of 0.504 – 504 $\mu g.ml^{-1}$ was prepared from the stock solution (section 2.4.2) as follows:

1. Five millilitres of the stock solution was diluted to 50 ml with phosphate buffer solution (pH 7.4) in a 50 ml volumetric flask in order to obtain a concentration of 50.4 $\mu g.ml^{-1}$.
2. From this solution, 5 ml was transferred to a 50 ml volumetric flask and filled to volume with phosphate buffer solution (pH 7.4) to attain a concentration of 5.04 $\mu g.ml^{-1}$.
3. Finally, 5 ml from the 5.04 $\mu g.ml^{-1}$ solution was diluted to 50 ml with phosphate buffer solution (pH 7.4) in order to obtain a concentration of 0.504 $\mu g.ml^{-1}$.

Therefore, the final concentrations used to determine the linearity were 504 $\mu g.ml^{-1}$; 50.4 $\mu g.ml^{-1}$; 5.04 $\mu g.ml^{-1}$; and 0.504 $\mu g.ml^{-1}$. These solutions were transferred into autosampler vials for analysis. A range of volumes (5 µl; 10 µl; 15 µl; 20 µl; and 25 µl) from each flask was analysed, in duplicate, by HPLC. Through using the peak areas and concentrations, linear regression analysis was used to determine the slope, y-intercept and regression coefficient (r^2). Linear regression analysis should yield a regression coefficient of ≥ 0.99 (Du Preez, 2010:4).

2.5.2 ACCURACY AND PRECISION

2.5.2.1 ACCURACY

Precision and accuracy together, determine the error of an analytical measurement. These are the primary criteria used in obtaining the "quality" of an analytical method and these parameters are considered together due to their interdependency in assessing the acceptability of a method (Karnes *et al.,* 1991:424).

The accuracy of an analytical procedure is defined as the closeness of agreement (the degree of scatter) between the true value or an accepted reference value; and the value found (ICH, 2005:4). This parameter estimates the extent to which systemic errors can influence an analytical method. It has, therefore, been highlighted as the most crucial step in any analytical method and is thus determined by replicate analysis of samples containing known amounts of the analytes. It is also referred to as trueness (Ermer, 2005a:3-4; Ermer, 2005b:21).

Three solutions of different concentrations were prepared to determine the accuracy of the HPLC method by correctly weighing 25 mg ibuprofen in a volumetric flask. The ibuprofen was dissolved in a small quantity of methanol and in order to ensure complete dissolution, the solution was sonicated in an ultrasonic bath for approximately 5 min. Subsequently, the volumetric flask was filled to 100 ml with phosphate buffer solution (pH 7.4). From this solution, 10 ml was diluted with phosphate buffer solution (pH 7.4) and filled to 50 ml in a volumetric flask. Furthermore, 10 ml from the latter solution was diluted with phosphate buffer solution (pH 7.4) and filled to 50 ml in a

volumetric flask. The three concentrations prepared, were 10; 50 and 250 $\mu g.ml^{-1}$. The solutions were analysed in duplicate by means of HPLC to determine system repeatability; and in order to be considered accurate, the recovery had to be between 98 and 102% (Du Preez, 2010:4).

The range of an analytical procedure is determined as the lowest and highest concentration between which the response remains linear, where acceptable precision is obtained, and is normally derived from linearity studies (Du Preez, 2010:4).

2.5.2.2 PRECISION

Precision, on the other hand, expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous samples (ICH, 2005:4). Precision considers three levels of analysis, i.e., repeatability (also known as intraday precision), intermediate precision (interday precision) and reproducibility (Ermer, 2005a:3-4; Ermer, 2005b:21; ICH, 2005:5).

2.5.2.2.1 Repeatability

Repeatability expresses the precision under the same operating conditions over a short period of time (ICH, 2005:5).

A sample was prepared as follows:

1. Ibuprofen (25.01 mg) was accurately weighed in a 100 ml volumetric flask.
2. The ibuprofen was dissolved in a small quantity of methanol. To ensure that it was completely dissolved, the solution was sonicated in an ultrasonic bath for approximately 5 min.
3. The volumetric flask was filled to 100 ml with phosphate buffer solution (pH 7.4).
4. Subsequently, 10 ml of this solution was diluted to 50 ml with phosphate buffer solution (pH 7.4) in order to attain a concentration of 50.02 $\mu g.ml^{-1}$.

The sample (50.02 $\mu g.ml^{-1}$) was analysed in triplicate by HPLC. The standard deviation (SD) and percentage relative standard deviation (%RSD) were calculated using the mean peak areas. The calculated %RSD must be 2% or less in order for the method to be considered repeatable (Du Preez, 2010:5).

2.5.2.2.2 Interday precision

Interday precision requires samples to be analysed by different analysts on different days, using different equipment (ICH, 2005:5). This test was performed over three consecutive days. The sample was prepared as described in section 2.5.2.3 and analysed in duplicate by HPLC. The mean peak area was used to determine the %RSD and SD. The calculated %RSD must be 5% or less in order for the method to be considered precise (Du Preez, 2010:5).

2.5.2.2.3 Reproducibility

Reproducibility expresses the precision between laboratories (ICH, 2005:5). This parameter was determined on day 3. The sample was prepared as described in section 2.5.5.2.1, and analysed in duplicate by HPLC. The mean peak area was used to determine the RSD and SD. The calculated %RSD must be 5% or less in order for the method to be considered reproducible (Du Preez, 2010:5).

2.5.3 RUGGEDNESS

Ruggedness has been defined by Araujo (2009:2232) as the measure in which test results can be reproduced under normal circumstances, whether using different laboratories or analysts. It can be viewed as a test for environmental or experimental factors or variables influencing the analytical method. Some of the possible variables include: column length; flow rate; mobile phase; composition; pH, and temperature. Ruggedness may, furthermore, be subdivided into two different categories, namely sample stability and system repeatability.

2.5.3.1 SAMPLE STABILITY

Sample stability was analysed through the preparation of a stock solution (section 2.5.1) and analysing it immediately at hourly intervals for a period of 24 h in order to determine the formation of degradation products and sample stability. Acceptance criteria stated that degradation may not be more than 2% (Du Preez, 2010:5).

2.5.3.2 SYSTEM REPEATABILITY

System repeatability was evaluated by preparing a standard solution (250.9 $\mu g.ml^{-1}$), as described in section 2.5.1. The samples were injected six times, consecutively. In order to comply, the RSD of the peak area and the retention time should be equal to, or less than 2% (Du Preez, 2010:6).

2.5.4 SPECIFICITY

Specificity may be defined as the capacity to evidently differentiate between an analyte and other compounds expected to be present in the sample, such as other active ingredients, degradation products, impurities, or matrix components (ICH, 2005:4). A method is considered selective when no interfering peaks with the same retention times as the APIs are detected (Du Preez, 2010:14). This was determined by adding water, hydrochloric acid (to decrease the pH), sodium hydroxide (to increase the pH) and hydrogen peroxide (to initiate oxidation) in a ratio of 1:1 to a prepared ibuprofen sample (section 2.4.2).

A stock solution was prepared by accurately weighing 25 mg ibuprofen in a 100 ml volumetric flask. The ibuprofen was dissolved in a small amount of methanol and sonicated in an ultrasonic bath for approximately 5 min in order to ensure complete dissolution. Phosphate buffer solution (pH 7.4) was used to fill the volumetric flask to volume and a concentration of 252 $\mu g.ml^{-1}$ was obtained. The stock solution was divided by placing 2 ml of the solution in four test tubes. Each of these stock solutions was diluted in a 1:1 ratio with either Milli-Q® water, 0.1 M hydrochloric acid (HCl); 0.1 M sodium hydroxide (NaOH); or 10% hydrogen peroxide (H_2O_2).

The test tubes were sealed and stored for 30 min at 25 °C to allow for possible degradation. Following the awaited time laps, the test tubes were neutralised as seen in table 2.2.

Table 2.2: *Neutralising agents used after 30 min*

Test tube	Dilution agent	Neutralising agent
1	Milli-Q® water	No neutralising agent
2	0.1 M HCl	2 ml 0.1 M NaOH
3	0.1 M NaOH	2 ml 0.1 M HCl
4	10 % H_2O_2	4 ml 0.1 M $NaHCO_3$

Each solution was transferred into an autosampler vial and injected, in duplicate, onto the HPLC. The run time was three times longer than the elution time of the ibuprofen peak in order to detect any possible additional peaks. A blank injection of phosphate buffer solution was also injected in order to test for any solvent interference.

2.5.5 ROBUSTNESS

The ICH (2005:5) defines the robustness of an analytical procedure as a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and therefore provides an indication of its reliability during normal usage. This parameter evaluates the constancy of the results when internal factors (no external factors as with ruggedness) such as flow rate, column temperature, injection volume, mobile phase composition (in liquid chromatography) or any other variable inherent to the method of analysis are varied, deliberately (Araujo, 2009:2232).

In this process deliberate changes were made to the injection volume, wavelength and flow rate (table 2.3). The influences of these deliberate changes on the chromatographic results were consequently determined.

Table 2.3: *Changes made to the chromatographic operating parameters*

Injection volume (µl)	Wavelength (nm)	Flow rate ($ml.min^{-1}$)
15	254	0.8
20	260	0.9
30	270	1.1

2.6 PHYSICOCHEMICAL PROPERTIES

In order to understand and predict the *in vivo* performance of a formulation, it is important to determine the solubility and the partition coefficient of the API within the formulation. This section deals with the experimental methods that were used to determine the solubility and partition coefficient of ibuprofen.

2.6.1 AQUEOUS SOLUBILITY

Three test tubes were each filled with 5 ml Milli-Q® water. Supersaturated solutions were obtained by adding excess amounts of ibuprofen to each test tube. The test tubes were submerged in a water bath (figure 2.2.a) set to a temperature of 37 °C and vigorously shaken for 24 h. Subsequently, the test tubes were centrifuged (figure 2.2.b) at 4 500 rpm for 10 min. The solutions were filtered (0.45 μm, Millipore® prefilter) and analysed by HPLC.

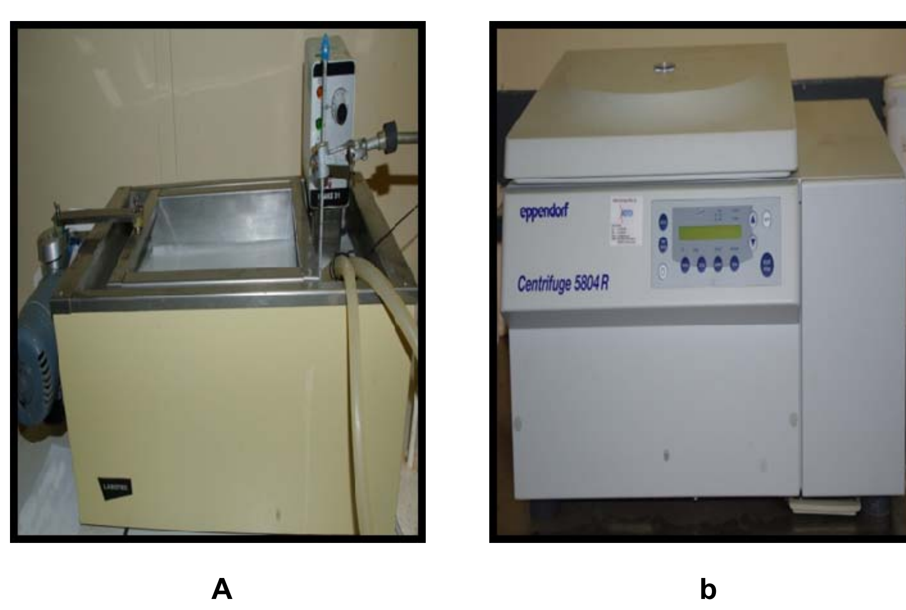


Figure 2.2: *(a) Water bath, (b) Eppendorf® Centrifuge 5804 R*

2.6.2 pH-SOLUBILITY PROFILE

The solubility of ibuprofen at a range of pH values (1 – 14) was determined to indicate the pH at which ibuprofen is most soluble. The pH of Milli-Q® water was adjusted with sodium hydroxide or phosphoric acid. Five millilitres (5 ml) of the adjusted solutions (pH 1 - 14) were filled in test tubes. Supersaturated solutions were obtained by adding excess amounts of ibuprofen to each test tube. The test tubes were submerged in a water bath set to a temperature of 37 °C and vigorously shaken for 24 h. Subsequently, the test tubes were centrifuged at 4 500 rpm for 10 min. The solutions were filtered (0.45 μm, Millipore® prefilter) and analysed by HPLC.

2.6.3 OCTANOL-WATER DISTRIBUTION COEFFICIENT (log P)

A mixture of *n*-octanol and Milli-Q® water, (pH 4.85) in a 1:1 ratio (200 ml : 200 ml) were vigorously stirred for 24 h to attain saturation. The mixture was transferred to a pear-shaped flask and left for 24 h to obtain phase separation. Subsequently the two phases were separated and stored in different containers. A stock solution was prepared by dissolving 2 mg ibuprofen in 100 ml saturated *n*-octanol (saturated with Milli-Q® water). Three test tubes were filled with 3 ml of the stock solution and 3 ml of the saturated Milli-Q® water (saturated with *n*-octanol). The test tubes were submerged in a water bath set to a temperature of 37 °C and vigorously shaken for 3 h. It was centrifuged at 4 500 rpm for 10 min. Analysis of the aqueous phase was determined by HPLC. The log P value was calculated by using the logarithmic ratio of the concentration in the aqueous phase, to the concentration in the *n*-octanol. After determination of the aqueous phase, the concentration of ibuprofen in the oil phase was determined by deducting the concentration of API in the aqueous phase from the original concentration.

2.6.4 OCTANOL-BUFFER DISTRIBUTION COEFFICIENT (log D)

A mixture of *n*-octanol and phosphate buffer solution, (pH 5 and 7.4, respectively) in a 1:1 ratio (200 ml : 200 ml) was vigorously stirred for 24 h to attain saturation. The mixtures (containing phosphate buffer solution at either pH 5 or pH 7.4) were transferred to pear-shaped flasks and left for 24 h to obtain phase separation. Subsequently, the two phases of each mixture were separated and stored in different containers. A stock solution for each mixture was subsequently prepared by dissolving either 2 mg ibuprofen in 50 ml saturated *n*-octanol (for the phosphate buffer solution at pH 5) or 2 mg ibuprofen in 100 ml saturated *n*-octanol (for the phosphate buffer solution at pH 7.4), respectively. Three test tubes for each mixture were filled with 3 ml of the stock solution and 3 ml of the saturated phosphate buffer. The test tubes were submerged in a water bath set to a temperature of 37 °C and vigorously shaken for 3 h. It was centrifuged at 4 500 rpm for 10 min. Analysis of the aqueous phase was determined by HPLC. The log D values were calculated by using the logarithmic ratio of the concentration in the aqueous phase, to the concentration in the *n*-octanol. After determination of the aqueous phase, the concentration of ibuprofen in the oil phase was determined by deducting the concentration of API in the aqueous phase from the original concentration.

2.7 FORMULATION OF SEMI-SOLID DOSAGE FORMS

2.7.1 INTRODUCTION

Semi-solid formulations contain dissolved or suspended APIs. Examples of semi-solid preparations include ointments, creams, gels, pastes, and lotions (Mahato, 2007:183). In this study gels and emulgels were formulated.

Gels are two-component semi-solid systems that are rich in liquid. Their one characteristic feature is the presence of a continuous structure providing solid-like properties (Barry, 2002:593). They produce a uniform external appearance, ranging from being transparent to semi-transparent (Mitsui, 1997:351). As transparency is an important feature in gels, dissolution and uniformity of raw materials should be considered during formulation (Mitsui, 1997:353).

An emulgel is an oil-in-water emulsion that is incorporated into a gel (Lopez-Cervantez *et al.,* 2009:1514). It is a system consisting of two immiscible liquid phases, one of which is dispersed throughout the other in the form of fine droplets. The phase that is present as fine droplets is called the dispersed phase, and the phase in which the droplets are suspended is the continuous phase (Attwood, 2002:92). Different types of emulsions exist, e.g., oil-in-water-, water-in-oil-, multiple- and micro-emulsions (Mahato, 2007:136). According to Mahato (2007:136), if the aqueous phase of an emulsion constitutes more than 45% of the total weight, an oil-in-water emulsion is formed. During this study oil-in-water emulsions were formulated as the aqueous phase constituted more than 45% of the total weight.

2.7.2 FORMULATION OF A GEL CONTAINING IBUPROFEN

Ingredients used in the formulation are presented in table 2.4.

Table 2.4: *Ingredients used in the gel formulations*

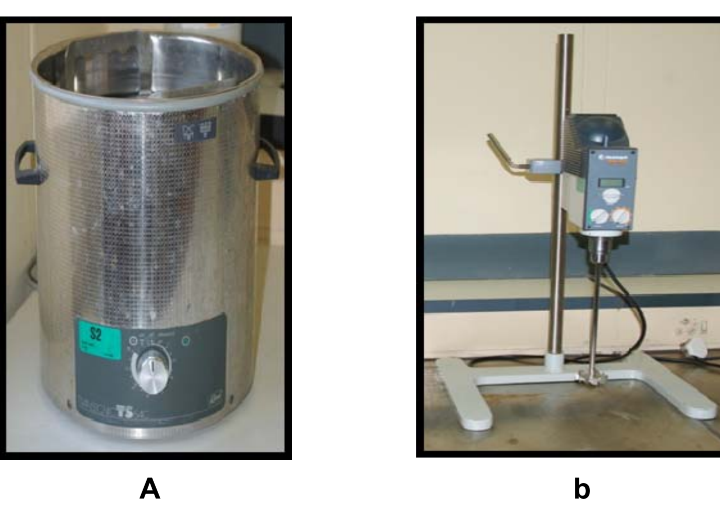
Ingredients	%m/m	Activity
A: Ibuprofen Propylene Glycol Ethanol	5% 20% 10%	Active ingredient Co-solvent Co-solvent / Preservative
B: Pluronic® F-127	20%	Viscosity enhancer
C: dH_2O	45%	Solvent

A, B and C = different phases and ingredients used

Preparation of the gel was conducted by means of the following procedure:

- Ibuprofen (5 g) was weighed and dissolved in 20 g propylene glycol and 10 g ethanol.
- The solution was sonicated in an ultrasonic bath (figure 2.3.a) for approximately 20 min to ensure complete dissolution.

- It was placed on a hot plate with a magnetic stirrer and heated to 70 °C.
- The solution was removed from the hot plate and blended with a mixer (figure 2.3.b) at low speed (300 rpm) for approximately 2 min.
  Pluronic® F-127 (20 g) was added to the solution and blended at low speed (300 rpm) for approximately 2 - 3 min.
- Finally, 45 ml dH_2O was heated to 70 °C and added to the mixture.



A **b**

Figure 2.3: *(a) Transonic TS 40 ultrasonic bath, (b) Heidolph RZR 2041 mixer*

2.7.3 FORMULATION OF AN EMULGEL CONTAINING IBUPROFEN

During preformulation studies a basic gel formulation containing ibuprofen was formulated in order to produce a homogenous, stable and attractive emulgel. The formulation was altered numerous times by varying the ingredients and concentrations. A formulation that adhered to all the requirements set was finally obtained and is presented in table 2.5.

Table 2.5: *Ingredients used in the emulgel formulations*

Ingredients	%m/m	Activity
A: Ibuprofen Polyethylene Glycol 400 Ethanol	5% 7.5% 9.3%	Active ingredient Co-solvent Co-solvent / Preservative
B: Isopropyl Myristate	20%	Oil phase
C: dH_2O	48.2%	Solvent
D: Pluronic® F-127	10%	Viscosity enhancer

A, B, C and D = different phases and ingredients used

Preparation of the emulgel was conducted by means of the following procedure:

- Ibuprofen (5 g) was weighed and dissolved in 7.5 g polyethylene glycol 400 and 9.3 g ethanol.

- The solution was sonicated in an ultrasonic bath for approximately 20 min to ensure complete dissolution.
- Isopropyl myristate (20 g) was added to the solution and sonicated for approximately 20 min.
- A volume of 48.2 ml dH_2O was added to the above solution and sonicated in an ultrasonic bath for approximately 20 min.
- The solution was placed on a hot plate with a magnetic stirrer and heated to 70 °C, after which it was removed and blended with a mixer at low speed (300 rpm) for approximately 2 min.
- Finally, 10 g Pluronic® F-127 was added and mixed at a high speed (900 rpm) until the formulation reached 25 °C ± 0.5 °C.

2.7.4 FORMULATION OF A PHEROID™ EMULGEL CONTAINING IBUPROFEN

Ingredients used in the formulation are presented in table 2.6. The Pheroid™ delivery system consists of three phases, namely, (1) a water phase, (2) an oil phase and (3) a gas phase. During this study, the oil phase was incorporated into the water phase and subsequently added to the nitrous oxide saturated water (gas phase). The function of the gas phase is to maintain stability and contribute to the self-assembly process of the Pheroid™ (Grobler *et al.,* 2008:289).

Table 2.6: *Ingredients used in the Pheroid™ emulgel formulations*

Ingredients	%m/m	Activity
A: Ibuprofen Polyethylene Glycol 400 Ethanol	5% 7.5% 9.3%	Active ingredient Co-solvent Co-solvent / Preservative
B: Isopropyl Myristate	20%	Oil phase
C: Cremophor® RH 40 Vitamin F Ethylester dl-α-Tocopherol	1% 2.8% 0.2%	Solubiliser Anti-oxidant Anti-oxidant
D: $N_2O.H_2O$	44.2%	Solvent
E: Pluronic® F-127	10%	Viscosity enhancer

A, B, C, D and E = different phases and ingredients used

Preparation of the Pheroid™ emulgel was conducted by means of the following procedure:

- Ibuprofen (5 g) was weighed and dissolved in 7.5 g polyethylene glycol 400 and 9.3 g ethanol.
- The solution was sonicated in an ultrasonic bath for approximately 20 min to ensure complete dissolution.
- Isopropyl myristate (20 g) was added to the solution and sonicated for approximately 20 min.

- The solution was removed from the ultrasonic bath and 1 g Cremophor® RH 40, 2.8 g Vitamin F Ethylester, and 0.2 g dl-α-tocopherol were added.
- This mixture was placed on a hot plate with a magnetic stirrer and heated to 70 °C.
- Subsequently, 44.2 ml $N_2O.H_2O$ was heated to 70 °C.
- Both the mixture and $N_2O.H_2O$ were removed from the hot plate; and the mixture was added to the $N_2O.H_2O$.
- A homogeniser (figure 2.4) was employed for 2 - 3 min at 13 500 rpm in order to ensure a homogenous mixture.
- The mixture was blended with a mixer at low speed (300 rpm) for approximately 2 min.
- Pluronic® F-127 (10 g) was added to the above mixture and blended at high speed (900 rpm) until the formulation reached 25 °C ± 0.5 °C.

Figure 2.4: *Heidolph Diax 600*

2.8 PERMEATION STUDIES

Permeation studies were performed to determine the rate and extent of ibuprofen skin permeation. Prior to the performance of skin permeation studies, membrane permeation studies were conducted to determine whether ibuprofen was in fact released from the formulations. Therefore, permeation studies were conducted in two categories, namely; membrane permeation studies and skin permeation studies. This section describes the experimental methods of these two categories.

2.8.1 SKIN PREPARATION

Ethical approval for the procurement and preparation of the skin was provided by the Research Ethics committee of the North-West University under the reference number NWU-00114-11-A5. Female Caucasian human skin obtained after abdominoplastic (tummy-tuck) surgery, was donated

by anonymous donors. Prior to obtaining the skin, informed consent was provided by the donor patients through their doctor in order to maintain confidentiality by concealing patient identity.

Full-thickness skin was removed from the abdomen of these patients. Within 24 h following surgical removal of the skin, it was frozen at a temperature of -20 °C for no longer than 6 months. Prior to the conduction of any studies, the full-thickness skin was taken from the freezer and left to thaw. The skin was first visually examined for any defects such as stretch marks, scarring or large hair follicles/holes. Subsequently, it was cut into pieces with the Zimmer® electric dermatome model 8821 (figure 2.5.a), removing only the subcutaneous fat layer of the skin. All prepared skin samples were placed on Whatman® filter paper with the stratum corneum facing upwards. Each skin piece was cut into circles of approximately 15 mm in diameter; with a punch and hammer (figure 2.5.b) and 400 µm in thickness using the Zimmer® Electric Dermatome. The skin circles (figure 2.5.c) on the filter paper were wrapped in aluminium foil, placed in a sealed plastic bag (Ziploc®) and stored in a freezer at -20 °C until utilised (within 24 h). Prior to the commencement of experiments, these skin samples were thawed at 25 ± 0.5 °C and each experiment was performed on skin obtained from a single donor.

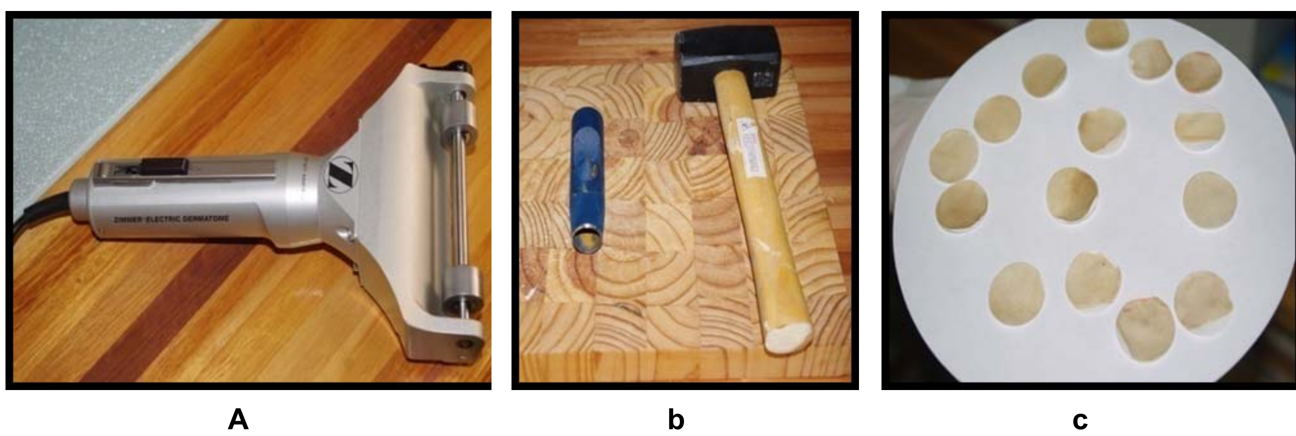


Figure 2.5: *(a) Zimmer® Electric Dermatome, (b) Punch and hammer, and (c) Skin circles prior to being wrapped in aluminium foil*

2.8.2 MEMBRANE RELEASE AND SKIN DIFFUSION STUDIES

In vitro transdermal studies evaluate the permeation of APIs over an artificial membrane or over dermatomed female Caucasian skin for a specified time period (6 h and 12 h, respectively). Franz cells consist of donor (top) and receptor (bottom) compartments. Either an artificial membrane (Polytetrafluoroethylene or PTFE membranes) or a skin sample was clamped between the two compartments; and if skin was used, it was placed with the stratum corneum facing the donor compartment. The formulation to be analysed is either placed on top of the artificial membrane or the skin sample in the donor compartment, whereas a phosphate buffer solution (pH 7.4,

simulating the blood) is placed in the receptor compartment and withdrawn at regular predetermined time intervals.

During the permeation studies, twelve vertical Franz diffusion cells (figure 2.6.a) with a receptor volume of approximately 2 ml and a diffusion area of 1.075 cm^2 were used to conduct the *in vitro* transdermal studies. Ten of these Franz cells contained a specific formulation that included ibuprofen; whereas the remaining two cells contained placebo formulations (gel, emulgel and Pheroid™ emulgel formulation with no API).

Every receptor compartment was filled with 2 ml phosphate buffer solution (pH 7.4) by tilting the Franz cells to ensure that no air bubbles were trapped beneath the membrane or skin circles. The temperature was maintained at 37 °C. A Teflon-coated magnetic stirring bar was placed in the receptor compartment in order to maintain stirring throughout the experiment, which imitates blood flow. Either an artificial polytetrafluoroethylene membrane (in order to conduct release studies) or the prepared skin circles (figure 2.6.b and figure 2.5.c) were mounted between the two compartments of each of the diffusion apparatus; the skin with the stratum corneum facing upwards in the direction of the donor compartment. A thin layer of Dow Corning® high vacuum grease (figure 2.6.c) was applied to the receptor and donor compartments of each Franz cell prior to assembling it. Furthermore, the sides of the cells were sealed with a thick layer of Dow Corning® high vacuum grease to prevent any leakage. A horseshoe clamp (figure 2.6.a) was employed to secure the receptor and donor compartments.

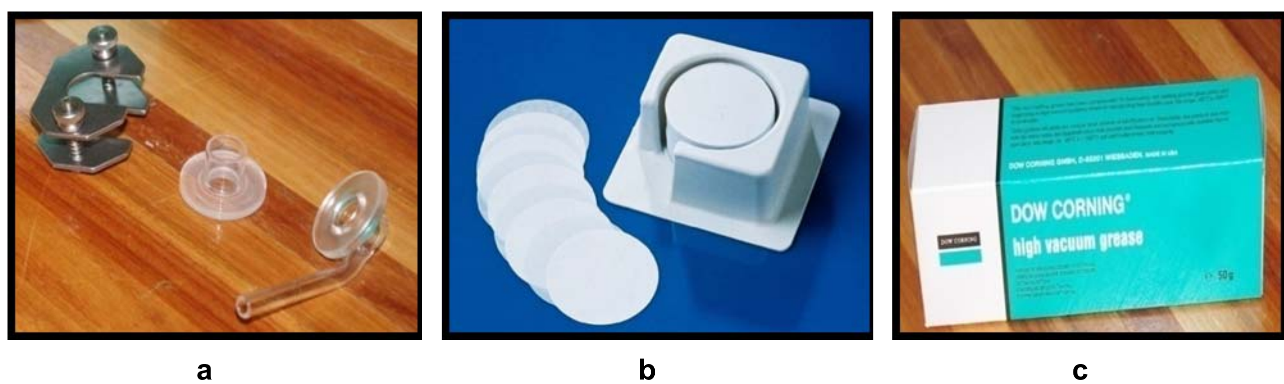
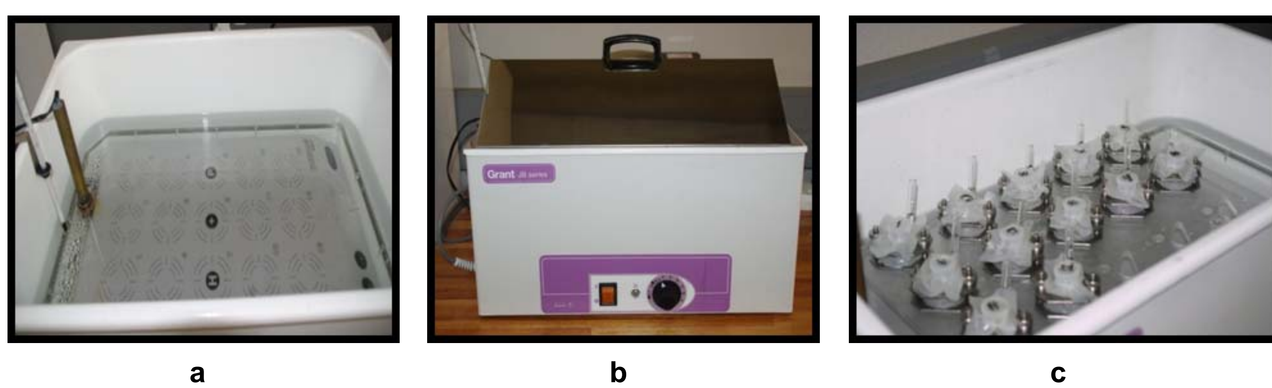


Figure 2.6: *(a) Horseshoe clamp, Donor compartment of Franz cell and Receptor compartment of Franz cell, (b) polytetrafluoroethylene (PTFE) membranes, (c) Dow Corning® high vacuum grease*

Subsequently, a sufficient amount of a specific formulation (approximately 1 ml), which was heated to 32 °C prior to commencement of the experiment in order to simulate skin surface temperature, was added to each of the donor compartments. This was done in order to ensure skin saturation. Each of the donor compartments was consequently covered with Parafilm® and a cap to prevent evaporation during the experiment. The Franz cells were placed on a stand which was set up on a Variomag® magnetic stirring plate (750 rpm) and submerged in a Grant® water bath set at 37 °C

(figure 2.7.a and b). At pre-determined time intervals the entire receptor volume was withdrawn and replaced with fresh buffer (37 °C) in order to ensure sink conditions existed throughout the experiment (figure 2.7.c). During the membrane release studies, samples were withdrawn every 2 h for 6 h. However, during skin diffusion studies, samples were withdrawn every 20 min for the first 2 h after which samples were withdrawn every 2 h for the following 10 h. All samples were directly assayed using an Agilent® isocratic HPLC system to determine the ibuprofen concentration in the receptor fluid. Hence, tape stripping was performed after the 12 h skin diffusion study.



a b c

Figure 2.7: *(a) Variomag® magnetic stirring plate, (b) Grant® waterbath (c) Permeation study in process*

Summaries of the experimental conditions and settings employed for the permeation studies are presented in tables 2.7 and 2.8.

Table 2.7: *Summary of membrane permeation studies*

Receptor medium	Phosphate buffer solution at pH 7.4
Temperature of experiment	37 °C
Run time	6 h
Withdrawal times	1, 2, 3, 4, 5, 6 h
Agitation	750 rpm
Franz cell volume	2 ml
Sample application	1 ml
Membrane	PTFE membrane (135 µm thickness)
Analysis of samples	HPLC

2.8.3 TAPE STRIPPING

After the final withdrawal was conducted during the skin diffusion experiment, the Franz cells were carefully dismantled and the skin pinned to a solid flat surface containing Whatman® filter paper. The exposed diffusion area (1.075 cm^3) was clearly demarcated by the imprints of the diffusion

cells (11.7 mm diameter). All excess formulation was dabbed from the skin with a clean paper towel. In order to perform tape stripping, sixteen strips of 3M Scotch® Tape were cut to a length that covered the diffusion area, but did not overlap the area outside the diffusion imprints. The first strip was discarded as it was considered part of the cleaning procedure and the remaining fifteen strips were used to strip the stratum corneum-epidermis off the diffusion area. Glistening of the viable epidermal layer indicated complete removal of the stratum corneum-epidermis (Pellet *et al.,* 1997:94). These strips were subsequently placed in a polytop containing 5 ml methanol (40%). The polytop was vigorously shaken to wet all the strips and retained overnight (approximately 12 h) at approximately 4 °C to be analysed by HPLC the following day.

Excess skin on the outside of the imprints of the diffusion area was trimmed and the remaining skin circle (dermis) was cut into pieces in order to enlarge the surface area in contact with the methanol solution. These skin pieces were placed in a polytop containing 5 ml methanol (40%) and was furthermore vigorously shaken after which it was stored overnight at 4 °C to be analysed by HPLC the following day.

Table 2.8: *Summary of skin permeation studies*

Receptor medium	Phosphate buffer solution at pH 7.4
Temperature of experiment	37 °C
Run time	12 h
Withdrawal times	0.33, 0.66, 1, 1.33, 1.66, 2, 4, 6, 8, 10, 12 h
Agitation	750 rpm
Franz cell volume	2 ml
Sample application	1 ml
Skin	Female Caucasian abdominal skin (400 µm thickness)
Tape stripping	• All Franz cells were dismantled, the skin was pinned on a solid surface and dabbed dry with a paper towel. • 16 strips of 3 M Scotch tape were cut. • The first strip was discarded and the remaining 15 strips were placed in a polytop with 5 ml methanol. • The edges of the skin were trimmed; the diffusion area was cut into pieces, and placed in a polytop with 5 ml methanol. • The polytops were stored overnight at 4 °C.
Analysis of samples	HPLC

2.9 STABILITY TESTING OF SEMI-SOLID FORMULATIONS

2.9.1 INTRODUCTION

The purpose of stability testing is to investigate how the quality of an API containing formulation varies with time under the influence of a variety of environmental factors, such as: (1) temperature, (2) humidity, and (3) light (ICH, 2003:2). The following characteristics were investigated:

- Physical properties,
- Chemical properties, and
- Microbial properties (ICH, 2003:2).

Commercial pharmaceutical products should possess a shelf life of at least 3 years. During the shelf life, the potency may not fall below an acceptable minimum. It is usually 95% under the recommended storage conditions without the generation of toxic degradation products. At the end of the shelf life, the product should still look and perform as it did when it was first manufactured (Wells & Aulton, 2002:351).

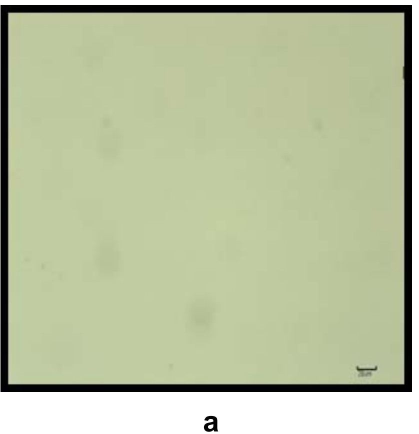
The stability of the formulated semi-solid products was examined over a period of 3 months. These products were stored at 25 °C/60% RH (relative humidity), 30 °C/60% RH and 40 °C/75% RH. According to Wells and Aulton (2002:352), a 10% increase in temperature may produce a 2 - 5 fold increase in decay.

All stability tests were conducted using calibrated or validated apparatus, where appropriate. The stability tests conducted on the different semi-solid formulations are presented in table 2.9.

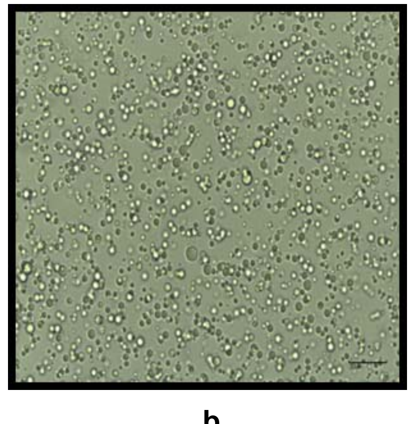
Table 2.9: *Stability tests conducted on the different semi-solid formulations*

Test	Emulgel	Pheroid™ emulgel	Gel
Assay	√	√	√
pH	√	√	√
Mass variation	√	√	√
Viscosity	√	√	√
Visual appearance	√	√	√
Zeta potential	√	√	X
Droplet size	√	√	X

The reason for not measuring zeta potential and droplet size for the gel formulations was due to the gels being clear solutions whereas the emulgels contained oil droplets. This is illustrated in figures 2.8 a and b.



a



b

Figure 2.8: *Micrograph images for: (a) Gel, (b) Emulgel*

For the purpose of this study, approximately 1 500 ml of each formulation was manufactured. The formulations were divided into 50 g glass containers. They were stored in Labcon® humidity chambers (Labex, Johannesburg, South Africa) (figure 2.9) at the different temperature and humidity conditions described. Each test required its own container. Tests were conducted at month 0, 1, 2, and 3.

Figure 2.9: *Labcon® humidity chamber*

2.9.2 VISUAL APPEARANCE

The visual appearance (any change in colour or texture) of each formulation stored at the specified conditions for 3 months was inspected. The colour of the formulations was matched to a graded colour chart obtained from a local paint store.

2.9.3 LIGHT MICROSCOPY

A sample of each formulation that was stored at the various specified conditions was placed on a glass slide and covered with a glass cover slip. Images were captured with a Nikon® Optiphot Microscope (Nikon®, Thailand, Bangkok) (figure 2.10). This was conducted in order to determine whether the formulations contained any crystals after exposure to the specified temperature and humidity conditions.

Figure 2.10: *Nikon® Optiphot microscope*

2.9.4 MASS VARIATION

A Mettler Toledo® balance (Mettler Toledo®, Schwerzenbach, Switzerland), was used to determine if any mass variation occurred during storage (figure 2.11). Prior to filling the formulations (50 g) into the glass containers, the weight of the empty glass container with the lid was determined. The weight of each formulation stored at the specified conditions was determined in triplicate. After the indicated time intervals, the change in mass for each formulation was determined and compared to the original mass of the formulation.

Figure 2.11: *Mettler Toledo® balance*

2.9.5 ASSAY

The purpose of the assay was to determine whether the ibuprofen concentration in the semi-solid formulations changed in relation to its initial or previous value during the course of storage. This was determined by means of HPLC analysis (Section 2.4.1).

2.9.5.1 PREPARATION OF STOCK SOLUTION

A stock solution was prepared as follows:

1. Ibuprofen (50 mg) was accurately weighed in a 100 ml volumetric flask.
2. The ibuprofen was dissolved in a small quantity of methanol. To ensure that it was completely dissolved, the solution was sonicated in an ultrasonic bath for approximately 5 min.
3. The volumetric flask was filled to 100 ml with methanol.

The stock solution was transferred into an autosampler vial for analysis. A range of volumes (5 µl; 10 µl; 15 µl; 20 µl; and 25 µl) was analysed, in duplicate, by HPLC.

2.9.5.2 PREPARATION OF SAMPLE

A sample was prepared as follows:

1. Each formulation (1 g) was accurately weighed in a 100 ml volumetric flask, using a syringe with a plastic tube attached to the tip.
2 The formulations were each dissolved in a small quantity of methanol. To ensure that the formulation was completely dispersed, it was sonicated in an ultrasonic bath for approximately 5 min.
3 Each volumetric flask was filled to 100 ml with methanol.

All of the samples were prepared in triplicate and transferred to HPLC vials. The samples were analysed in duplicate by HPLC at the default injection volume (20 µl).

2.9.6 pH

A Mettler Toledo® Inlab 410 pH-meter (Mettler Toledo®, Schwerzenbach, Switzerland) as depicted in figure 2.12 was used to measure the pH of each formulation. Prior to measurement, the apparatus was calibrated with Mettler Toledo® buffer solutions at pH 4.01, 7.00 and 10.01 at 25 °C. The pH of each formulation was measured in triplicate and the average pH determined.

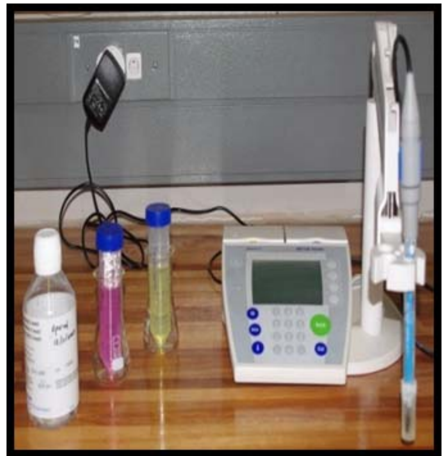


Figure 2.12: *Mettler Toledo® Inlab 410 pH-meter*

2.9.7 VISCOSITY

Viscosity is a measure of a fluid's resistance to flow (Marriot, 2002:42). The viscosity of each formulation stored at the specified conditions was measured using a Brookfield® Viscometer model DV-II+ (Stoughton, United States of America) as seen in figure 2.13. The temperature of the water circulating in the water bath was controlled by a Brookfield® temperature controller. Appropriate Helipath and LV spindles were selected to ensure that an optimum torque existed depending on the consistency of the semi-solid formulations.

Figure 2.13: *Brookfield® Viscometer*

A Helipath stand D20733 is a motorised stand to which any Brookfield® Viscometer can be attached. The stand slowly raises and lowers the Viscometer (at a rate of 7/8-inch per minute) whilst a special T-bar spindle (figure 2.14.a) rotates in the formulation. The cross bar of the spindle continuously cuts into fresh sample material, describing a helical path through the sample as it rotates. The LV spindles (vane spindles, figure 2.14.b) are immersed directly into the formulation without causing a disturbance. A formulation trapped between the vanes moves as the spindle rotates. The net effect is that a virtual cylinder of formulation in which the vane spindle is immersed, will flow at defined rotational speeds (Brookfield Engineering Labs, 2010a:4).

Figure 2.14: *(a) LV spindle, (b) T-bar spindle*

The viscosity readings were measured every 10 sec for 5 min in revolutions per minutes (rpm). Approximately 32 measurements were obtained and the average viscosity was determined. Viscosity parameters for the different semi-solid formulations are presented in table 2.10.

Table 2.10: *Viscosity parameters for the different semi-solid formulations*

Formulation	Spindle	Entry code	rpm	Temperature
Emulgel	T-F	96	0.3	25°C, 30°C, 40°C
Pheroid™ emulgel	T-F	96	0.3	25°C, 30°C, 40°C
Gel	LV	62	30	25°C, 30°C, 40°C

2.9.8 ZETA POTENTIAL

Electrical charges that exist at the particle-liquid interface of suspensions, colloidal dispersions, emulsions and other related systems, strongly influence the stability of these systems (Malvern® Instruments, 2000:1.1). In order to determine the electrical charges, zeta potential was measured.

A sample was prepared as follows:

1. Each formulation (0.5 g) was accurately weighed in a 25 ml volumetric flask, using a syringe with a plastic tube attached to the tip.
2. The formulations were each dissolved in a small quantity of 0.01 M potassium chloride. To ensure that the formulation was completely dispersed, it was sonicated in an ultrasonic bath for approximately 5 min.
3. Each of the volumetric flasks was filled to 25 ml with 0.01 M potassium chloride.

All of the samples were prepared and measured in triplicate by means of injecting the samples into a Malvern® Zetasizer 2000 (Malvern® Instruments, Worcestershire, United Kingdom) as demonstrated in figure 2.15.

Figure 2.15: *Malvern® Zetasizer 2000*

2.9.9 DROPLET SIZE

Approximately 0.5 g of each formulation stored at the specified conditions was mixed with approximately 3 ml Milli-Q® water to form a homogenous dispersion. These mixtures were made up with approximately 3 ml Milli-Q® water, mixed well and injected into a Malvern® Mastersizer 2000 (figure 2.16), fitted with a wet cell Hydro 2000 SM dispersion unit (Malvern® Instruments, Worcestershire, United Kingdom).

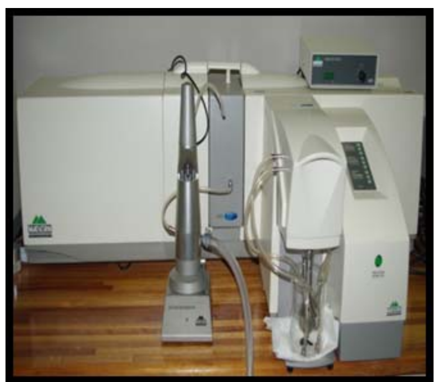


Figure 2.16: *Malvern® Mastersizer 2000, equipped with a wet cell Hydro 2000 SM dispersion unit*

2.10 STATISTICAL METHODS

Inventions, formulation of new theories, and refinement of existing processes depend on scientific research. During scientific research, relevant data is collected, analysed, and evaluated in terms of the scientific method of investigation. Statistics entails the development and application of techniques for interpreting numerical data. Therefore, it is considered an essential link in the research process (Steyn *et al.,* 1994:1). This section deals with the statistical methods of analysis where membrane and skin diffusion data were analysed in such ways that assumptions for all procedures were met.

One-way analysis of variances (ANOVA) was performed to determine whether statistical significant differences existed between the mean values of the different formulations and Nurofen® gel in general. Levene's test was performed to assure equality of variances in each ANOVA's case. In the case of inequality of variances, Welch tests were conducted. Normal probability plots on the residuals were performed to assure that the data was fairly normally distributed. Tukey's post-hoc multiple comparison tests were performed to determine statistically significant differences between all the different formulations. Dunnet's tests were conducted to determine which of the mean values of the formulations differed statistically significant from the mean of the Nurofen® gel formulation. These procedures were performed if the assumption of normal distributed data was valid.

In cases where the normal probability plots yielded data that could not be seen as normally distributed, non-parametric tests were performed using the Kruskal Wallis test. This test was performed to determine if statistically significant differences existed between the mean values of the different formulations and Nurofen® gel in general. Dunn's post-hoc multiple comparison tests were conducted to determine statistically significant differences between all the formulations. Bonferoni corrections on Dunn's p-values were calculated in order to determine which of the test formulations mean values differed statistically significantly from the mean of Nurofen® gel.

These procedures were performed using the statistical data analysis software system, Statistica. All tests were performed at a significant level of 0.05.

CHAPTER 3
RESULTS AND DISCUSSION

3.1 INTRODUCTION

The delivery of an active pharmaceutical ingredient (API) through the skin has long been a promising concept due to its large surface area, ease of access, vast exposure to the circulatory and lymphatic networks, and non-invasive nature of the therapy. This is true whether a local or systemic pharmacological effect is desired (Aukunuru *et al.,* 2007:856).

The amount of an API that can be transported into or through the skin (dermal or transdermal delivery) depends on its ability to permeate the skin at a rate; and in a quantity sufficient to achieve an effective concentration at the target site. Prior to the development of pharmaceutical dosage forms, it is essential to evaluate the physicochemical properties of an API. These properties indicate the suitability of the type of dosage form, as well as any potential problems associated with instability, poor permeation and the target site to be reached (Wells & Aulton, 2002:337).

To determine the recovery of ibuprofen in the different semi-solid formulations that were prepared, the validation of an analytical method was performed. Subsequently, artificial membranes were used to determine whether or not any ibuprofen was released from these formulated products. *In vitro* skin permeation studies followed in order to determine the amount of ibuprofen that diffused into, and through the skin. Tape stripping was performed to obtain ibuprofen concentrations in the stratum corneum and epidermis. The results were analysed and compared to determine whether topical or transdermal delivery was achieved.

Lastly, accelerated stability experiments were conducted at selected time intervals to investigate the stability of the formulated products. This was performed by varying the (1) temperature and (2) humidity conditions over a period of three months.

This chapter deals with the analytical method validation, physicochemical properties, permeation studies and stability testing. The interpretation of the results obtained by these evaluations will provide some insight into the choice of excipients and conditions when preparing formulations for topical/transdermal delivery.

3.2 VALIDATION OF THE ANALYTICAL METHOD

3.2.1 LINEARITY

Linearity of an analytical method is an indication of (1) how well a calibration plot of peak area *versus* concentration produces an approximate straight line, or (2) how well the data fit to the linear equation (equation 2.1; section 2.5.1). Figure 3.1 depicts the linear regression curve of peak areas *versus* ibuprofen concentration (table A.1; annexure A). Through using the peak area ratios and concentrations, a linear regression analysis was used to determine the slope, y-intercept and regression coefficient (table 3.1).

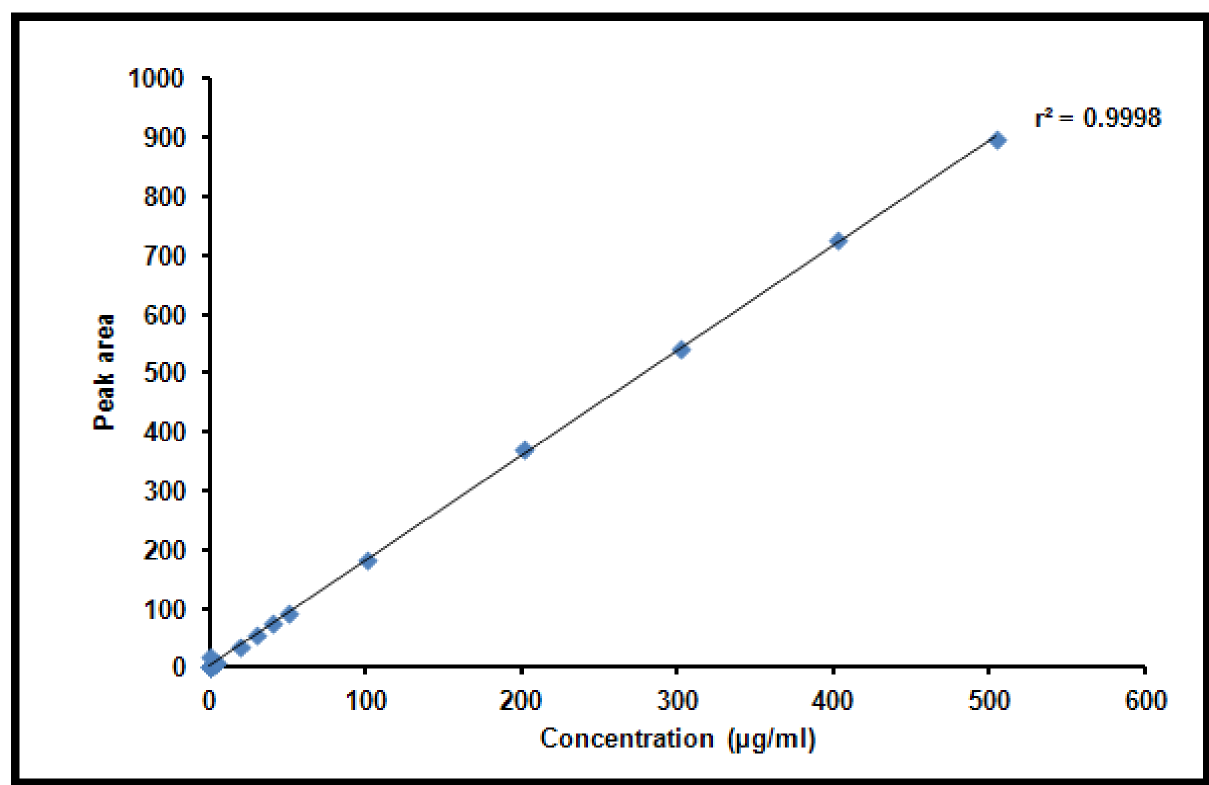


Figure 3.1: *Linearity with peak area as a function of. concentration (µg/ml)*

Table 3.1: *Regression results*

Slope	1.7878
y-intercept	1.6718
r^2	0.9998

r^2 = Correlation coefficient

The obtained regression value ($r^2 = 0.9998$) indicated a linear relationship between ibuprofen and the peak areas over the tested concentration range of 0.504µg.ml^{-1} - 504µg.ml^{-1}.

3.2.2 ACCURACY

The accuracy of an analytical procedure is the closeness of agreement (the degree of scatter) between the true value or an accepted reference value (ICH, 2005:4). According to Du Preez (2010:4), the percentage recovery must be between 98 – 102%. The recovered ibuprofen percentage ranged between 93.70% and 101.70% (table A.2, annexure A). The average ibuprofen recovered was 98.50%, which was within the acceptable range (table 3.2).

Table 3.2: *The mean ibuprofen recovery (%), standard deviation (SD) and coefficient of variation (%RSD)*

Mean (%)	98.50
SD*	3.122
RSD**	3.170

***Standard deviation**
****Relative standard deviation**

3.2.3 PRECISION

Precision considered three levels of analysis, i.e., repeatability (also known as intraday precision), intermediate precision (interday precision) and reproducibility.

3.2.3.1 REPEATABILITY

According to Du Preez (2010:5), the acceptable %RSD for repeatability must be 2.00% or less. The mean, SD and %RSD are presented in table 3.3. A %RSD of 0.200 was obtained, which was within the acceptable range (table A.3, annexure A). This is in an indication that the method was repeatable.

Table 3.3: *The mean ibuprofen recovery (%), standard deviation (SD) and coefficient of variation (%RSD)*

Mean (%)	96.85
SD*	0.190
%RSD**	0.200

*** Standard deviation**
****Relative standard deviation**

3.2.3.2 INTERDAY PRECISION AND REPRODUCIBILITY

The calculated %RSD for interday precision and reproducibility must be 5.00% or less (Du Preez, 2010:5). The mean, SD and %RSD are presented in table 3.4. A %RSD of 1.210 was obtained for interday precision and 0.510 for reproducibility (table A.4 and A.5, annexure A). These results were, thus, within the acceptable range and indicated that the method was precise and reproducible.

Table 3.4: *The mean ibuprofen recovery (%), standard deviation (SD) and coefficient of variation (%RSD)*

	Reproducibility	Interday precision
Mean (%)	99.3	98.5
SD*	0.510	1.200
%RSD*	0.510	1.210

***Standard deviation**
****Relative standard deviation**

3.2.4 RUGGEDNESS

Ruggedness is subdivided into two different categories, namely sample stability and system repeatability.

3.2.4.1 SAMPLE STABILITY

In order to determine the formation of degradation products, sample stability was tested. Acceptance criteria stated that degradation of an API may not be more than 2.00% (Du Preez, 2010:5). The obtained %RSD of 0.200 was within the accepted range (table 3.5). Therefore, ibuprofen exhibited acceptable stability over a period of 24 h (table A.6; annexure A).

Table 3.5: *The mean ibuprofen recovery (%), standard deviation (SD) and coefficient of variation (%RSD)*

Mean (%)	100.0
***SD**	0.200
****%RSD**	0.200

*** Standard deviation**
****Relative standard deviation**

3.2.4.2 SYSTEM REPEATABILITY

In order to comply, the %RSD of the peak areas and retention times of an API should be equal to, or less than 2.00% (Du Preez, 2010:6). As seen in table 3.6, the variation between the obtained values (table A.7; annexure A) proved to be acceptable with a %RSD of 0.100 for peak area and 0.410 for retention time.

Table 3.6: *The mean, standard deviation (SD) and coefficient of variation (%RSD) obtained for the peak areas and retention times of ibuprofen*

	Peak area (mAU)	Retention time (min)
Mean	460.1	5.200
SD*	0.600	0.020
%RSD**	0.100	0.410

***Standard deviation**
****Relative standard deviation**

3.2.5 SPECIFICITY

Figures 3.2 – 3.5 illustrate the chromatograms obtained for specificity.

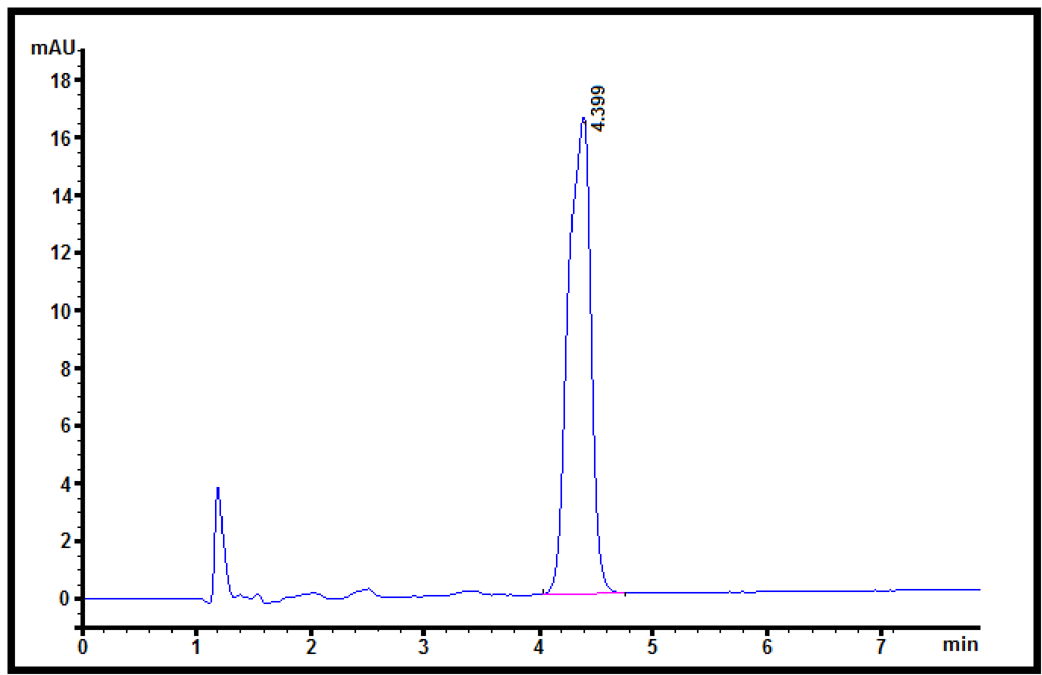


Figure 3.2: *Chromatogram of an ibuprofen sample exposed to water*

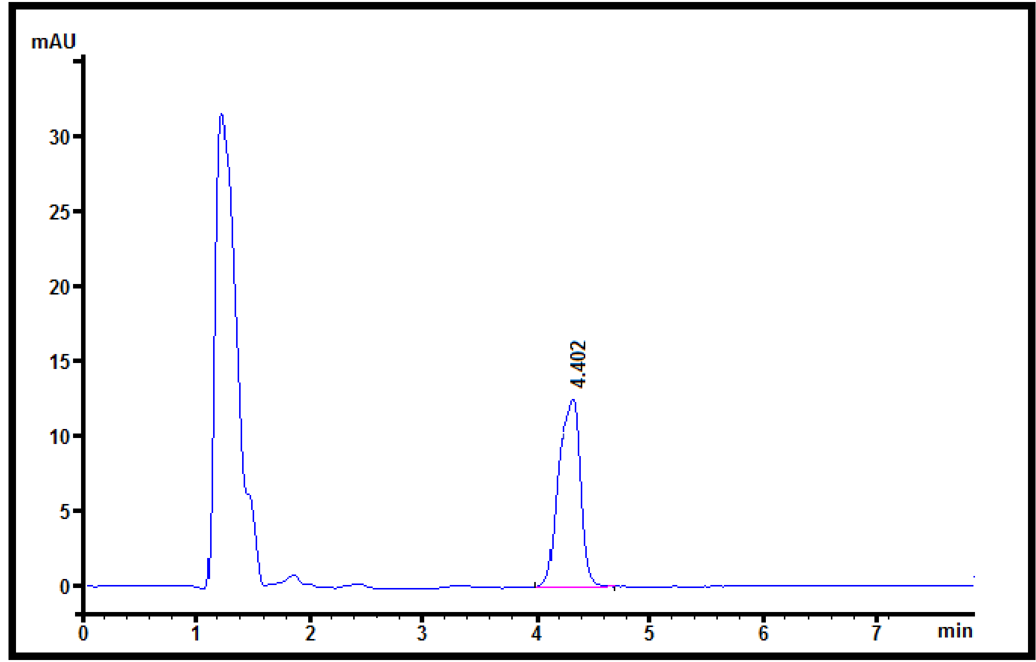


Figure 3.3: *Chromatogram of an ibuprofen sample exposed to 0.1 M hydrochloric acid*

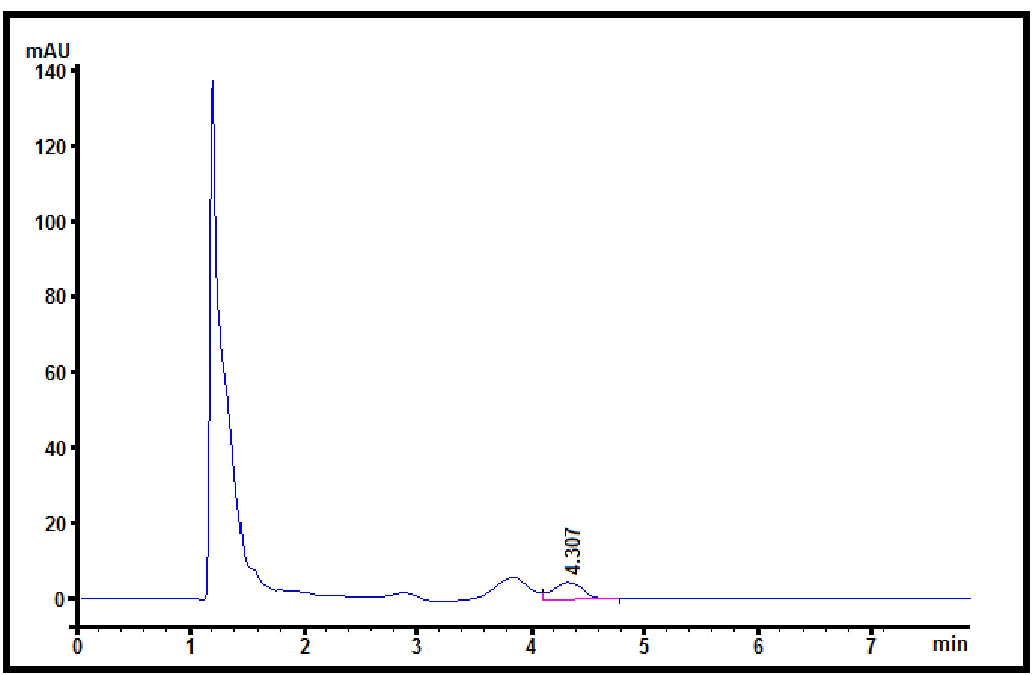


Figure 3.4: *Chromatogram of an ibuprofen sample exposed to 0.1 M sodium hydroxide*

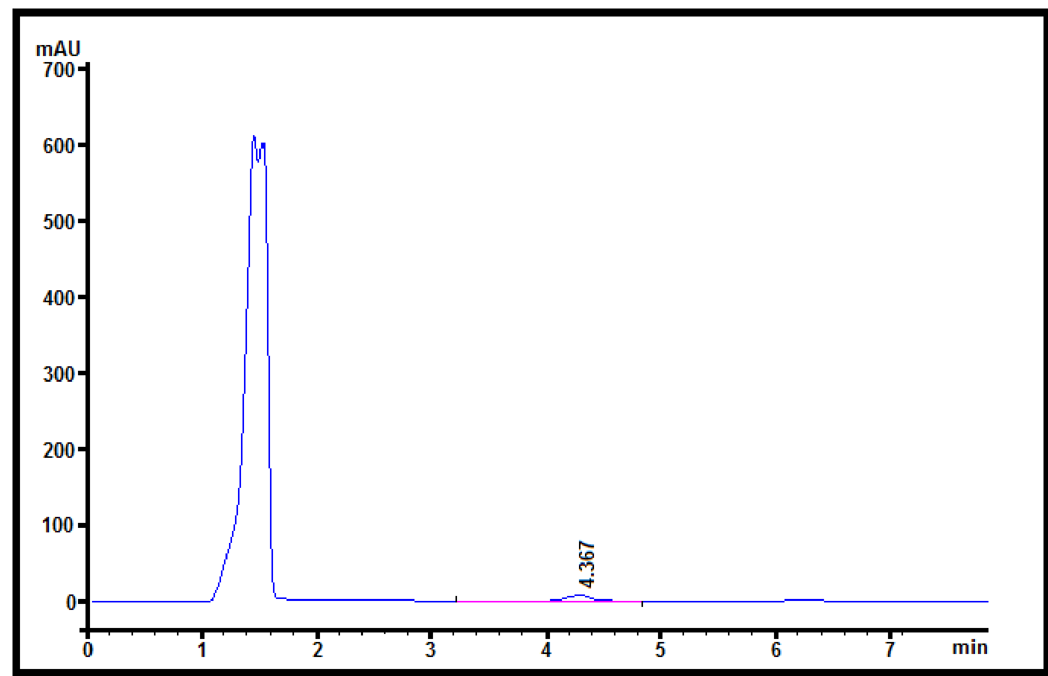


Figure 3.5: *Chromatogram of an ibuprofen sample exposed to 10% hydrogen peroxide*

From these figures it is clear that the formulation excipients and degradation products did not interfere with the ibuprofen peak that eluted at approximately 4.3 min, and this proves the specificity of the analytical method for ibuprofen.

3.2.6 ROBUSTNESS

ICH (2005:5) defines robustness of an analytical procedure as a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and therefore provides an indication of its reliability during normal storage. In this process deliberate changes were made to the injection volume, wavelength and flow rate. Figures 3.6 – 3.8 illustrate the chromatograms.

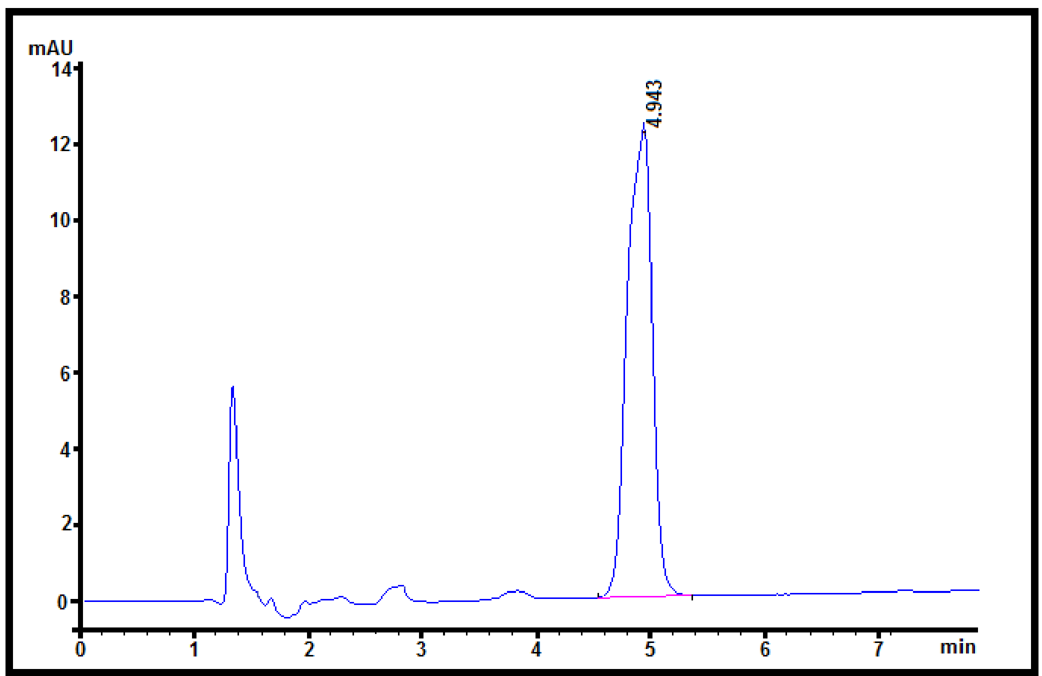


Figure 3.6: *Chromatogram of an ibuprofen stock solution analysed at a flow rate of 0.8 ml.min^{-1}, UV wavelength of 254 nm and an injection volume of 15 µl*

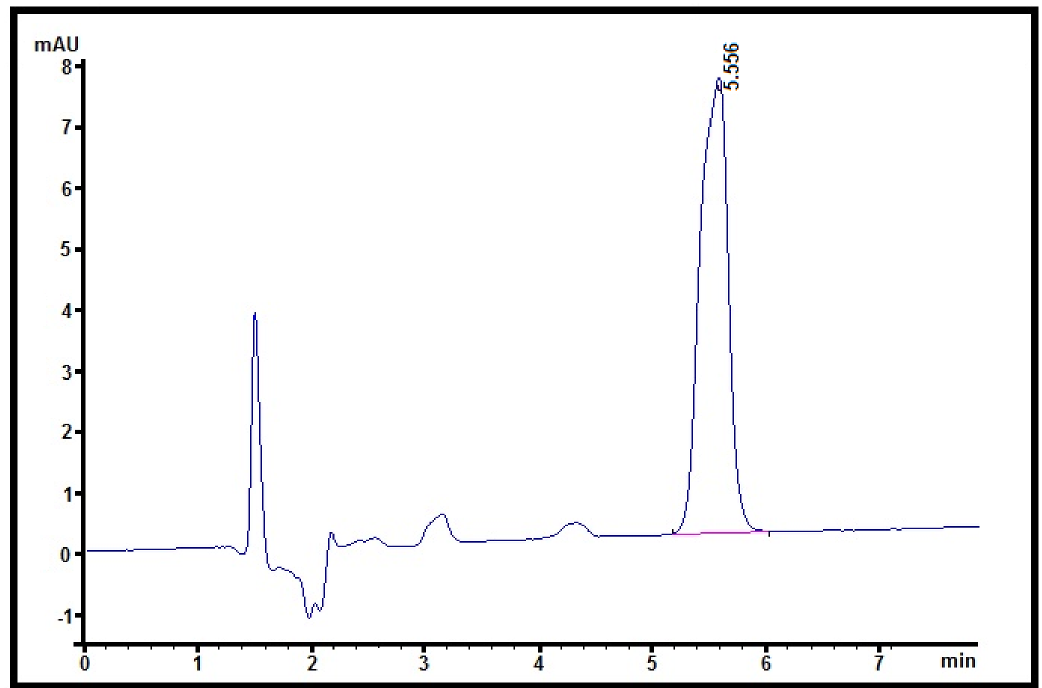


Figure 3.7: *Chromatogram of an ibuprofen stock solution analysed at a flow rate of 0.9 ml.min^{-1}, a UV wavelength of 260 nm and an injection volume of 20 µl*

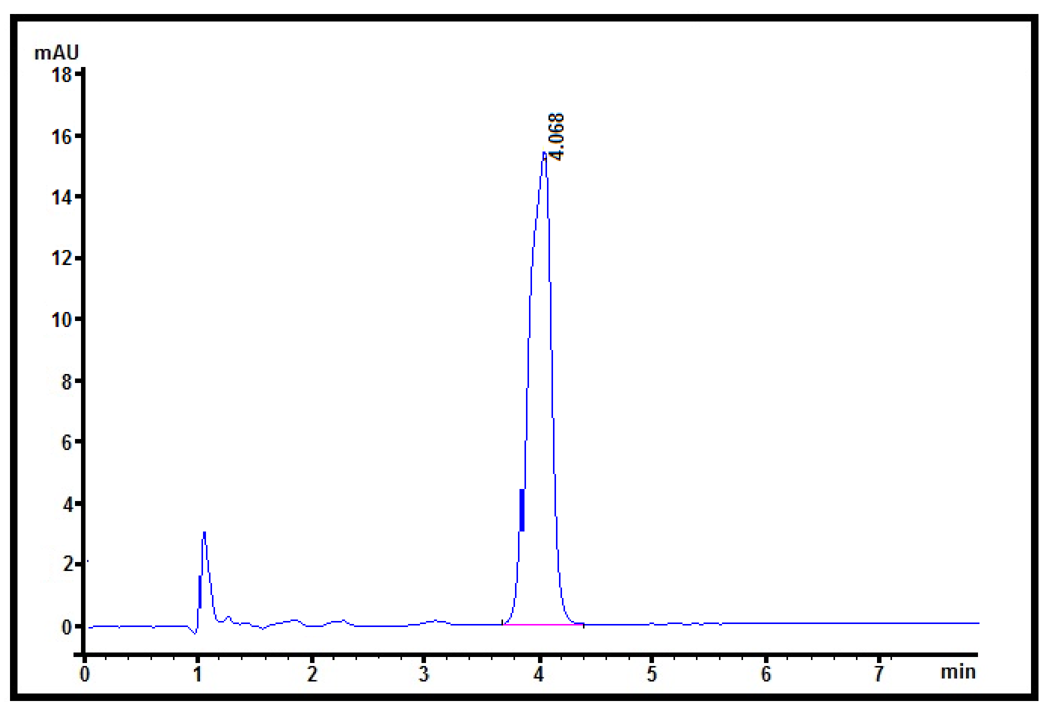


Figure 3.8: *Chromatogram of an ibuprofen stock solution analysed at a flow rate of 1.1 ml.min^{-1}, UV wavelength of 270 nm and an injection volume of 30 µl*

The method was able to tolerate small changes in the chromatographic conditions and therefore it was accepted that the method should perform well under normal use.

3.2.7 CONCLUSION

The results obtained during this validation process proved that the HPLC method for the determination of ibuprofen was sensitive and reliable enough in order to determine the ibuprofen concentration in the different semi-solid formulations.

3.3 PHYSICOCHEMICAL PROPERTIES

Aqueous solubility is an important factor that influences the concentration of API that is presented at the absorption site. The absorption process is strongly influenced by the partition coefficient, which is an indication of the lipophilicity of a molecule. The partition coefficient can be used as a prediction of the ability of an API to cross a biological membrane (Ansel & Popovich, 1990:309, Ashford, 2002:307). This section deals with the results obtained from determining the aqueous solubility, pH-solubility profile, log P-value and log D-values (pH 5 and 7.4) of ibuprofen. If water is used in conjunction with the octanol, the resulting partition coefficient is called log P, whereas if a buffer of a given pH is used, the resulting distribution coefficient is called log D. The log D-value accounts for the ionisation of the molecule at that pH, and is therefore liable to produce an improved prediction of the APIs ability to cross the lipid stratum corneum. The log P-value however, does not consider the degree of ionisation (Ashford, 2002:307).

3.3.1 AQUEOUS SOLUBILITY

According to Naik *et al.,* (2000:319) the ideal aqueous solubility of APIs for transdermal delivery should be more than 1 $mg.ml^{-1}$. The aqueous solubility of ibuprofen was determined as 0.096 $mg.ml^{-1}$ ± 25.483. Thus, ibuprofen is considered practically insoluble in water and would therefore be rendered unsuitable for transdermal delivery if only considering the aqueous solubility (British Pharmacopeia, 2013).

3.3.2 pH-SOLUBILITY PROFILE

Results obtained for the solubility as a function of pH (pH-solubility profile) is illustrated in figure 3.9 (table A.8; annexure A).

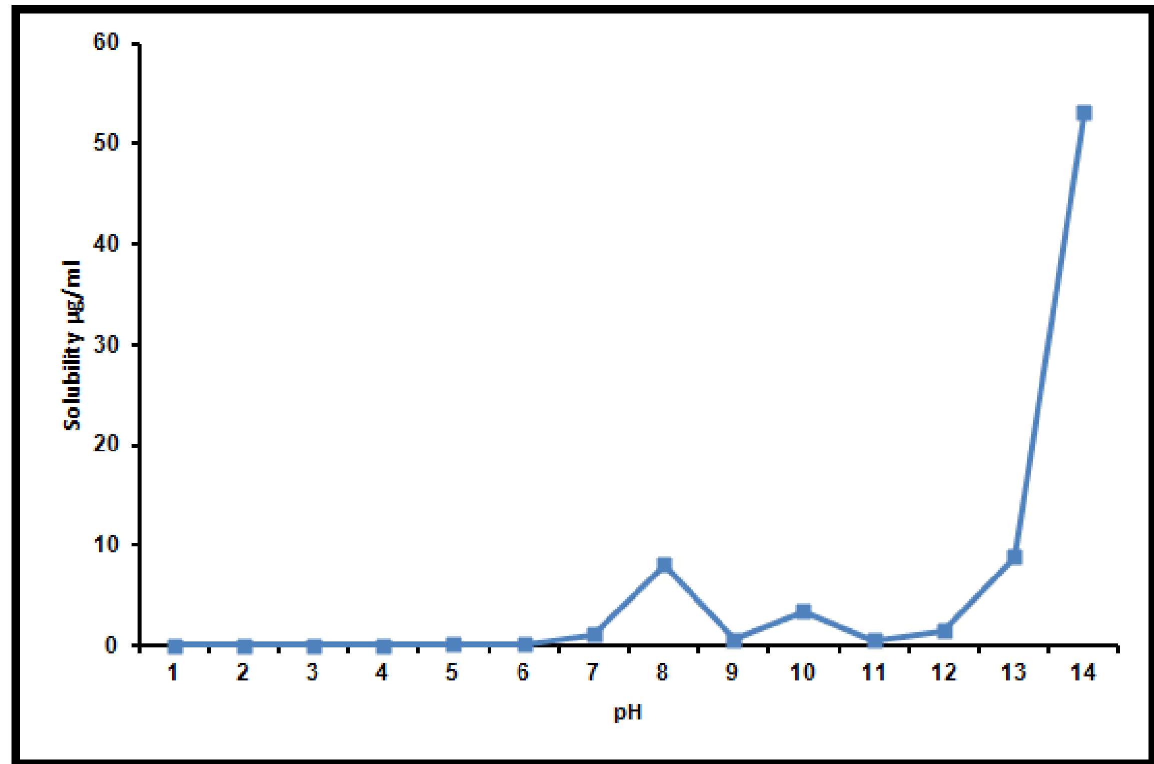


Figure 3.9: *pH-solubilty profile of ibuprofen*

From the graph it is clear that ibuprofen is less soluble at pH 5 than at pH 7.4. In fact, it is practically insoluble at pH 5. However, results obtained between pH 9 and pH 11 were unexpected and therefore requires further investigation. Absorption of an API is possible only when it is present in solution (Mahato, 2007:11). This indicates that ibuprofen will be more soluble at pH 7.4 when compared to pH 5. This is due to the fact that ibuprofen is a weak acidic compound, and for every 3 units away from the pK_a, the solubility changes 10-fold (Mahato, 2007:14).

3.3.3 OCTANOL-WATER DISTRIBUTION COEFFICIENT (log P)

Previous research indicated that the ideal log P-values for transdermal API permeation of NSAIDs are between 2 and 3 (Swart *et al.,* 2005:72). The log P-value of ibuprofen was determined as 4.238. This value is not included in the ideal range, which is an indication that the lipophilic/hydrophilic properties are not ideal and this will therefore contribute to poor ibuprofen penetration through the skin.

3.3.4 OCTANOL-BUFFER DISTRIBUTION COEFFICIENT (log D)

Higgins *et al.,* (2001:280) determined the log D-profile of ibuprofen at a range of pH-values (figure 3.10). They established that the log D-values at pH 5 and 7 were 3.030 and 1.1500, respectively. The obtained log D-values at pH 5 and 7.4 were 3.105 and 0.386, respectively.

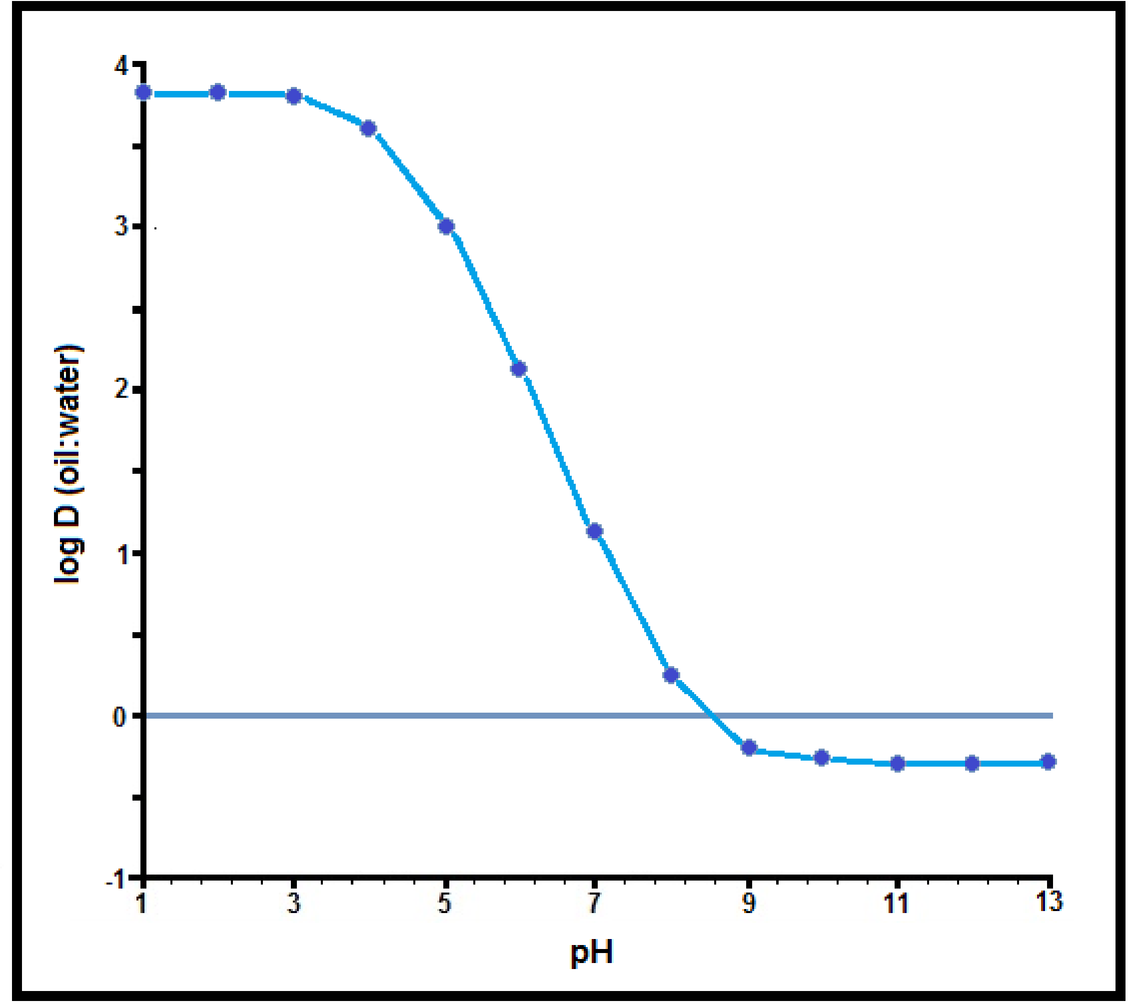


Figure 3.10: *Log D profile of ibuprofen (Higgins et al., 2001:280)*

3.4 PERMEATION STUDIES

Permeation studies were performed to determine the rate and extent of ibuprofen skin permeation. Prior to the performance of skin permeation studies, membrane permeation studies were conducted to determine whether ibuprofen was in fact released from the formulations. Therefore, permeation studies were conducted in two categories, namely; membrane permeation studies and skin permeation studies. This section discusses the results obtained of these two categories.

3.4.1 MEMBRANE PERMEATION STUDIES

An artificial membrane was used to conduct the membrane permeation studies over a period of 6 h. Table B.1 in annexure B shows a summary of the average cumulative amount of ibuprofen released from the formulations and that permeated the membranes over 6 h. From these results figure 3.11 was constructed in order to illustrate the data obtained.

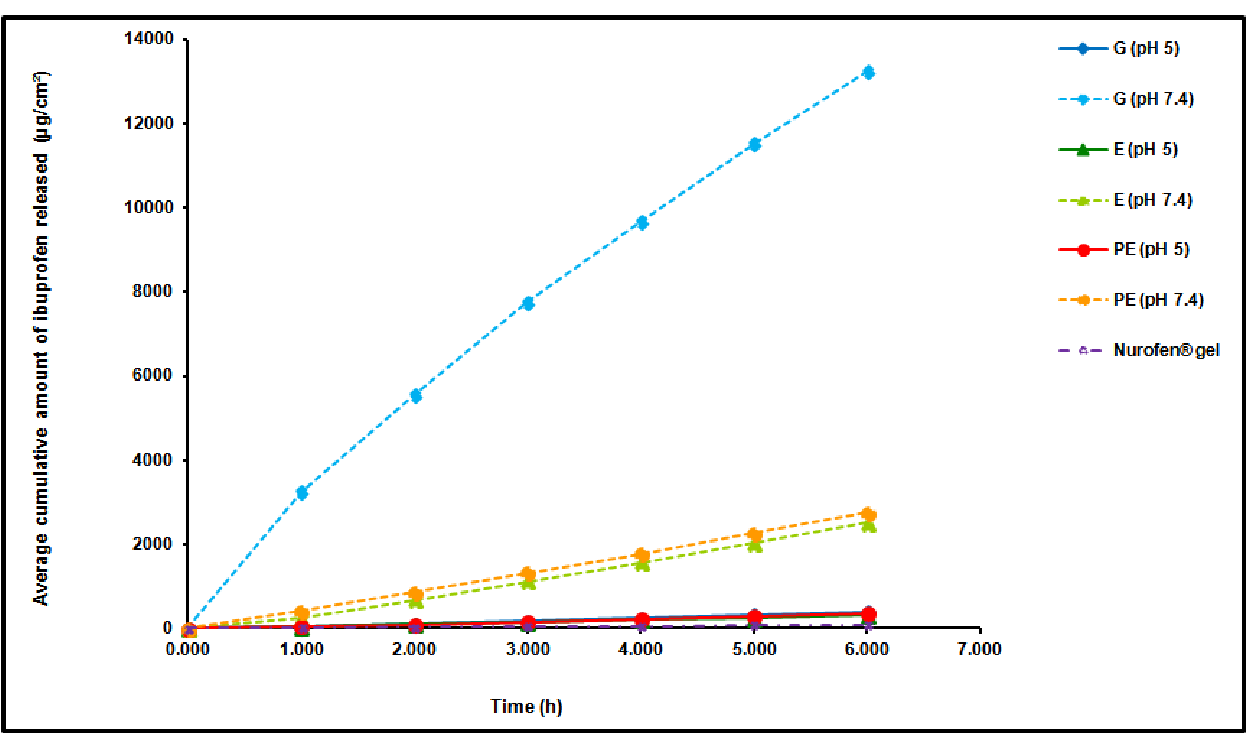


Figure 3.11: *Average cumulative amount of ibuprofen released from the formulations that permeated the membrane over 6 h (E = emulgel, G = gel, PE = Pheroid™ emulgel)*

It is evident from figure 3.11 that:

All the formulations, when compared to Nurofen® gel, exhibited an increase in the average cumulative amount of ibuprofen released from the formulations and that permeated the membrane. This increase was statistically significant ($p < 0.05$) for the emulgel, gel and Pheroid™ emulgel at pH 7.4. The gel at pH 7.4 exhibited the highest average cumulative amount of ibuprofen that was released and that permeated the membrane.

On the other hand, the products formulated at a pH of 5, did not differ significantly from Nurofen® when the average cumulative amount of ibuprofen that permeated the membrane was compared. The following rank order for the average cumulative amount of ibuprofen released from the formulations could be established: Gel (pH 7.4) >>>> Pheroid™ emulgel (pH 7.4) > Emulgel (pH 7.4) >>> Gel (pH 5)> Pheroid™ emulgel (pH 5) ≈ Emulgel (pH 5) > Nurofen® gel. From this rank order it was clear that a trend was followed where both, the type of formulation and pH played a role in releasing the ibuprofen from the formulations.

The pH of a formulation plays an important role with respect to API permeation. As previously discussed, the reported pK_a value of ibuprofen is 4.4 (Dollery, 1999:I1); and by application of the Henderson-Hasselbach equation, at pH 5, 20.08% of ibuprofen is present in its unionised form and at pH 7.4, 0.1% of ibuprofen is present in its unionised form. Since the unionised form of APIs is more lipid soluble than the ionised form, unionised forms of APIs permeate more readily across the lipid membranes (Surber & Smith, 2000:27). Therefore, it would be expected that formulations at pH 5 would exhibit a higher average cumulative amount of ibuprofen permeation than formulations at pH 7.4. However, this did not correspond to the data obtained in figure 3.11. This may be attributed to the solubility of ibuprofen in the different formulations. According to the pH-solubility profile (figure 3.10), ibuprofen is more soluble at pH 7.4 than at pH 5. This is due to the fact that ibuprofen is a weak acidic compound, and for every 3 units away from the pK_a, the solubility changes 10-fold (Mahato, 2007:14).

Flux (apparent release constant) is defined as the total quantity of permeant that will diffuse through the membrane (Surber & Smith, 2000:25). A relationship existed between the flux and the release rate of the ibuprofen from the different formulations (table B.2, annexure B). The release rate is obtained as the slope of the graph when the cumulative amount of ibuprofen is constructed against time. In order to investigate the nature of this relationship, a graph was constructed, and can be seen in figure 3.12.

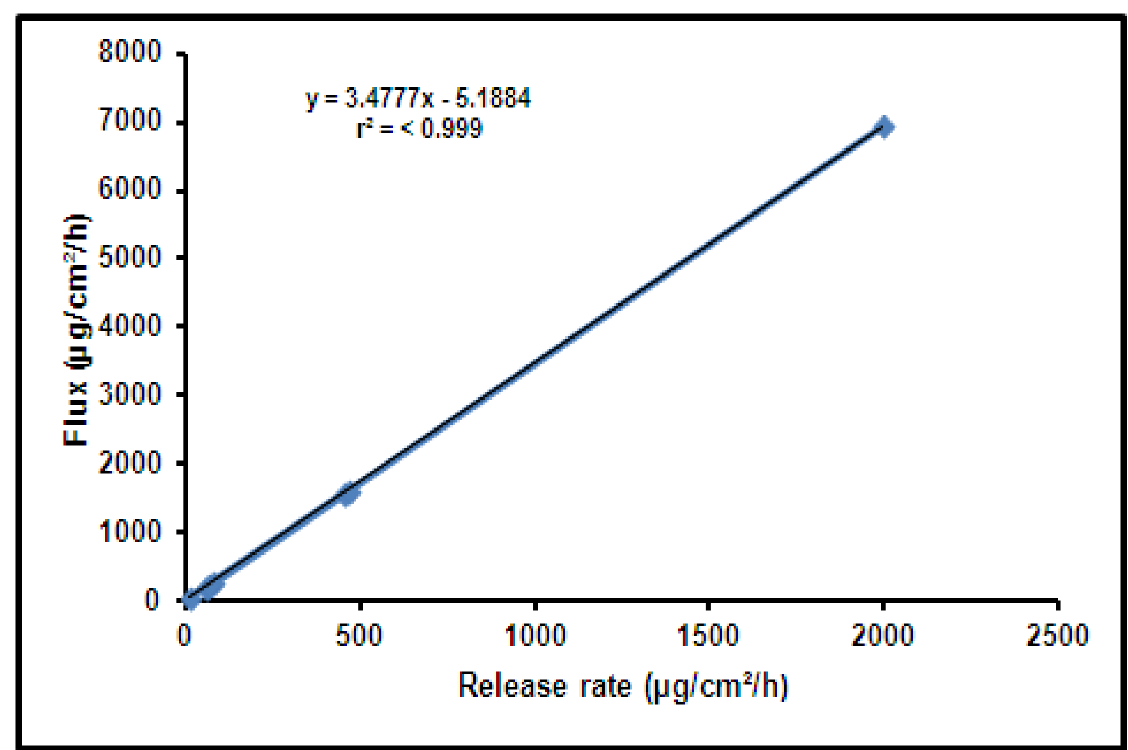


Figure 3.12: *Nature of the relationship between flux and release rate obtained for membrane permeation studies*

The graph in figure 3.12 illustrates a linear relationship ($r^2 < 0.999$). This indicates that as the ibuprofen was released, the flux increased correspondingly. All the formulations, when compared to Nurofen® gel, exhibited a higher release rate and flux. This was statistically significant for the emulgel, gel and Pheroid™ emulgel at pH 7.4. The gel at pH 7.4 exhibited the highest release rate and flux (table B.2, annexure B).

As previously stated, Higuchi described API dissolution from several types of modified release pharmaceutical dosage forms, such as transdermal systems and matrix tablets with water soluble APIs by making use of the Higuchi equation which describes a "square root of time" release kinetics (equation 1.25 and 1.26, section 1.5.3). If the rate of API released obeys this law, the amount of API released is a linear function of the square root of time ($t^{1/2}$).

In order to determine whether the obtained results (table B.3, annexure B) fitted the Higuchi model, the cumulative amount of ibuprofen released per square centimeter was plotted against the square root of time (figure 3.13).

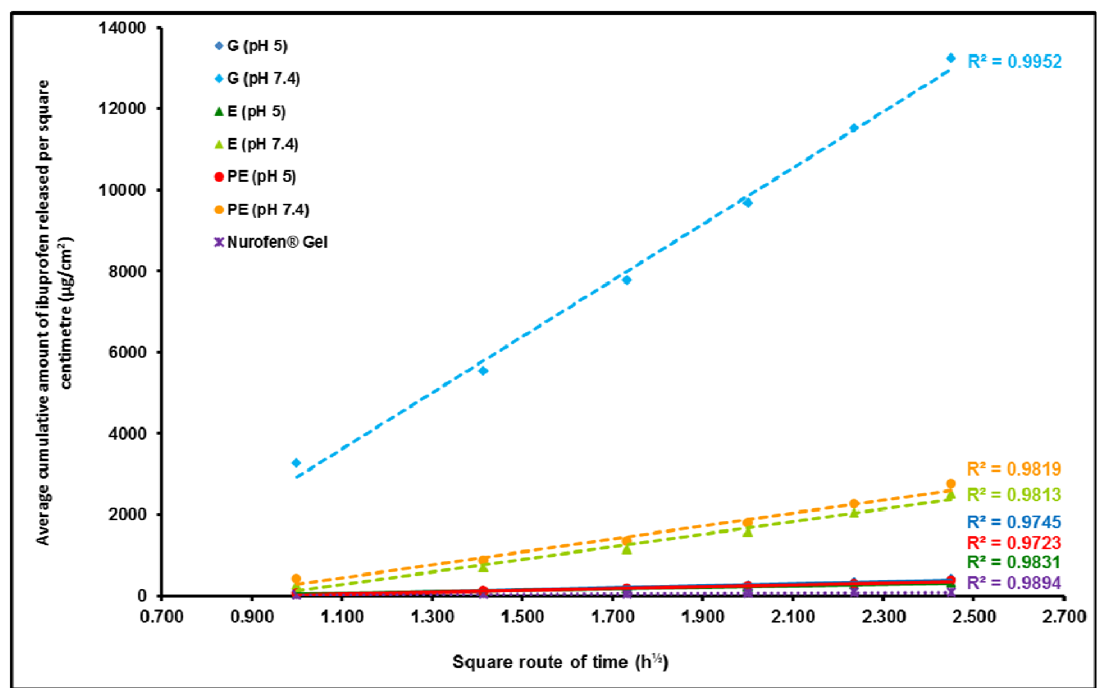


Figure 3.13: *Higuchi plot obtained for membrane permeation studies (E = emulgel, G = gel, PE = Pheroid™ emulgel)*

All the formulations presented a correlation coefficient of (r^2 = 0.972 – 0.995), indicating that the release of ibuprofen from each of the formulations could be described by the Higuchi model.

Considering the lag time, all the formulations, when compared to Nurofen® gel, exhibited a longer lag time (table 3.7). This was statistically significant ($p < 0.05$) for the emulgel at pH 7.4, the gel and Pheroid™ emulgel at pH 5. Longer lag time values indicated that the ibuprofen was retained for a longer time by the base. All the formulations exhibited a prolonged lag time compared to Nurofen® gel which indicated that the ibuprofen was retained for a longer time by the base. This was statistically significant ($p < 0.05$) for the emulgel at pH 7.4, the gel and Pheroid™ emulgel at pH 5. The gel at pH 7.4 exhibited a lag time closest to that of Nurofen® gel and this difference could not be classified as statistically significant ($p > 0.286$).

Table 3.7: *Number of cells used (n), the average lag time values, standard deviations and p-values obtained for membrane permeation studies for the various formulations tested*

Formulation	n*	Average lag time (h)	Standard deviation	p-value	
				Kruskal-Wallis	Bonferoni on Dunn
E (pH 5)	8	0.799	0.050		0.286
E (pH 7.4)	8	0.943	0.068		0.002*
G (pH 5)	7	0.961	0.066		0.0008*
G (pH 7.4)	7	0.573	0.108	<0.001*	0.286
PE (pH 5)	9	0.920	0.034		0.005*
PE (pH 7.4)	10	0.833	0.064		0.286
Nurofen® gel	7	0.556	0.401		

(E = emulgel, G = gel, PE = Pheroid™ emulgel)
n* = data from leaking cells was discarded

3.4.2 SKIN PERMEATION STUDIES

Skin circles were used to conduct the skin permeation studies over a period of 12 h. Table B.4 in annexure B shows a summary of the average cumulative amount of ibuprofen that permeated the skin over 12 h. From these results figure 3.14 was constructed in order to illustrate the data obtained.

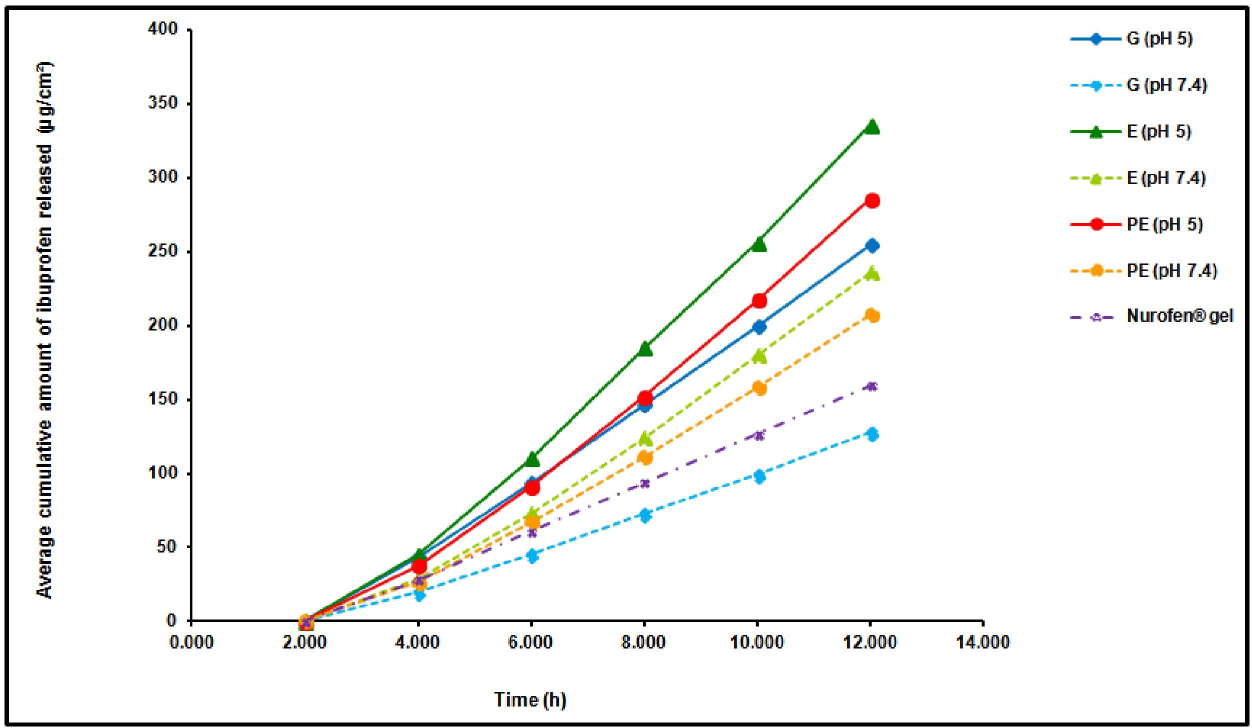


Figure 3.14: *Average cumulative amount of ibuprofen that permeated the skin over 12 h (E = emulgel, G = gel, PE = Pheroid™ emulgel)*

All the formulations (except the gel at pH 7.4), when compared to Nurofen® gel, exhibited an increase in the average cumulative amount of ibuprofen that permeated the skin. This increase

was statistically significant ($p < 0.05$) for the emulgel, gel and Pheroid™ emulgel at pH 5; as well as the emulgel and Pheroid™ emulgel at pH 7.4. The emulgel at pH 5 exhibited the highest average cumulative amount of ibuprofen that permeated the skin. The following rank order for the average cumulative amount of ibuprofen that permeated the skin from the formulations could be established: Emulgel (pH 5) >> Pheroid™ emulgel (pH 5) > Gel (pH 5) > Emulgel (pH 7.4) > Pheroid™ emulgel (pH 7.4) >> Nurofen® gel > Gel (pH 7.4). From this rank order it was clear that a trend was followed where the pH of formulation also played a role in ibuprofen skin permeation, and was thus in accordance with Corrigan *et al.,* (2003:148) who stated that NSAIDs are less soluble and more permeable at low pH values, and more soluble and less permeable at high pH values. This was most probably due to the fact that unionised species, although possessing a lower aqueous solubility than the ionised species, resulted in enhanced skin permeation due to being more lipid-soluble.

Furthermore, as with the membrane release studies, a relationship existed between the flux and the release rate of the ibuprofen from the different formulations (table B.5, annexure B). In order to investigate the nature of this relationship, a graph was constructed, and the nature of the relationship can be seen in figure 3.15.

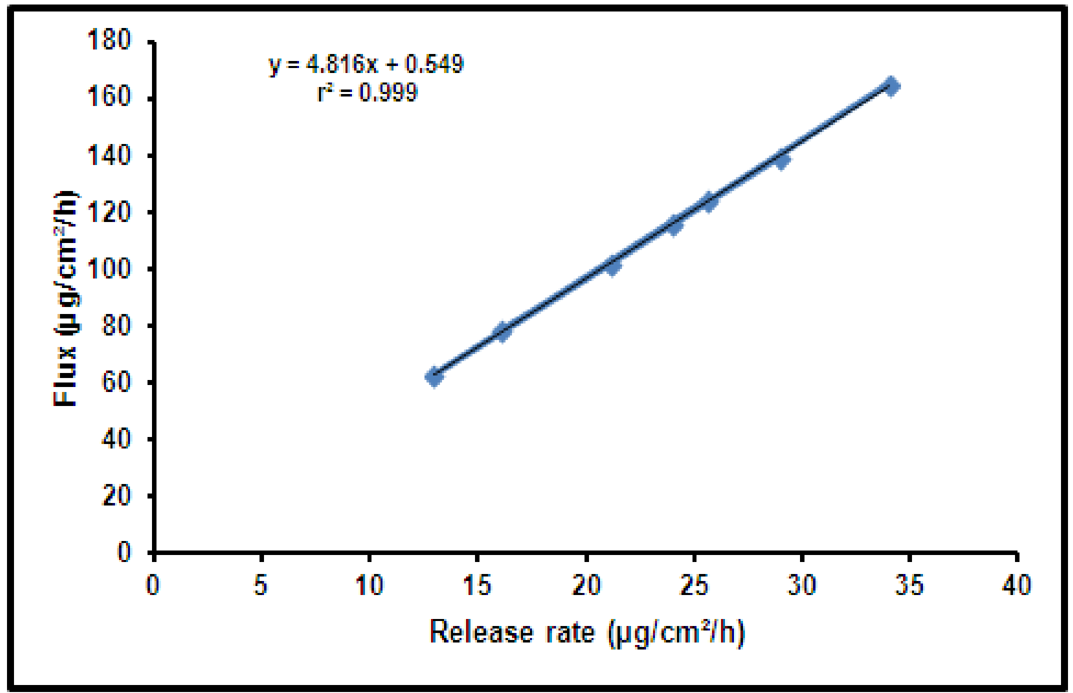


Figure 3.15: *Nature of the relationship between flux and release rate obtained for skin permeation studies*

The graph in figure 3.15 illustrates a linear relationship ($r^2 > 0.999$). This indicated that as the ibuprofen was released, the flux increased correspondingly. All the formulations (except the gel at pH 7.4), when compared to Nurofen® gel, exhibited a higher release rate and flux. This was statistically significant ($p < 0.05$) for the emulgel, gel and Pheroid™ emulgel at pH 5, as well as

for the emulgel and Pheroid™ emulgel at pH 7.4. The emulgel at pH 5 exhibited the highest release rate and flux.

In order to determine whether the obtained results (table B.6, annexure B) fitted the Higuchi model, the cumulative amount of ibuprofen released per square centimeter was plotted against the square root of time (figure 3.16).

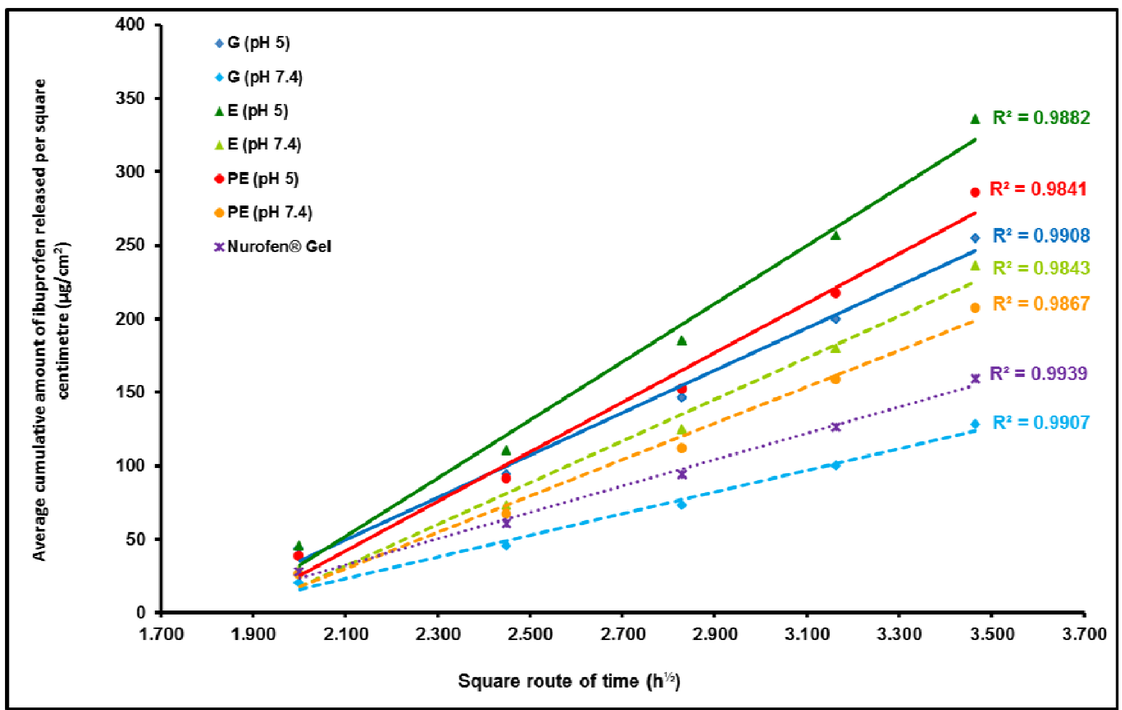


Figure 3.16: *Higuchi plot obtained for skin permeation studies (E = emulgel, G = gel, PE = Pheroid™ emulgel)*

Similar to the membrane permeation studies, the skin permeation studies were also described by the Higuchi model. All the formulations presented a correlation coefficient of (r^2 = 0.950 – 0.978), indicating that the release of ibuprofen from the formulations could be described by the Higuchi model.

Considering the lag time, all the formulations, when compared to Nurofen® gel, exhibited a delayed release of ibuprofen from the dosage form (table 3.8). This was statistically significant ($p < 0.05$) for the gel, emulgel and Pheroid™ emulgel at pH 7.4, as well the emulgel and Pheroid™ emulgel at pH 5. Longer lag time values indicate that the ibuprofen was retained for a longer time by the base. The gel formulation at pH 5 exhibited a lag time closest to that of Nurofen® gel and this difference could not be classified as statistically significant ($p > 0.924$). Despite the statistical differences, all the formulations exhibited a lag time within 2 h. Therefore ibuprofen would be retained for approximately 2 h by the base, thus commencement of action will be delayed for approximately 2 h.

Table 3.8: Number of cells used (n), the average lag time values, standard deviations and p-values obtained for skin permeation studies for the various formulations tested

Formulation	n*	Average lag time (h)	Standard deviation	p-value	
				Welch	Dunnet
E (pH 5)	9	1.609	0.006		0.000009*
E (pH 7.4)	10	1.630	0.007		0.000008*
G (pH 5)	8	1.563	0.005		0.923507
G (pH 7.4)	7	1.582	0.008	<0.001*	0.034251*
PE (pH 5)	9	1.617	0.008		0.000008*
PE (pH 7.4)	9	1.620	0.007		0.000008*
Nurofen® gel	10	1.556	0.0038		

(E = emulgel, G = gel, PE = Pheroid™ emulgel)
n* = data from leaking cells was discarded

3.4.3 TAPE STRIPPING

Nurofen® gel exhibited the highest ibuprofen concentration in the stratum corneum (table B.7; annexure B). However, considering the other formulations, the following rank order could be established: Gel (pH 5) ≈ Gel (pH 7.4) >> Emulgel (pH 5) > Pheroid™ emulgel (pH 7.4) > Pheroid™ emulgel (pH 5) > Emulgel (pH 7.4). The gel formulations showed the highest ibuprofen concentration in the stratum corneum. Since ibuprofen is considered poorly water soluble, the gel formulations contained propylene glycol as a co-solvent. Propylene glycol has a dramatic effect on the solubilising potential of the aqueous systems for lipophilic APIs. Therefore, ibuprofen would exhibit a higher saturation solubility in the co-solvent system compared to pure water; and one would therefore expect ibuprofen permeation rates to be proportionally higher from the co-solvent system, due to the fact that an API needs to be in solution to be absorbed (Surber & Smith, 2000: 27).

Furthermore, Nurofen® gel exhibited the highest ibuprofen concentration in the epidermis as well (table B.8; annexure B). Considering the other formulations, again the following rank order could be established: Emulgel (pH 5) > Gel (pH 7.4) >> Emulgel (pH 7.4) ≈ Gel (pH 5) > Pheroid™ emulgel (pH 7.4) ≈ Pheroid™ emulgel (pH 5). From this rank order it could be established that the Pheroid™ emulgel formulations did not deliver ibuprofen to the same extent to the epidermis, compared to the other formulations.

3.5 STABILITY TESTING OF SEMI-SOLID FORMULATIONS

The stability of a pharmaceutical dosage form relates to the various changes that may occur during preparation and storage, as well as the impact of these changes on its suitability for use. Most formulations are composite physicochemical systems, and are subjected to deterioration by diverse chemical, physical, and microbial reactions. The interaction between an API, excipients and a container may occur, leading to either (1) the inactivation of the API through decomposition or (2) loss of the API by its conversion to a diminished physical or chemical form (Shaikh & Sial, 1996:83).

No pharmaceutical formulation is stable indefinitely and certainly the majority of formulations are stable only for a limited period of time. Instability may be due to degradation of the API or excipients. Poor formulation, packaging and storage conditions may also lead to degradation. Pharmaceutical formulations may be considered instable as a result of (Shaikh & Sial, 1996:83):

1. loss of API,
2. loss of vehicle,
3. loss of content uniformity,
4. loss of elegance,
5. reduction in bioavailability, and
6. production of toxic materials.

Pharmaceutical dosage forms should be stored in compatible, good quality containers. The packaging should be appropriately designed in order to maintain the chemical integrity of the API during the shelf-life of the formulation (Shaikh & Sial, 1996:86).

Stability evaluation simulates ambient stresses to which pharmaceutical products might be exposed to during storage as well as various other conditions other than that in the factory itself. Some of these simulated stresses include the exposure of materials or pharmaceutical products to elevated temperatures and high relative humidites. Evaluation of semi-solid formulations under three sets of conditions (25 °C/60%; 30 °C/60% and 40 °C/75% relative humidity, respectively) can simulate the aging of the products under the conditions for which they were manufactured in the first place (e.g., air-conditioned pharmacy) or for exceptionally unsuitable conditions, i.e., storage in extreme humidity (de Kock, 2005:102).

3.5.1 VISUAL APPEARANCE

The visual appearance of each formulation at all the stability testing conditions were assessed by means of taking photos with a digital camera and comparing the initial colour of the formulation to the colour of the formulations over the three months. Results obtained for the variation of colour can be seen in tables 3.9 - 3.14.

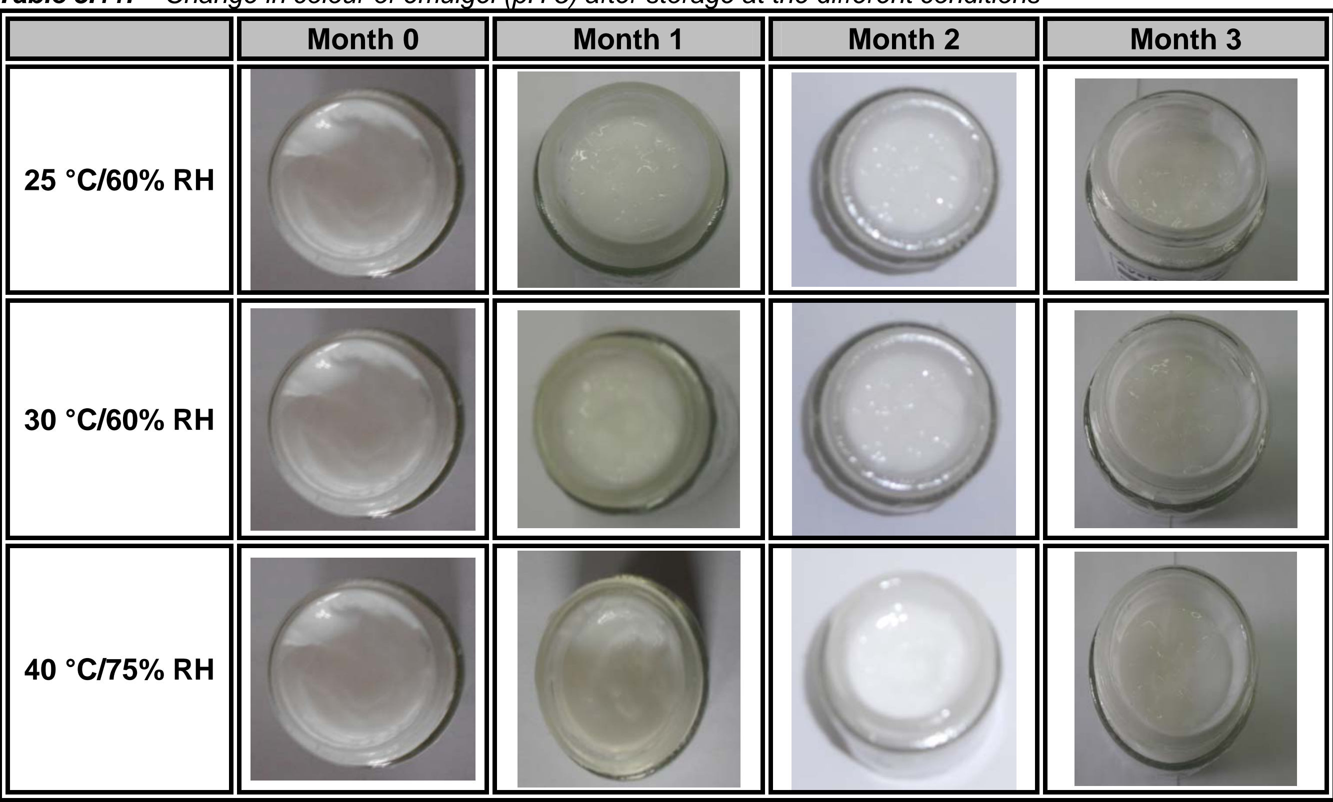
Table 3.9: *Change in colour of gel (pH 5) after storage at the different conditions*

	Month 0	Month 1	Month 2	Month 3
25 °C/60% RH				
30° C/60% RH				
40 °C/75% RH				

Table 3.10: *Change in colour of gel (pH 7.4) after storage at the different conditions*

	Month 0	Month 1	Month 2	Month 3
25 °C/60% RH				
30 °C/60% RH				
40 °C/75% RH				

Table 3.11: *Change in colour of emulgel (pH 5) after storage at the different conditions*

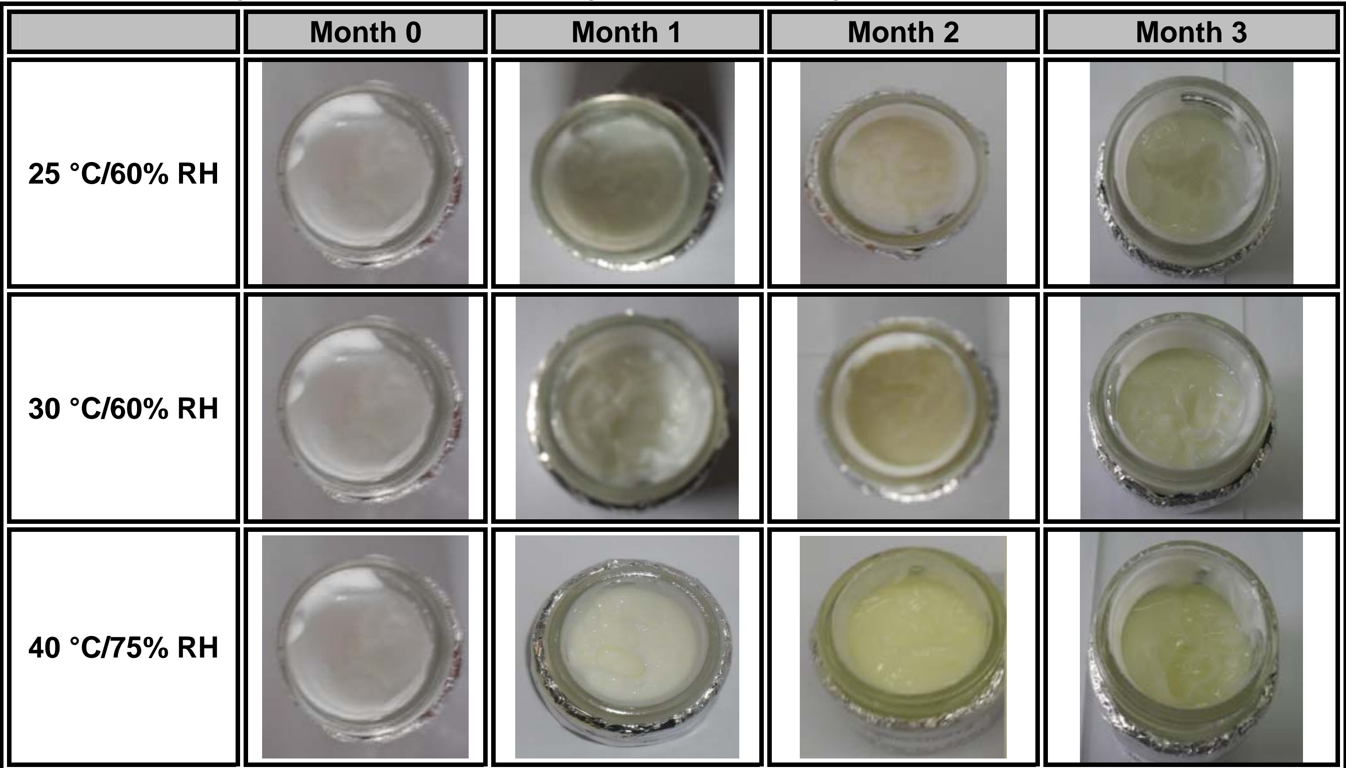


	Month 0	Month 1	Month 2	Month 3
25 °C/60% RH				
30 °C/60% RH				
40 °C/75% RH				

Table 3.12: *Change in colour of emulgel (pH 7.4) after storage at the different conditions*

	Month 0	Month 1	Month 2	Month 3
25 °C/60% RH				
30 °C/60% RH				
40 °C/75% RH				

Table 3.13: *Change in colour of Pheroid™ emulgel (pH 5) after storage at the different conditions*



	Month 0	Month 1	Month 2	Month 3
25 °C/60% RH				
30 °C/60% RH				
40 °C/75% RH				

Table 3.14: *Change in colour of Pheroid™ emulgel (pH 7.4) after storage at the different conditions*

	Month 0	Month 1	Month 2	Month 3
25 °C/60% RH				
30 °C/60% RH				
40 °C/75% RH				

No significant change in colour was observed for the gel and emulgel formulations at pH 5 and 7.4 over the three months at all the storage conditions. However, it was observed that the formulations containing Pheroid™ showed a radical change in colour at all the storage conditions. This may be due to oxidation of certain components such as vitamin E present in the Pheroid™ system. Consequently, further investigation is necessary to find the cause of the discolouration and a method to prevent it.

3.5.2 LIGHT MICROSCOPY

Light microscopy was conducted in order to determine whether the formulations contained any crystals after exposure to the specified temperature and humidity conditions over the three months. Results obtained can be seen in table 3.15.

Table 3.15: *Light microscopy images of formulations after exposure to different storage conditions*

	Month 0	25 C/60% RH	30 C/60% RH	40 C/75% RH
Gel (pH 5)				
Gel (pH 7.4)				
Emulgel (pH 5)				
Emulgel (pH 7.4)				
Pheroid™ emulgel (pH 5)				
Pheroid™ emulgel (pH 7.4)				

From table 3.15, it is clear that the gel formulated at pH 5 depicted the formation of crystals. No significant change was observed for the emulgel and Pheroid™ emulgel formulated at pH 5 and 7.4.

3.5.3 MASS VARIATION

Mass variation is considered an indication of the quality of the containers rather than the stability of the API (Taylor, 2002:627). Results obtained for the variation in mass is seen in table C.1, annexure C. No significant changes were observed for any of the semi-solid formulations exposed to the different stability conditions (< 0.7 g; %RSD < 0.9) during the time of exposure. Therefore, it could be concluded that appropriate containers were used to store the various semi-solid formulations with regards to mass variation.

3.5.4 ASSAY

To determine whether the ibuprofen concentration in the different formulations changed in relation to its initial or previous value during the course of stability testing, an assay was performed by means of HPLC analysis (section 2.4.1). This data is presented in table C.2 in annexure C. Figures 3.17 – 3.22 illustrate the percentage of ibuprofen present in the different formulations over the three month period.

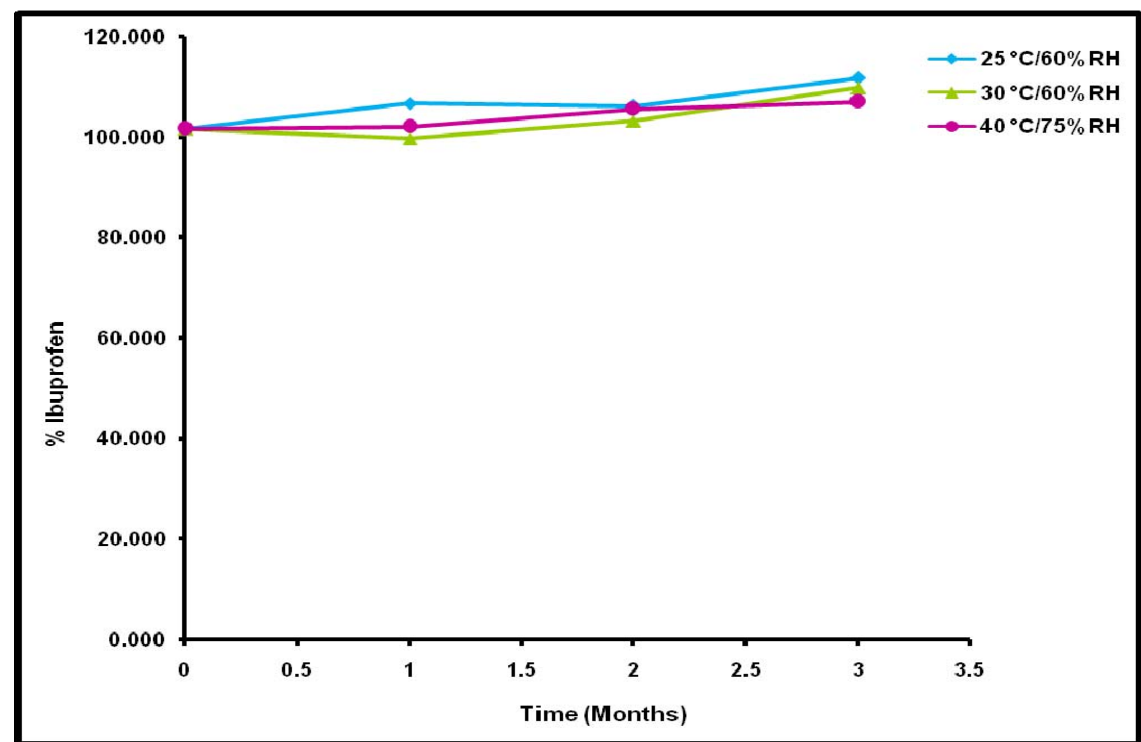


Figure 3.17: *Percentage of ibuprofen present in the gel (pH 5) at the specified conditions after each time interval*

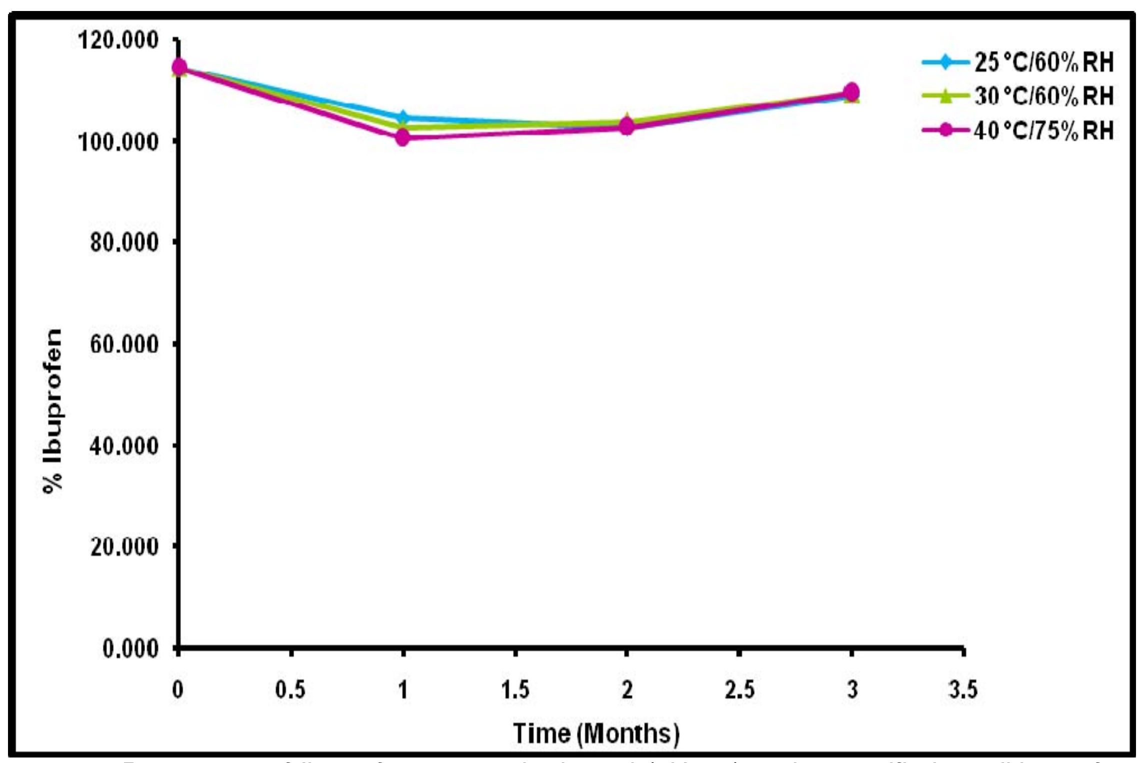


Figure 3.18: *Percentage of ibuprofen present in the gel (pH 7.4) at the specified conditions after each time interval*

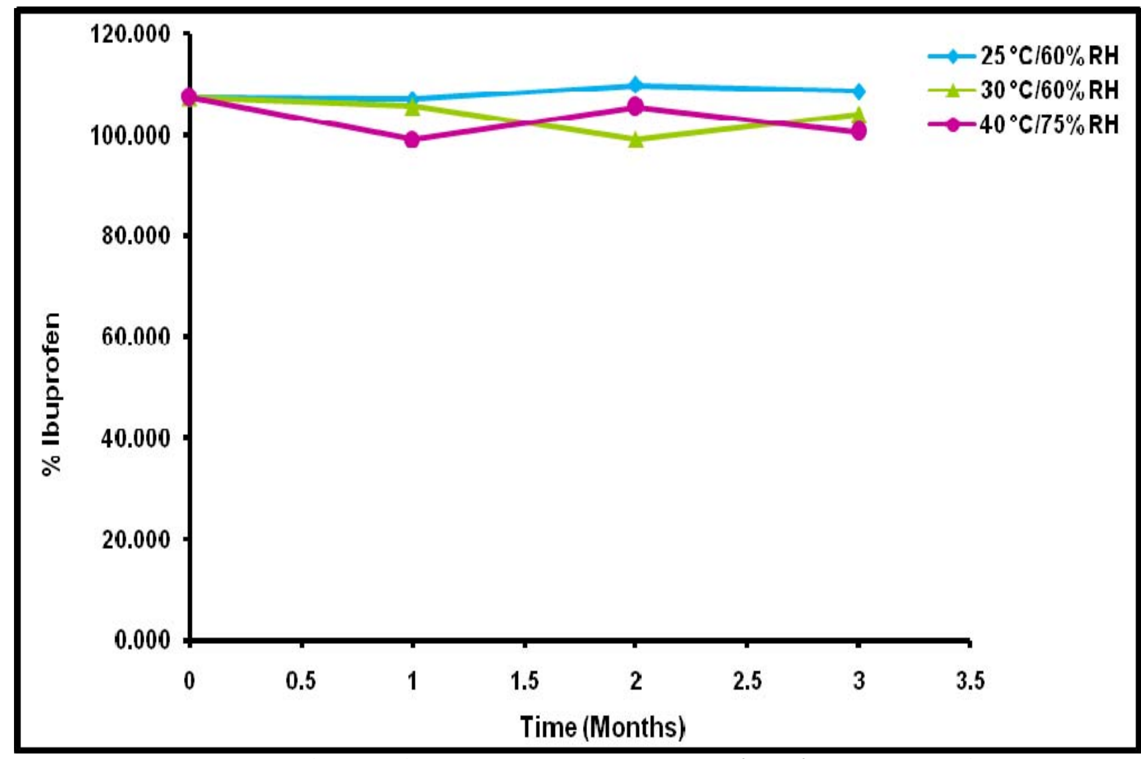


Figure 3.19: *Percentage of ibuprofen present in the emulgel (pH 5) at the specified conditions after each time interval*

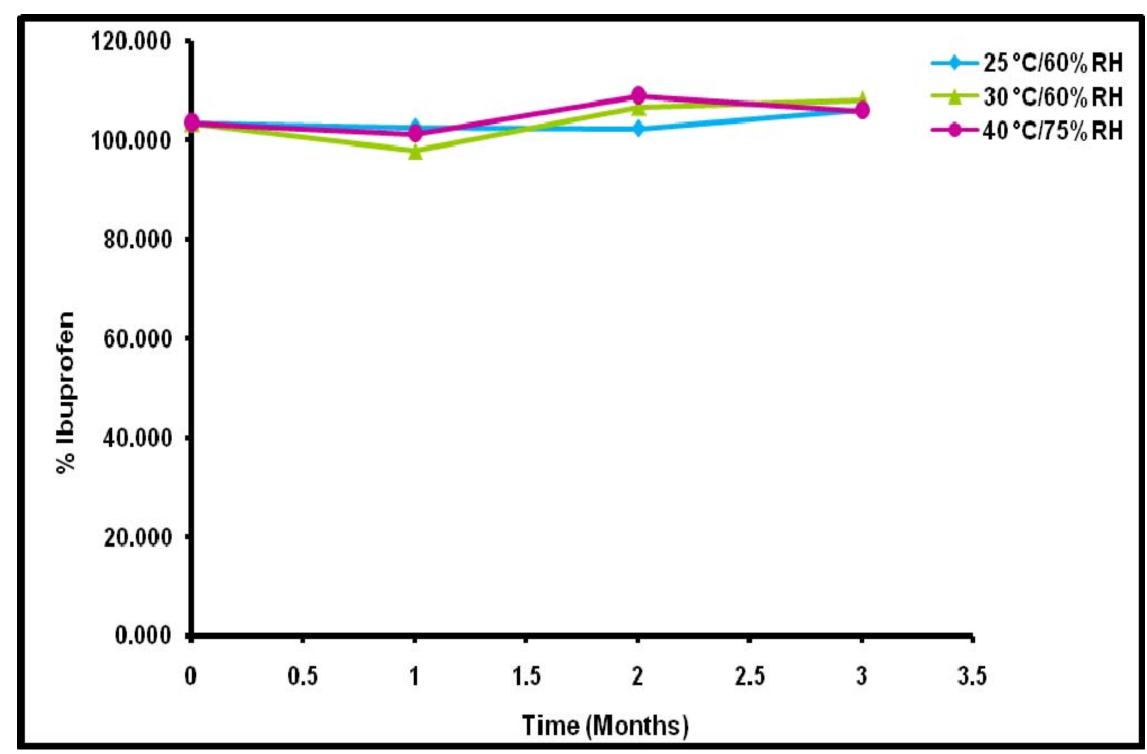


Figure 3.20: *Percentage of ibuprofen present in the emulgel (pH 7.4) at the specified conditions after each time interval*

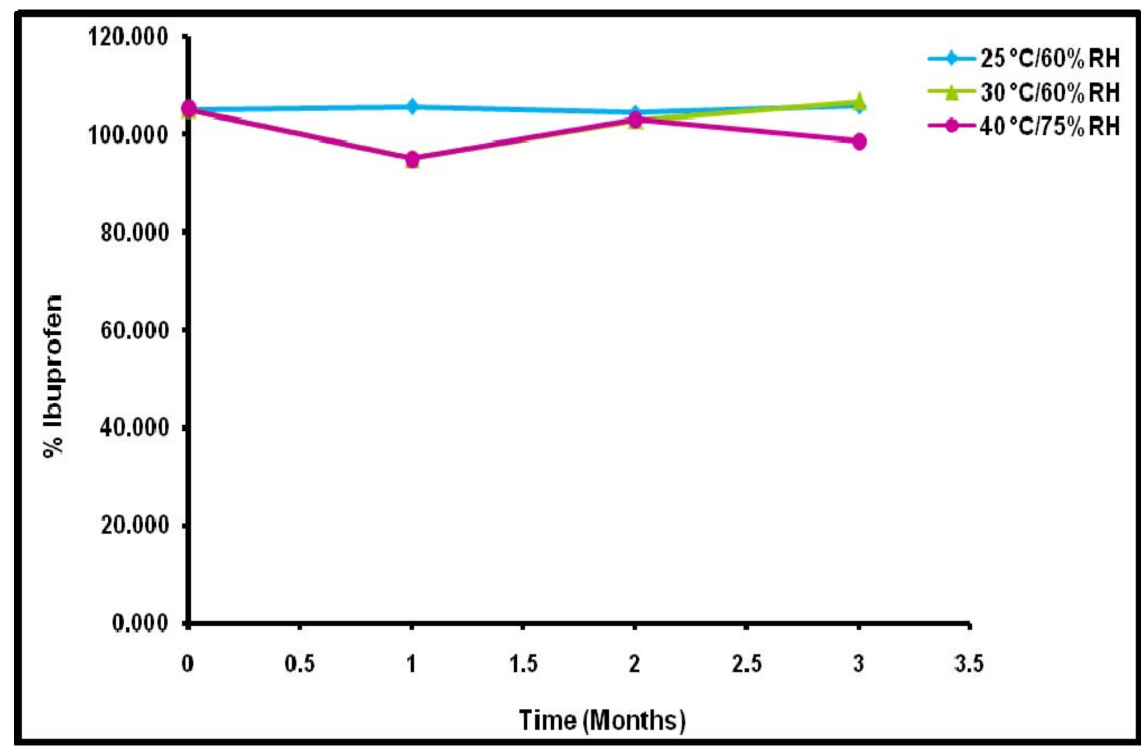


Figure 3.21: *Percentage of ibuprofen present in the Pheroid™ emulgel (pH 5) at the specified conditions after each time interval*

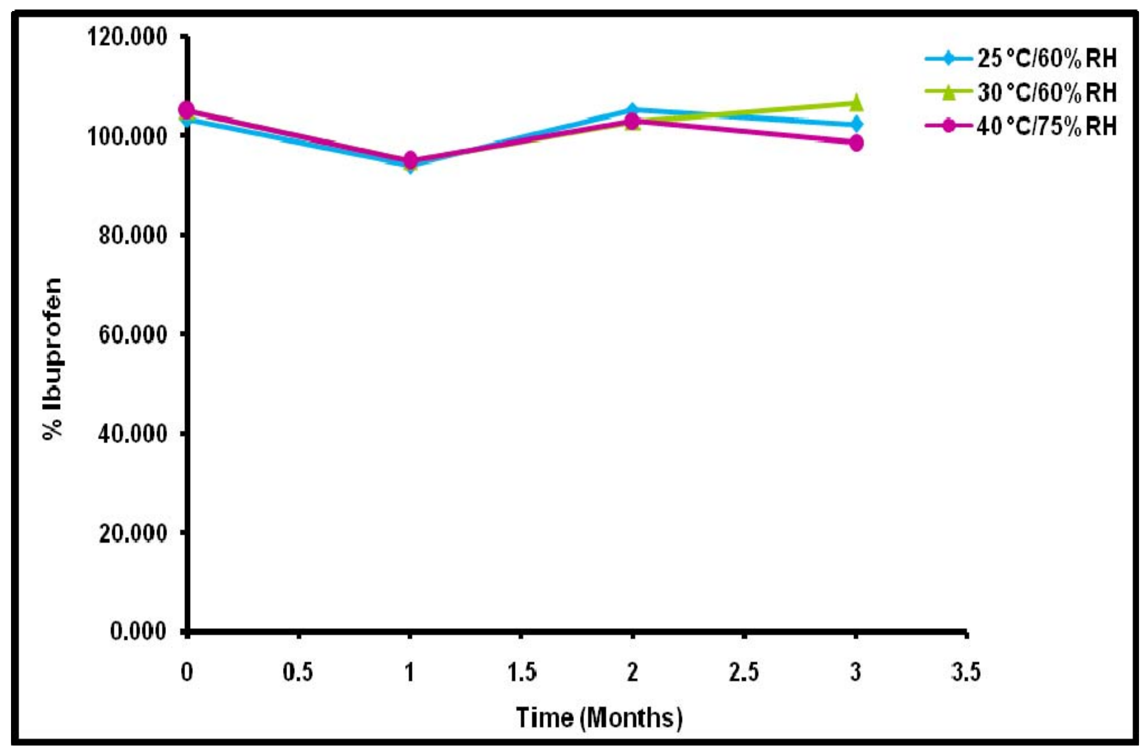


Figure 3.22: *Percentage of ibuprofen present in the Pheroid™ emulgel (pH 7.4) at the specified conditions after each time interval*

According to the ICH (2005:9) a change in concentration of more than 5% indicates instability of the product. Considering all of the formulations, the gel at pH 5 showed the most pronounced change in ibuprofen content at all of the storage conditions. This may be due to the fact that the solubility of ibuprofen was exceeded, leading to it precipitating from the formulation, thereby causing the formation of crystals. A contributing factor to the values obtained might have been due to the withdrawal of a non-homogenous sample. On the other hand, the emulgel and Pheroid™ emulgel at pH 5 depicted relative instability (according to the ICH) only at 40 °C/75% RH with a change in ibuprofen content of more than 5% (6.78 and 6.46%, respectively). The gel, emulgel and Pheroid™ emulgel at pH 7.4 exhibited the least variation in the change of ibuprofen content at all of the storage conditions.

3.5.5 pH

The pH of the various semi-solid formulations was measured each month for three months. The results obtained are summarised in table C.3 of annexure C. Figures 3.23 – 3.25 depict the pH-variation over the 3 month time period for the various semi-solid formulations.

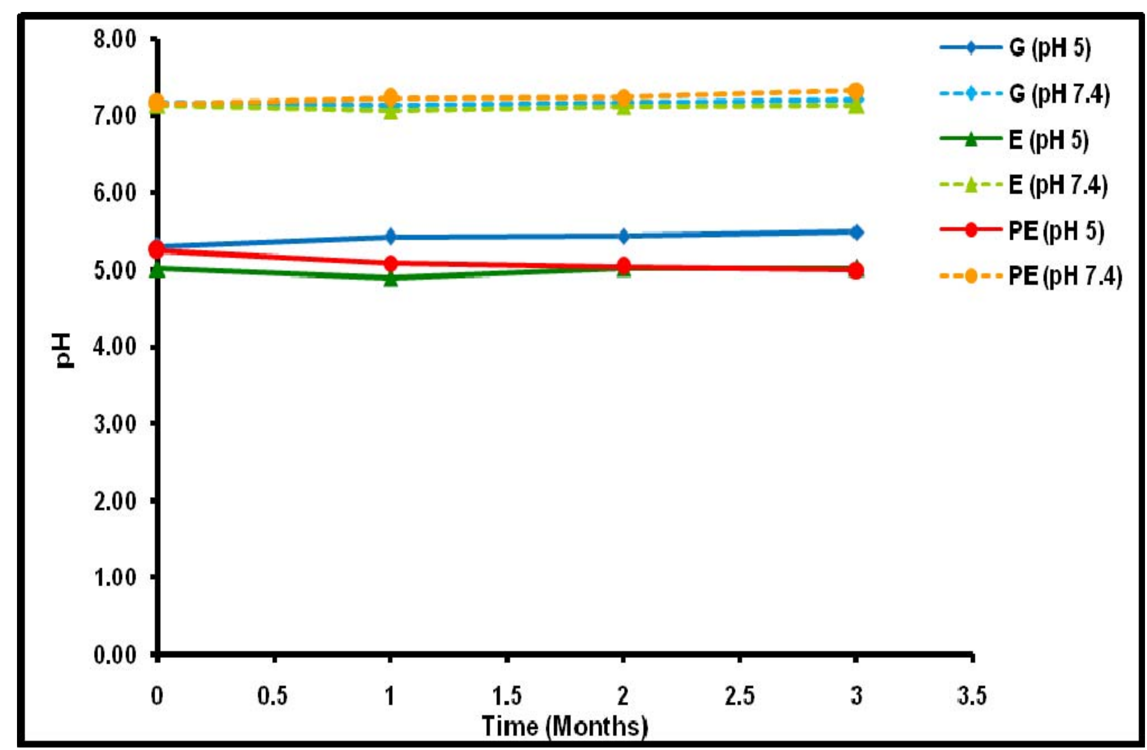


Figure 3.23: *pH of ibuprofen formulations at 25 °C/60% RH (relative humidity) after each time interval*

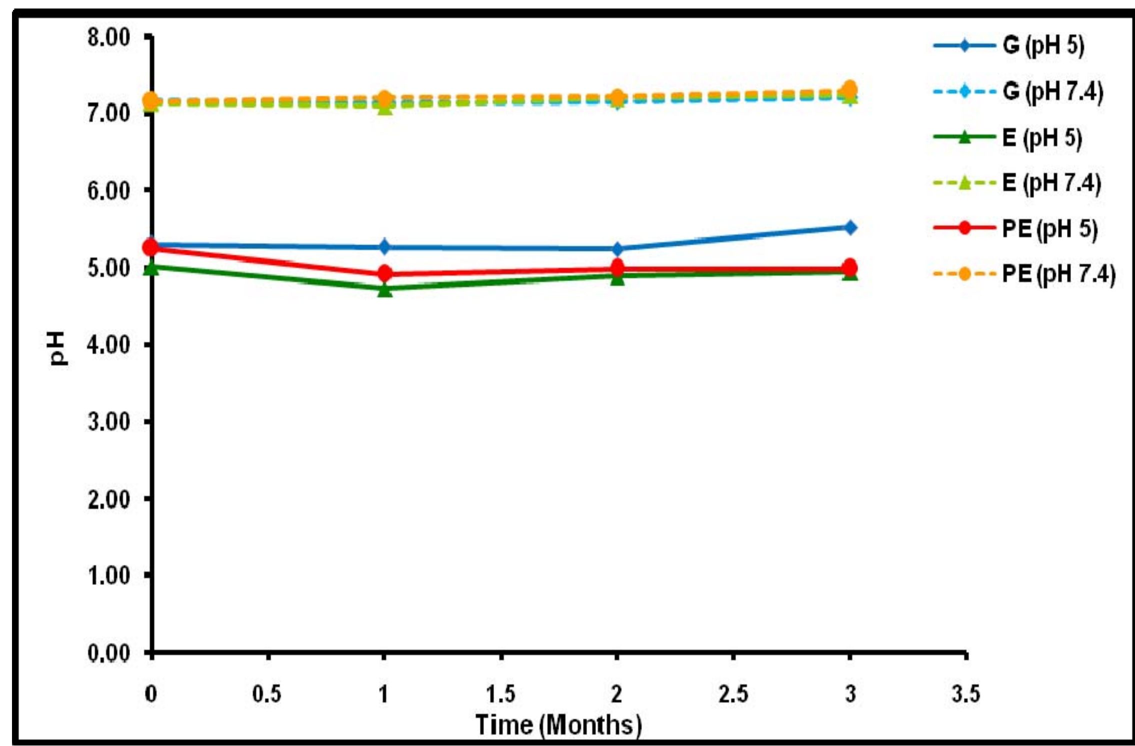


Figure 3.24: *pH of ibuprofen formulations at 30 °C/60% RH (relative humidity) after each time interval*

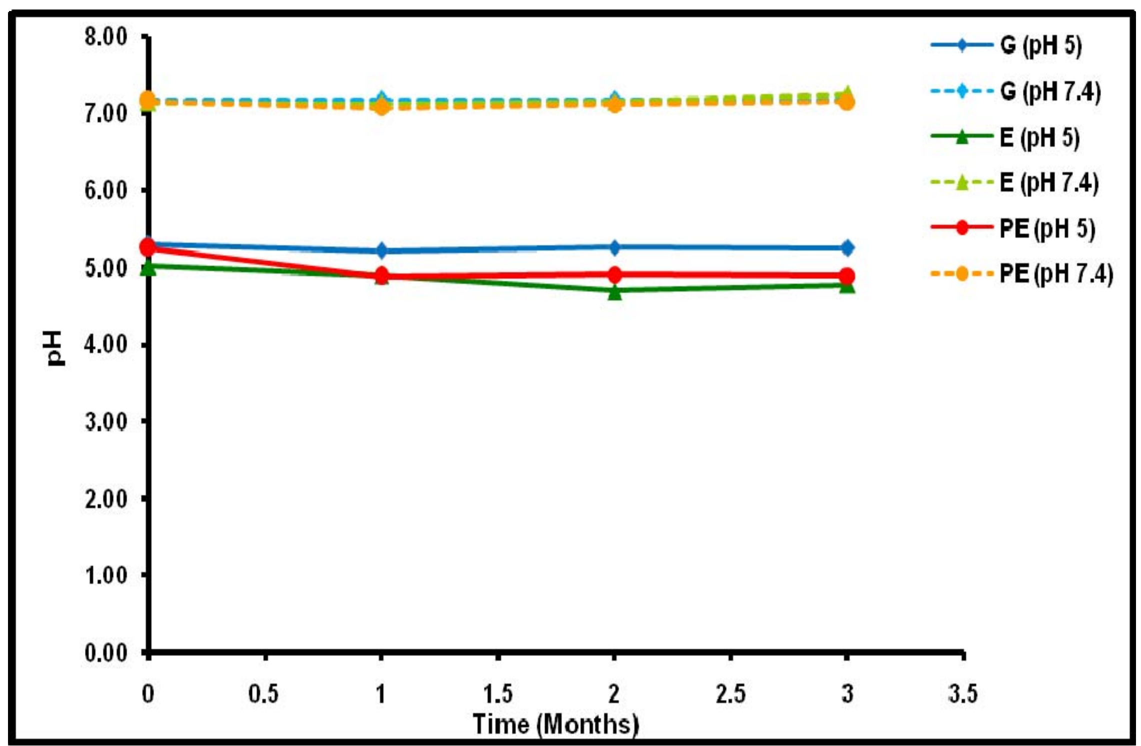


Figure 3.25: *pH of ibuprofen formulations at 40 °C/75% RH (relative humidity) after each time interval*

No significant change ($p > 0.05$) was observed over the three months for any of the different formulations stored at the different storage conditions. Thus, considering pH-change, all of the semi-solid formulations were considered physically stable (< 5% change). However, differences between the formulations could still be established. The gel, emulgel and Pheroid™ emulgel at pH 7.4 showed the least variation in pH at all of the different storage conditions, with a maximum change (for all three stability conditions) in the pH-values of 0.04, 0.38 and 1.13, respectively. Again, these results indicated that the pH at which a semi-solid formulation is formulated will have an influence on the stability of the product, though in this case, it was not significant ($p > 0.05$). Furthermore, the formulations at pH 5 exhibited a higher variation in pH at the various storage conditions. Thus, all of these formulations at all of the conditions were still considered stable with a change in pH-values of less than 5%. A trend could be depicted between the various formulations formulated at the different pH values, which could be placed in the following rank order: Gel > Emulgel > Pheroid™ emulgel. Interestingly, an increase in temperature and humidity did not play a significant role in changing the pH of the semi-solid formulations.

3.5.6 VISCOSITY

Viscosity is a measure of a fluid's resistance to flow. In order to determine whether any of the formulations stored at the different stability conditions exhibited a change in its resistance to flow, viscosity was measured. This data is presented in table C.4 in annexure C.

Figure 3.26 – 3.31 illustrate the change in viscosity of the different formulations over the three month period.

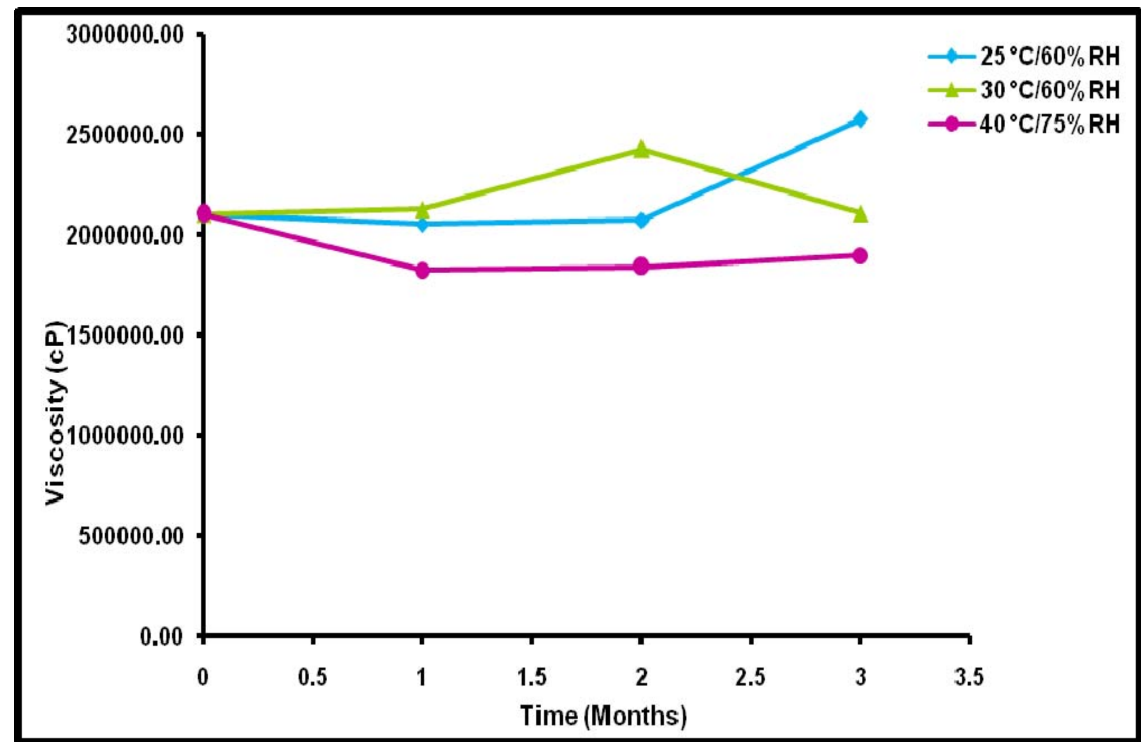


Figure 3.26: *Change in viscosity of the gel (pH 5) at the specified conditions after each time interval*

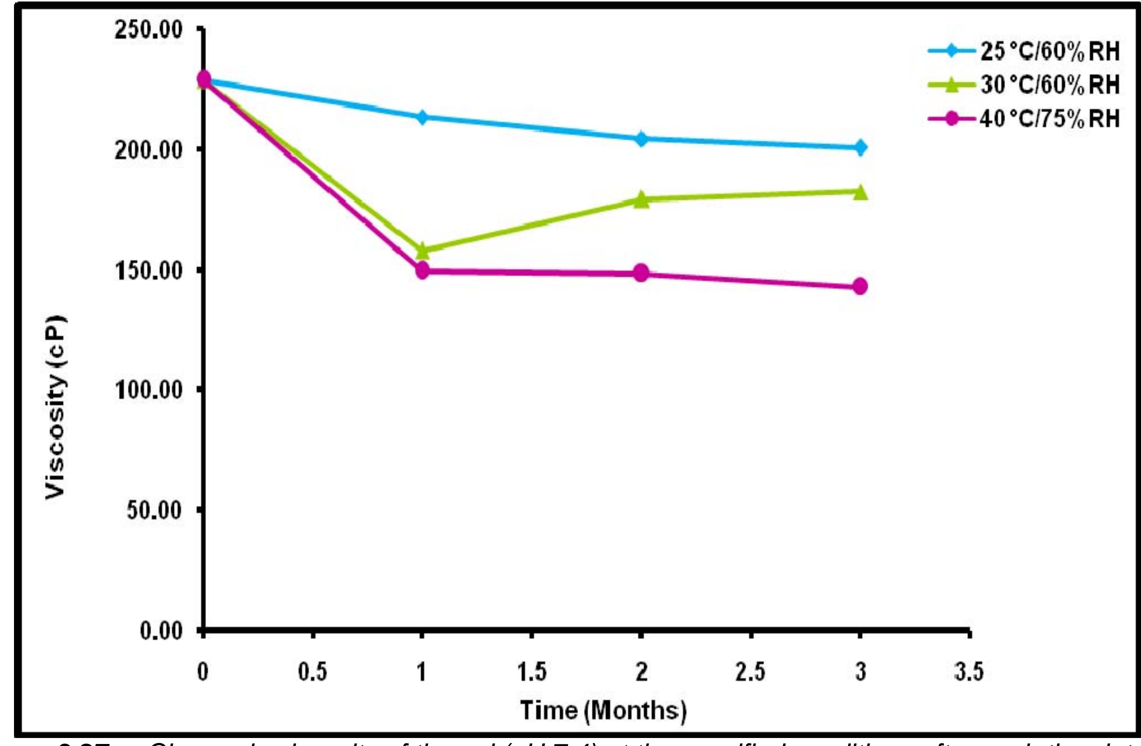


Figure 3.27: *Change in viscosity of the gel (pH 7.4) at the specified conditions after each time interval*

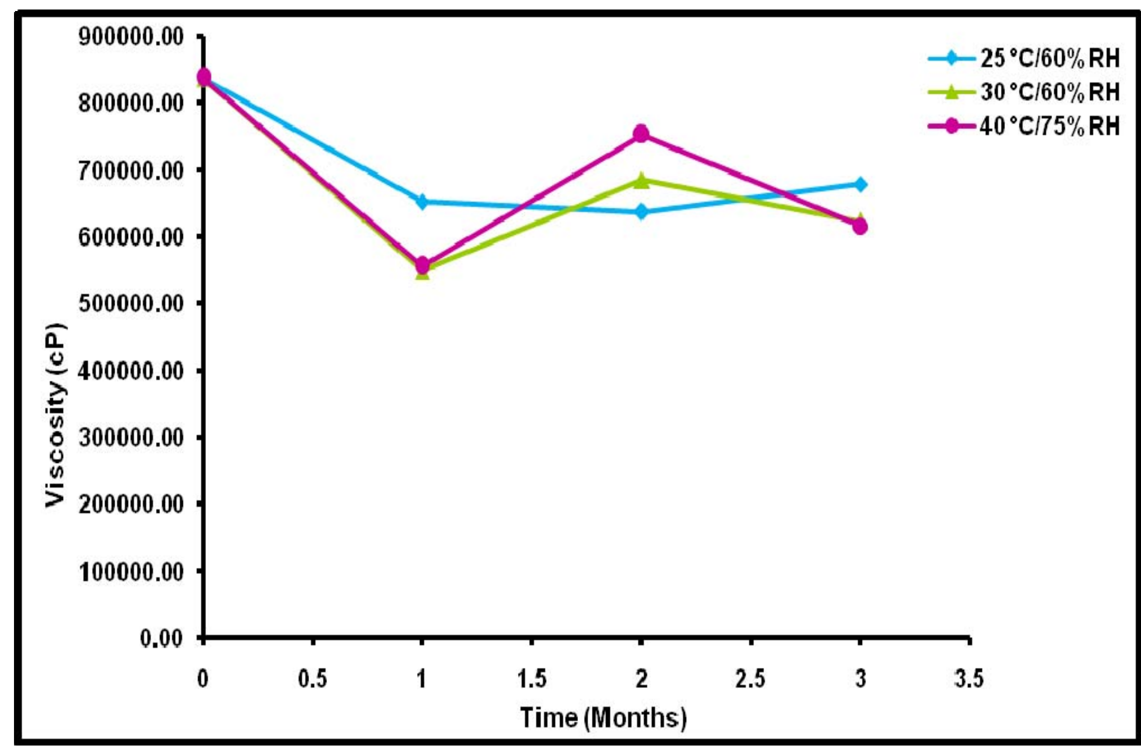


Figure 3.28: *Change in viscosity of the emulgel (pH 5) at the specified conditions after each time interval*

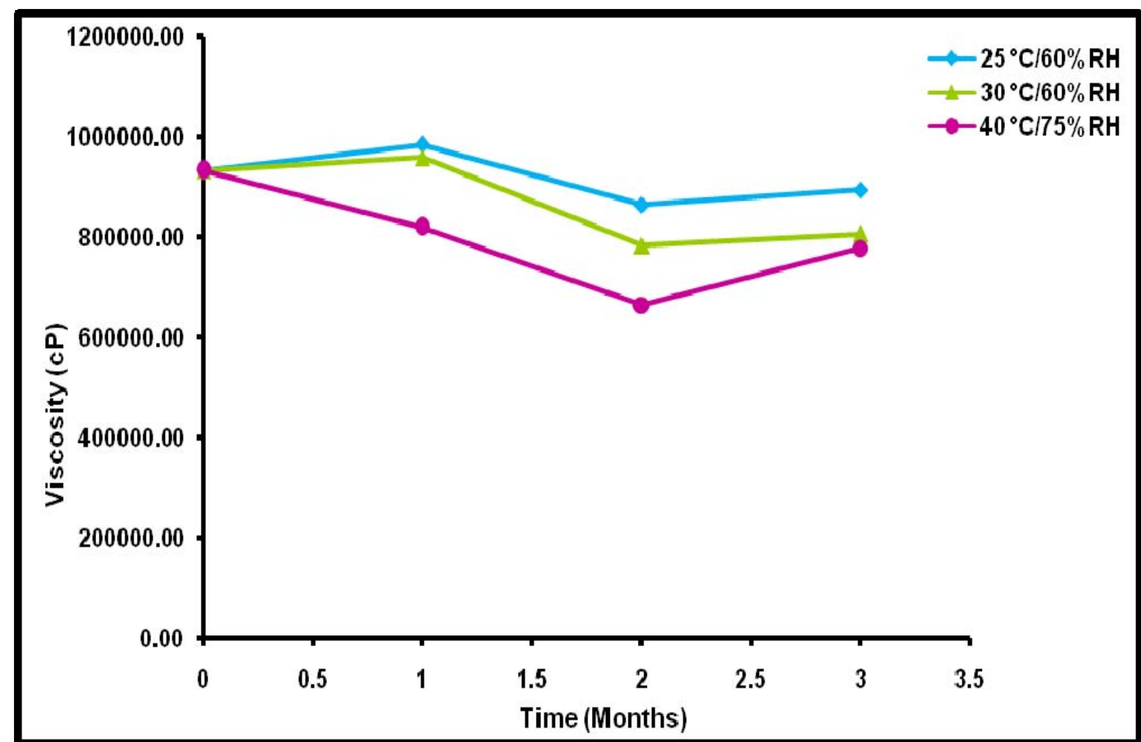


Figure 3.29: *Change in viscosity of the emulgel (pH 7.4) at the specified conditions after each time interval*

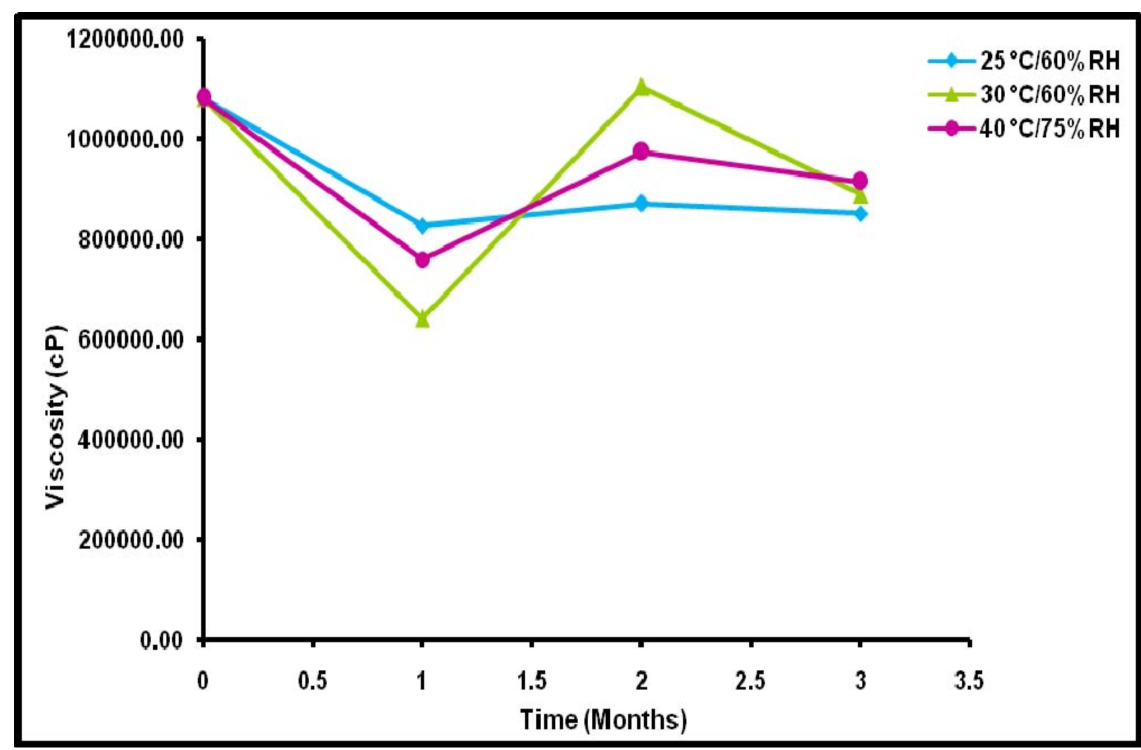


Figure 3.30: *Change in viscosity of the Pheroid™ emulgel (pH 5) at the specified conditions after each time interval*

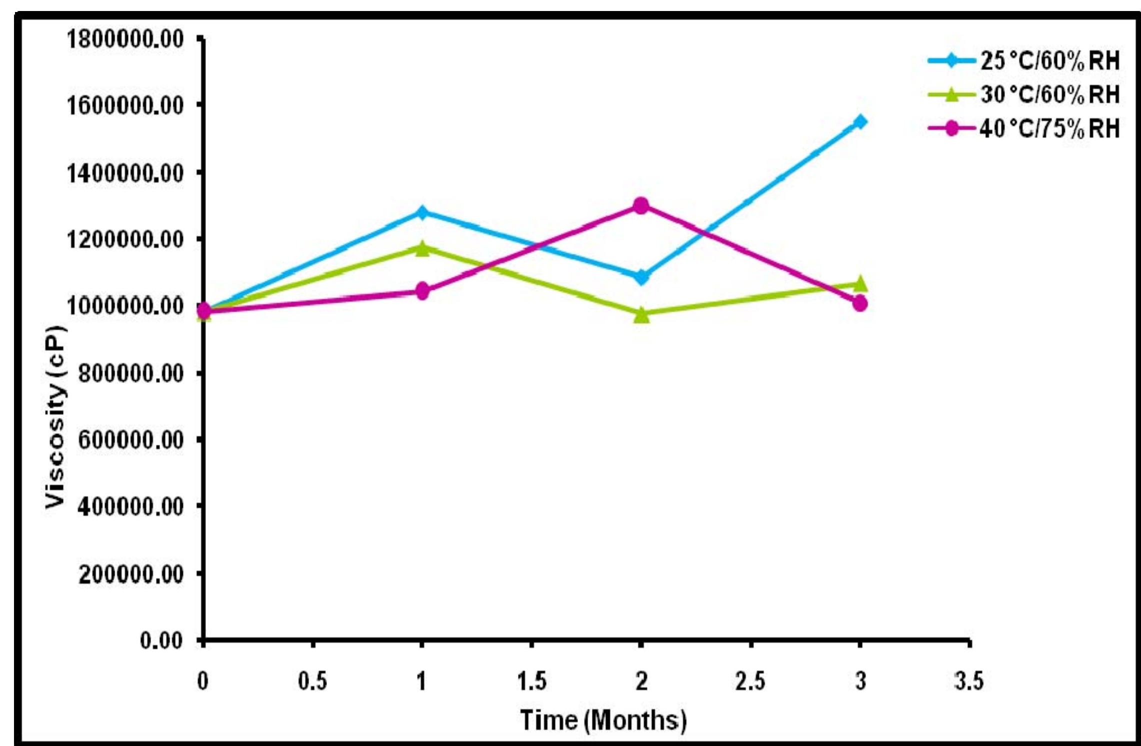


Figure 3.31: *Change in viscosity of the Pheroid™ emulgel (pH 7.4) at the specified conditions after each time interval*

No significant ($p > 0.05$) changes in viscosity (%RSD < 5) was observed for the gel and emulgel formulated at pH 7.4 and stored at 25 °C/60% RH. The remaining formulations at all the specified storage conditions exhibited a significant change ($p < 0.05$) in viscosity (%RSD > 5) with a decrease in viscosity being more pronounced at the higher temperature and humidity storage conditions. A possible contributing factor to the change in viscosity over three months at the specified storage conditions might have been due to the use of Pluronic® F-127 (surfactant). This surfactant possesses a melting point of approximately 56 °C (BAST Corporation. s.a). The problem with this might have been the temperature (70 °C) at which the formulations were prepared. The higher preparation temperature might have caused the Pluronic® F-127 to degrade, thereby losing its ability to function appropriately as a viscosity enhancer.

3.5.7 ZETA POTENTIAL

The stability of particle suspensions, colloidal dispersions, emulsions and other related systems is strongly influenced by electrical charges that exist at the particle-liquid interface of these systems (Malvern® Instruments, 2000:1.1). In order to determine the electrical charges of the oil droplets, zeta potential was measured. Zeta potential was not conducted on the gel formulations due to the gels being clear solutions whereas the emulgels contained oil droplets (figure 2.7.a and b). Results obtained for the variation in zeta potential is seen in table C.5, annexure C. No significant changes ($p > 0.05$) were observed for any of the semi-solid formulations exposed to the different storage conditions (%RSD < 2.5) during the time of exposure. Therefore, it could be concluded that the different storage conditions and pH of the formulations provided no significant ($p > 0.05$) effect on the zeta potential of the semi-solid formulations, implying that the formulations were generally stable due to electrical charges that may exist.

3.5.8 DROPLET SIZE

Droplet size was not conducted on the gel formulations due to the gels being clear solutions whereas the emulgels contained oil droplets (figure 2.7.a and b). Results obtained for the variation in droplet size ranged from 0.01 – 0.74 μm. This indicates a relatively small change in the droplet size over the three months exposure to different temperature and humidity conditions.

CHAPTER 4
ARTICLE FOR PUBLICATION IN THE INTERNATIONAL JOURNAL OF PHARMACEUTICS

Influence of selected formulation factors on the transdermal delivery of ibuprofen

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Abstract

The aim of this study was to determine the influence of selected formulation factors on the transdermal delivery of ibuprofen. The physicochemical properties of ibuprofen were thus determined. A gel, emulgel and Pheroid™ emulgel were formulated at pH 5 and 7.4. Obtained diffusion results of these formulations were subsequently compared to a commercial product (Nurofen® gel). Membrane permeation studies were conducted prior to skin permeation studies. Membrane and skin permeation studies were performed using Franz cells over a period of 6 h and 12 h, respectively. Tape stripping was performed after completion of the skin permeation studies. Finally, stability tests at various conditions were conducted.

All the formulations exhibited an increase in the average cumulative amount of ibuprofen that permeated the skin when compared to Nurofen® gel. The following rank order could be established: Emulgel (pH 5) >> Pheroid™ emulgel (pH 5) > Gel (pH 5) > Emulgel (pH 7.4) > Pheroid™ emulgel (pH 7.4) ≈ Emulgel (pH 7.4) >> Nurofen® gel > Gel (pH 7.4). Correlation coefficients of 0.950 – 0.978 for skin permeation studies were obtained for all the formulations. Nurofen® gel exhibited the lowest release rate and flux, the shortest lag time, the highest ibuprofen concentration in the stratum corneum and in the epidermis. The gel formulated at pH 7.4 performed the best of all the formulations. It was considered stable and depicted the least variation during stability testing.

Keywords: Ibuprofen, physicochemical properties, transdermal diffusion, pH, solubility

1 Introduction

Delivery of an active pharmaceutical ingredient (API) through the skin has long been a promising concept due to its large surface area, ease of access, vast exposure to the circulatory and lymphatic networks, and non-invasive nature of the therapy. This is true whether a local or systemic pharmacological effect is desired (Aukunuru *et al.,* 2007:856). However, ibuprofen administered orally is highly metabolised in the liver (first pass metabolism) and in the gastrointestinal tract (Bouwstra *et al.,* 2003:2) resulting in decreased bioavailability. Furthermore, it also causes gastric mucosal damage, bleeding, and ulceration (Rhee *et al.,* 2008:14). Another obstacle associated with oral API delivery is that some APIs require continuous delivery which is difficult to achieve (Bouwstra *et al.,* 2003:3). Therefore, there is significant interest to develop topical dosage forms for ibuprofen to avoid oral side effects and to provide relatively consistent API levels at the application site for prolonged periods (Rhee *et al.,* 2003:14).

A pharmaceutical dosage form is an entity that is administered to patients so that they receive an effective dose of an active pharmaceutical ingredient (API). The proper design and formulation of a dosage form requires a thorough understanding of the physiological factors affecting percutaneous penetration and the physicochemical characteristics of the API, as well as that of the pharmaceutical excipients that are used during formulation. The API and pharmaceutical excipients must be compatible with one another to produce a formulation that is stable, efficacious, attractive, easy to administer, and safe (Mahato, 2007:11). Amongst others, these properties indicate the suitability of the type of dosage form, as well as any potential problems associated with instability, poor permeation and the target site to be reached (Wells & Aulton, 2002:337). Therefore, when developing new or improved dosage forms, it is of utmost importance to evaluate the factors influencing design and formulation to provide the best possible dosage form and its formulation. Thus, the aim of this study was to determine the influence of selected formulation factors on the transdermal delivery of ibuprofen.

2 Materials and methods

2.1 Materials

Ibuprofen was obtained from DB fine chemicals® (Johannesburg, South Africa). The other excipients used in this study were: Cremophor® RH 40, dl-α-Tocopherol, vitamin F ethylester (BASF Aktiengesellschaft, Ludwigshafen, Germany), ethanol, isopropyl myristate, polyethylene glycol 400, potassium chloride, propylene glycol, sodium hydroxide (Merck Chemicals®, Midrand, South Africa), octan-1-ol (Associated Chemical Enterprises, Johannesburg, South Africa), Pluronic® F-127 (Sigma Aldrich, Johannesburg, South Africa), and potassium dihydrogen orthophosphate (Labchem, Johannesburg, South Africa).

2.2 Methods

2.2.1 Preparation of phosphate buffer solution (pH 7.4)

Sodium hydroxide pearls (1.571 g) were dissolved in 393 ml Milli-Q® water and added to a solution prepared by dissolving potassium dihydrogen orthophosphate (6.82 g) in 250 ml Milli-Q® water. The pH was measured, and if necessary, it was adjusted to 7.4 with phosphoric acid (British Pharmacopoeia, 2013).

2.2.2 Preparation of phosphate buffer solution (pH 5)

Potassium dihydrogen phosphate (2.72 g) was dissolved in 800 ml Milli-Q® water. The pH was measured, and if necessary, it was adjusted to 5.0 with sodium hydroxide. Milli-Q® water was used to fill the beaker to volume (1 000 ml) (British Pharmacopoeia, 2013).

2.2.3 Chromatographic conditions and apparatus

HPLC analysis of ibuprofen was performed using an Agilent® 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA) in a controlled laboratory environment at 25 °C. This instrument was equipped with a G1322A degasser, G1311A quaternary pump, G1313A autosampler injection mechanism, G1316A column oven and a G1315A multi wavelength diode array detector. Chromatograms were processed using Chemstation Rev. A.10.03 software. All validation calculations were carried out with Microsoft® Excel™ 2007, Office Package. A high performance silica based, reversed phase C18-2 column with a 5 μm particle size, endcapped,

150 x 4.60 mm (Venusil XBP Agela Technologies, Newark, DE) was used. The mobile phase consisted of a filtered and degassed mixture of 1% acetic acid, 70% acetonitrile and 30% HPLC grade (Milli-Q®) water (1:70:30). Phosphate buffer solution (pH 7.4) was used as solvent for the validation of ibuprofen and methanol was used as solvent to analyse the ibuprofen concentration for the assay during stability testing. The flow rate and detection was set to 1 $ml.min^{-1}$ and 265 nm, respectively. The retention time was approximately 5.2 min for the validation of ibuprofen, whereas it was approximately 5.5 min for the assay determination during stability testing. The run time was approximately 10.0 min for the validation of ibuprofen and approximately 7 min for the assay during stability testing.

2.2.4 Physicochemical properties

This section deals with the experimental methods that were used to determine the solubility and partition coefficient of ibuprofen.

2.2.4.1 Aqueous solubility

Three test tubes were each filled with 5 ml Milli-Q® water. Supersaturated ibuprofen solutions were obtained by adding excess amounts of ibuprofen to each test tube. The test tubes were submerged in a water bath set to a temperature of 37 °C and vigorously shaken for 24 h. Subsequently, the test tubes were centrifuged at 4 500 rpm for 10 min. The solutions were filtered and analysed by HPLC.

2.2.4.2 pH-Solubility profile

The solubility of ibuprofen at a range of pH values (1 – 14) was determined to indicate the pH at which ibuprofen is most soluble. Sodium hydroxide or phosphoric acid was used to adjust the pH of Milli-Q® water. Five milliliters (5 ml) of the adjusted solutions (pH 1 - 14) were filled in test tubes. Supersaturated ibuprofen solutions were obtained by adding excess amounts of ibuprofen to each test tube. The test tubes were submerged in a water bath set to a temperature of 37 °C and vigorously shaken for 24 h. Subsequently, the test tubes were centrifuged at 4 500 rpm for 10 min. The solutions were filtered and analysed by HPLC.

2.2.4.3 Octanol-water distribution coefficient (log P)

A mixture of *n*-octanol and Milli-Q® water, (pH 4.85) in a 1:1 ratio (200 ml : 200 ml) were vigorously stirred for 24 h to attain saturation. The mixture was transferred to a pear-shaped flask and left for 24 h to obtain phase separation. Subsequently the two phases were separated and stored in different containers. A stock solution was prepared by dissolving 2 mg ibuprofen in 100 ml saturated *n*-octanol (saturated with Milli-Q® water). Three test tubes were filled with 3 ml of the stock solution and 3 ml of the saturated Milli-Q® water (saturated with *n*-octanol). The test tubes were submerged in a water bath set to a temperature of 37 °C and vigorously shaken for 3 h. It was centrifuged at 4 500 rpm for 10 min. Analysis of the aqueous phase was determined by HPLC. The log P value was calculated by using the logarithmic ratio of the concentration in the aqueous phase, to the concentration in the *n*-octanol. After determination of the aqueous phase, the concentration of ibuprofen in the oil phase was determined by deducting the concentration of API in the aqueous phase from the original concentration.

2.2.4.4 Octanol-buffer distribution coefficient (log D)

A mixture of *n*-octanol and phosphate buffer solution, (pH 5 and 7.4, respectively) in a 1:1 ratio (200 ml : 200 ml) was vigorously stirred for 24 h to attain saturation. The mixtures (containing phosphate buffer solution at either pH 5 or pH 7.4) were transferred to pear-shaped flasks and left for 24 h to obtain phase separation. Subsequently, the two phases of each mixture were separated and stored in different containers. A stock solution for each mixture was subsequently prepared by dissolving either 2 mg ibuprofen in 50 ml saturated *n*-octanol (for the phosphate buffer solution at pH 5) or 2 mg ibuprofen in 100 ml saturated *n*-octanol (for the phosphate buffer solution at pH 7.4), respectively. Three test tubes for each mixture were filled with 3 ml of the stock solution and 3 ml of the saturated phosphate buffer. The test tubes were submerged in a water bath set to a temperature of 37 °C and vigorously shaken for 3 h. It was centrifuged at 4 500 rpm for 10 min. Analysis of the aqueous phase was determined by HPLC. The log D values were calculated by using the logarithmic ratio of the concentration in the aqueous phase, to the concentration in the *n*-octanol. After determination of the aqueous phase, the concentration of ibuprofen in the oil phase was determined by deducting the concentration of API in the aqueous phase from the original concentration.

2.2.5 Formulations of semi-solid dosage forms

2.2.5.1 Formulation of a gel containing ibuprofen

Ibuprofen (5 g) was weighed and dissolved in 20 g propylene glycol and 10 g ethanol. The solution was sonicated in an ultrasonic bath (figure 2.3.a) for approximately 20 min to ensure complete dissolution. It was placed on a hot plate with a magnetic stirrer and heated to 70 °C. The solution was removed from the hot plate and blended with a mixer (figure 2.3.b) at low speed (300 rpm) for approximately 2 min. Pluronic® F-127 (20 g) was added to the solution and blended at low speed (300 rpm) for approximately 2 - 3 min. Finally, 45 ml dH_2O was heated to 70 °C and added to the mixture.

2.2.5.2 Formulation of an emulgel containing ibuprofen

Ibuprofen (5 g) was weighed and dissolved in 7.5 g polyethylene glycol 400 and 9.3 g ethanol. The solution was sonicated in an ultrasonic bath for approximately 20 min to ensure complete dissolution. Isopropyl myristate (20 g) was added to the solution and sonicated for approximately 20 min. A volume of 48.2 ml dH_2O was added to the above solution and sonicated in an ultrasonic bath for approximately 20 min. The solution was placed on a hot plate with a magnetic stirrer and heated to 70 °C, after which it was removed and blended with a mixer at low speed (300 rpm) for approximately 2 min. Finally, 10 g Pluronic® F-127 was added and mixed at a high speed (900 rpm) until the formulation reached 25 °C ± 0.5 °C.

2.2.5.3 Formulation of a Pheroid™ emulgel containing ibuprofen

Ibuprofen (5 g) was weighed and dissolved in 7.5 g polyethylene glycol 400 and 9.3 g ethanol. The solution was sonicated in an ultrasonic bath for approximately 20 min to ensure complete dissolution. Isopropyl myristate (20 g) was added to the solution and sonicated for approximately 20 min. The solution was removed from the ultrasonic bath and 1 g Cremophor RH 40, 2.8 g Vitamin F Ethylester, and 0.2 g dl-α-tocopherol were added. This mixture was placed on a hot plate with a magnetic stirrer and heated to 70 °C. Subsequently, 44.2 ml $N_2O.H_2O$ was heated to 70 °C. Both the mixture and $N_2O.H_2O$ were removed from the hot plate; and the mixture was added to the $N_2O.H_2O$. A homogeniser (figure 2.4) was employed for 2 - 3 min at 13 500 rpm in order to ensure a homogenous mixture. The mixture was blended with a mixer at low speed (300 rpm) for approximately 2 min. Pluronic® F-127 (10 g) was added

to the above mixture and blended at high speed (900 rpm) until the formulation reached 25 °C ± 0.5 °C.

2.2.6 Permeation studies

Permeation studies were performed to determine the rate and extent of ibuprofen skin permeation. Prior to the performance of skin permeation studies, membrane permeation studies were conducted to determine whether ibuprofen was in fact released from the formulations. Therefore, permeation studies were conducted in two categories, namely; membrane permeation studies and skin permeation studies. This section describes the experimental methods of these two categories.

2.2.6.1 Skin preparation

Ethical approval for the procurement and preparation of the skin was provided by the Research Ethics committee of the North-West University under the reference number NWU-00114-11-A5. Female Caucasian human skin obtained after abdominoplastic (tummy-tuck) surgery, was donated by anonymous donors. Prior to obtaining the skin, informed consent was provided by the donor patients through their doctor in order to maintain confidentiality by concealing patient identity.

Full-thickness skin was removed from the abdomen of these patients. Within 24 h following surgical removal of the skin, it was frozen at a temperature of -20 °C for no longer than 6 months. Prior to the conduction of any studies, the full-thickness skin was taken from the freezer and left to thaw. The skin was first visually examined for any defects such as stretch marks, scarring or large hair follicles/holes. Subsequently, it was cut into pieces with a Zimmer® electric dermatome model 8821, removing only the subcutaneous fat layer of the skin. All prepared skin samples were placed on Whatman® filter paper with the stratum corneum facing upwards. Each skin piece was cut into circles of approximately 15 mm in diameter; with a punch and hammer (figure 2.5.b) and 400 µm in thickness using the Zimmer® Electric Dermatome. The skin circles on the filter paper were wrapped in aluminium foil, placed in a sealed plastic bag (Ziploc®) and stored in a freezer at -20 °C until utilised (within 24 h). Prior to the commencement of experiments, these skin samples were thawed at 25 ± 0.5 °C and each experiment was performed on skin obtained from a single donor.

2.2.6.2 Membrane release and skin permeation studies

During the permeation studies, twelve vertical Franz diffusion cells with a receptor volume of approximately 2 ml and a diffusion area of 1.075 cm^2 were used to conduct the *in vitro* transdermal studies. Ten of these Franz cells contained a specific formulation that included ibuprofen; whereas the remaining two cells contained placebo formulations (gel, emulgel and Pheroid™ emulgel formulation with no API).

Every receptor compartment was filled with 2 ml phosphate buffer solution (pH 7.4). The temperature was maintained at 37 °C. A Teflon-coated magnetic stirring bar was furthermore placed in the receptor compartment in order to maintain stirring throughout the experiment. Either an artificial membrane (in order to conduct release studies) or the prepared skin circles were mounted between the two compartments of each of the diffusion apparatus; the skin with the stratum corneum facing upwards in the direction of the donor compartment. A thin layer of Dow Corning® high vacuum grease was applied to the receptor and donor compartments of each Franz cell prior to assembling it. Furthermore, the sides of the cells were sealed with Dow Corning® high vacuum grease to prevent any leakage. A horseshoe clamp was employed to secure the receptor and donor compartments.

Subsequently, a sufficient amount of a specific formulation (approximately 1 ml), which was heated to 32 °C prior to commencement of the experiment in order to simulate skin surface temperature, was added to each of the donor compartments. This was done in order to ensure skin saturation. Each of the donor compartments was consequently covered with Parafilm® and a cap to prevent evaporation during the experiment. The Franz cells were placed on a stand which was set up on a Variomag® magnetic stirring plate (750 rpm) and submerged in a Grant® water bath set at 37 °C. At pre-determined time intervals the entire receptor volume was withdrawn and replaced with fresh buffer (37 °C) in order to ensure sink conditions existed throughout the experiment. During the membrane release studies, samples were withdrawn every 2 h for 6 h. However, during skin diffusion studies, samples were withdrawn every 20 min for the first 2 h after which samples were withdrawn every 2 h for the following 10 h. All samples were directly assayed using HPLC analysis to determine the ibuprofen concentration in the receptor fluid. Hence, tape stripping was performed after the 12 h skin diffusion study.

2.2.6.3 Tape stripping

Tape stripping was conducted after completion of the skin permeation studies to determine whether topical or transdermal delivery of ibuprofen was achieved. After the final withdrawal was conducted during the skin diffusion experiment, the Franz cells were carefully dismantled and the skin pinned to a solid flat surface containing Whatman® filter paper. The exposed diffusion area (1.075 cm^3) was clearly demarcated by the imprints of the diffusion cells (11.7 mm diameter). All excess formulation was dabbed from the skin with a clean paper towel. In order to perform tape stripping, sixteen strips of 3M Scotch® Tape were cut to a length that covered the diffusion area, but did not overlap the area outside the diffusion imprints. The first strip was discarded as it was considered part of the cleaning procedure and the remaining fifteen strips were used to strip the stratum corneum-epidermis off the diffusion area. Glistening of the viable epidermal layer indicated complete removal of the stratum corneum-epidermis (Pellet *et al.,* 1997:94). These strips were consequently placed in a polytop containing 5 ml methanol (40%). The polytop was vigorously shaken to wet all the strips and retained overnight (approximately 12 h) at approximately 4 °C to be analysed by HPLC the following day.

Excess skin on the outside of the imprints of the diffusion area was trimmed and the remaining skin circle (dermis) was cut into pieces in order to enlarge the surface area in contact with the methanol solution. These skin pieces were placed in a polytop containing 5 ml methanol (40%) and was furthermore vigorously shaken after which it was stored overnight at 4 °C to be analysed by HPLC the following day.

2.2.7 Stability testing of semi-solid formulations

The stability of the formulated semi-solid products was examined over a period of 3 months. These products were stored at 25 °C/60% RH (relative humidity), 30 °C/60% RH and 40 °C/75% RH. Approximately 1 500 ml of each formulation was manufactured. The formulations were divided into 50 g glass containers. They were stored in Labcon® humidity chambers (Labex, Johannesburg, South Africa) at the different temperature and humidity conditions described. Each test required its own container. Tests were conducted at month 0, 1, 2, and 3. Stability tests included: Visual appearance, light microscopy, mass variation, assay, pH, viscosity, zeta potential and droplet size

2.2.7.1 Visual appearance

The visual appearance (any change in colour or texture) of each formulation stored at the specified conditions for 3 months was inspected. The colour of the formulations was matched to a graded colour chart obtained from a local paint store.

2.2.7.2 Light microscopy

A sample of each formulation that was stored at the various specified conditions was placed on a glass slide and covered with a glass cover slip. Images were captured with a Nikon® Optiphot Microscope (Nikon® Thailand, Bangkok). This was conducted in order to determine whether the formulations contained any crystals after exposure to the specified temperature and humidity conditions.

2.2.7.3 Mass variation

A Mettler® Toledo scale (Mettler® Toledo, Schwerzenbach, Switzerland), was used to determine if any mass variation occurred during storage. Prior to filling the formulations (50 g) into the glass containers, the weight of the empty glass container with the lid was determined. The weight of each formulation stored at the specified conditions was determined in triplicate each month. Mass variation for each formulation was determined and compared.

2.2.7.4 Assay

The purpose of the assay was to determine whether the ibuprofen concentration in the semi-solid formulations changed in relation to its initial or previous value during the course of storage.

Ibuprofen (50 mg) was accurately weighed in a 100 ml volumetric flask. The ibuprofen was dissolved in a small quantity of methanol. To ensure that it was completely dissolved, the solution was sonicated in an ultrasonic bath for approximately 5 min. The volumetric flask was filled to 100 ml with methanol. This stock solution was transferred into an autosampler vial for analysis. A range of volumes (5 µl; 10 µl; 15 µl; 20 µl; and 25 µl) was analysed, in duplicate, by HPLC.

Each formulation (1 g) was accurately weighed in a 100 ml volumetric flask, using a syringe with a plastic tube attached to the tip. The formulations were each dissolved in a small quantity of

methanol. To ensure that the formulation was completely dispersed, it was sonicated in an ultrasonic bath for approximately 5 min. Each volumetric flask was filled to 100 ml with methanol. All of the samples were prepared in triplicate and transferred to HPLC vials. The samples were analysed in duplicate by HPLC at the default injection volume (20 µl).

2.2.7.5 pH

A Mettler® Toledo Inlab 410 pH-meter (Mettler® Toledo, Schwerzenbach, Switzerland) was used to measure the pH of each formulation. Prior to measurement, the apparatus was calibrated at 25 °C with Mettler Toledo buffer solutions at pH 4.01, 7.00 and 10.0. The pH of each formulation was measured in triplicate and the average pH determined.

2.2.7.6 Viscosity

Viscosity is a measure of a fluid's resistance to flow (Marriot, 2002:42). The viscosity of each formulation stored at the specified conditions was measured using a Brookfield Viscometer model DV-II+ (Stoughton, United States of America). The temperature of the water circulating in the water bath was controlled by a Brookfield temperature controller. Appropriate Helipath and LV spindles were selected to ensure that an optimum torque existed depending on the consistency of the semi-solid formulations. The viscosity readings were measured every 10 sec for 5 min in revolutions per minutes (rpm). Approximately 32 measurements were obtained and the average viscosity was determined. Viscosity parameters for the different semi-solid formulations are presented in table 4.1.

Table 1: *Viscosity parameters for the different semi-solid formulations*

2.2.7.7 Zeta potential

Electrical charges that exist at the particle-liquid interface of suspensions, colloidal dispersions, emulsions and other related systems, strongly influence the stability of these systems (Malvern® Instruments, 2000:1.1). In order to determine the electrical charges of the oil droplets, zeta potential was measured. Each formulation (0.5 g) was accurately weighed in a 25 ml volumetric flask, using a syringe with a plastic tube attached to the tip. The formulations were each dissolved in a small quantity of 0.01 M potassium chloride. To ensure that the formulation was completely dispersed, it was sonicated in an ultrasonic bath for approximately 5 min. Each of the volumetric flasks was filled to 25 ml with 0.01 M potassium chloride. All of

the samples were prepared and measured in triplicate by means of injecting the samples into a Malvern® Zetasizer 2000 (Malvern® Instruments, Worcestershire, United Kingdom).

2.2.7.8 Droplet size

Approximately 0.5 g of each formulation stored at the specified conditions was mixed with approximately 3 ml Milli-Q® water to form a homogenous dispersion. These mixtures were made up with approximately 3 ml Milli-Q® water, mixed well and injected into a Malvern® Mastersizer 2000, fitted with a wet cell Hydro 2000 SM dispersion unit (Malvern® Instruments, Worcestershire, United Kingdom).

2.2.8 Statistical methods

One-way analysis of variances (ANOVA) was performed to determine whether statistical significant differences existed between the mean values of the different formulations and Nurofen® gel in general. Levene's test was performed to assure equality of variances in each ANOVA's case. In the case of inequality of variances, Welch tests were conducted. Normal probability plots on the residuals were performed to assure that the data was fairly normally distributed. Tukey's post-hoc multiple comparison tests were performed to determine statistically significant differences between all the different formulations. Dunnet's tests were conducted to determine which of the mean values of the formulations differed statistically significant from the mean of the Nurofen® gel. These procedures were performed if the assumption of normal distributed data was valid.

In cases where the normal probability plots yielded data that could not be seen as normally distributed, non-parametric tests were performed using the Kruskal Wallis test. This test was performed to determine if statistically significant differences existed between the mean values of the different formulations and Nurofen® gel in general. Dunn's post-hoc multiple comparison tests were performed to determine statistically significant differences between all the formulations. Bonferoni corrections on Dunn's p-values were calculated in order to determine which of the test formulations mean values differed statistically significant from the mean of Nurofen® gel.

These procedures were performed using the statistical data analysis software system, Statistica. All tests were performed at a significant level of 0.05.

3 Results and discussion

3.1 Physicochemical properties

3.1.1 Aqueous solubility

According to Naik *et al.,* (2000:319) the ideal aqueous solubility of APIs for transdermal delivery should be more than 1 $mg.ml^{-1}$. The aqueous solubility of ibuprofen was determined as 0.096 $mg.ml^{-1}$ ± 25.483. Thus, ibuprofen is considered practically insoluble in water and would therefore be rendered unsuitable for transdermal delivery if only considering the aqueous solubility (British Pharmacopoeia, 2013).

3.1.2 pH-solubility profile

Results obtained for the solubility of ibuprofen as a function of pH (pH-solubility profile) are illustrated in figure 1.

Figure 1: *pH-solubility profile of ibuprofen*

From the graph it was clear that ibuprofen is less soluble at pH 5 than at pH 7.4. In fact, it is poorly soluble at pH 5. Absorption of an API is possible only when it is present in solution (Mahato, 2007:11). This indicated that ibuprofen would be more soluble at pH 7.4 when compared to pH 5. This is due to the fact that ibuprofen is a weak acidic compound, and for every 3 units away from the pK_a-value (Ibuprofen possesses a pK_a of 4.4), the solubility changes 10-fold (Mahato, 2007:11, Mahato, 2007:14, Dollery, 1999:I1).

3.1.3 Octanol-water distribution coefficient (log P)

Previous research indicated that the ideal log P-values for transdermal API permeation of NSAIDs are between 2 and 3 (Swart *et al.,* 2005:72). A log P-value of 4.238 for ibuprofen was obtained. This value is not included in the ideal range, which is an indication that the lipophilic/hydrophilic properties are not ideal and this will therefore contribute to poor ibuprofen penetration through the skin.

3.1.4 Octanol-buffer distribution coefficient (log D)

Higgins *et al.,* (2001:280) determined the log D-profile of ibuprofen at various pH-values (figure 2). They established that the log D-values at pH 5 and 7 were 3.030 and 1.1500, respectively. The obtained log D-values in this study at pH 5 and 7.4 were 3.105 and 0.386, respectively. Therefore, it would be expected that dosage forms formulated at a pH of 5 would more readily permeate the skin compared to those formulated at pH 7.4.

Figure 2: *Log D profile of ibuprofen (Higgins et al., 2001:280)*

3.2 Membrane and skin permeation studies

All the formulations exhibited an increase in the average cumulative amount of ibuprofen released from the formulations and that permeated the membrane when compared to Nurofen® gel (figure 3). This increase was statistically significant ($p < 0.05$) for the gel, emulgel and Pheroid™ emulgel at pH 7.4. The gel at pH 7.4 exhibited the highest cumulative amount of ibuprofen that was released and that permeated the membrane. Preparations formulated at a pH of 5, did not differ significantly from Nurofen® when the average cumulative amount of ibuprofen that was released and that permeated the membrane were compared. The following rank order for the average cumulative amount released from the formulations could be established: Gel (pH 7.4) >>>> Pheroid™ emulgel (pH 7.4) > Emulgel (pH 7.4) >>> Gel (pH 5)> Pheroid™ emulgel (pH 5) ≈ Emulgel (pH 5) > Nurofen® gel.

Figure 3: *Average cumulative amount of ibuprofen released from the formulations and permeated the membrane over 6 h*

On the other hand, all the formulations exhibited an increase in the average cumulative amount of ibuprofen that permeated the skin when compared to Nurofen® gel (figure 4). This increase was statistically significant ($p < 0.05$) for the gel, emulgel and Pheroid™ emulgel at pH 5, as well as the emulgel and Pheroid™ emulgel at pH 7.4. The emulgel at pH 5 exhibited the highest cumulative amount of ibuprofen that permeated the skin. The following rank order for the average cumulative amount released from the formulations and that permeated the skin could be established: Emulgel (pH 5) >> Pheroid™ emulgel (pH 5) > Gel (pH 5) > Emulgel (pH 7.4)> Pheroid™ emulgel (pH 7.4) ≈ Emulgel (pH 7.4) >> Nurofen® gel > Gel (pH 7.4). From

this rank order it was clear that a trend was followed where the pH of formulation also played a role in ibuprofen permeation.

Figure 4: *Average cumulative amount of ibuprofen that permeated the skin over 12 h*

All the formulations exhibited a higher release rate and flux when compared to Nurofen® gel. This was statistically significant ($p < 0.05$) for the emulgel, gel and Pheroid™ emulgel at pH 7.4. The gel at pH 7.4 exhibited the highest release rate and flux (figures 5 and 6). This was observed for the membrane and skin permeation studies. All the formulations (including Nurofen® gel) presented a correlation coefficient (r^2) of 0.972 – 0.995 for membrane permeation studies, and 0.950 – 0.978 for skin permeation studies; indicating that the release of ibuprofen from each of the formulations could be described by the Higuchi model. Furthermore, all the formulations exhibited a prolonged lag time compared to Nurofen® gel which indicated that the ibuprofen was retained for a longer time by the base. This was statistically significant ($p < 0.05$) for the emulgel at pH 7.4, the gel and Pheroid™ emulgel at pH 5. The gel at pH 7.4 exhibited a lag time closest to that of Nurofen® gel and this difference could not be classified as statistically significant ($p > 0.286$). This was observed for the membrane and skin permeation studies (tables 2 and 3).

Figure 5: *Nature of the relationship between flux and release rate obtained for membrane permeation studies*

Figure 6: *Nature of the relationship between flux and release rate obtained for skin permeation studies*

Table 2: *Number of cells used (n), the average lag time values, standard deviations and p-values obtained for the membrane permeation studies for the various formulations tested*

Table 3: *Number of cells used (n), the average lag time values, standard deviations and p-values obtained for the skin permeation studies for the various formulations tested*

Nurofen® gel exhibited the highest ibuprofen concentration in the stratum corneum as well as in the epidermis followed by the gel at pH 7.4. However, results obtained for all the formulations indicated that topical as well as transdermal delivery of ibuprofen were achieved.

The pH of a formulation plays an important role with respect to API permeation. As previously discussed, the reported pK_a value of ibuprofen is 4.4 (Dollery, 1999:I1); and by application of the Henderson-Hasselbach equation, at pH 5, 20.08% of ibuprofen is present in its unionised form and at pH 7.4, 0.1% of ibuprofen is present in its unionised form. Since the unionised form of APIs is more lipid soluble than the ionised form, unionised forms of APIs permeate more readily across the lipid membranes (Surber & Smith, 2000:27). Therefore, it would be expected that formulations at pH 5 would be more permeable than formulations at pH 7.4. However, this did not correspond to the data obtained in figure 2. This may be attributed to the solubility of ibuprofen in the different formulations. According to the pH-solubility profile (figure 1), ibuprofen was more soluble at pH 7.4 than at pH 5. This is due to the fact that ibuprofen is a weak acidic compound, and for every 3 units away from the pK_a, the solubility changes 10-fold (Mahato, 2007:14). However, with regard to skin permeation studies, enhanced permeation was obtained with the formulations prepared at pH 5. This was in accordance with Corrigan *et al.,* (2003:148) who stated that NSAIDs are less soluble and more permeable at low pH values, and more soluble and less permeable at high pH values. This was most probably due to the fact that unionised species, although possessing a lower aqueous solubility than the ionised species, resulted in enhanced skin permeation due to being more lipid-soluble.

The graph in figures 5 and 6 illustrates a linear relationship ($r^2 > 0.999$). This indicates that as the ibuprofen was released, the flux increased correspondingly.

Figure 7: *Higuchi plot obtained for membrane permeation studies*

Figure 8: *Higuchi plot obtained for skin permeation studies*

Higuchi described API dissolution from several types of modified release pharmaceutical dosage forms, such as transdermal systems and matrix tablets with water soluble APIs by making use of the Higuchi equation which describes a "square root of time" release kinetics. If the rate of API released obeys this law, the amount of API released is a linear function of the square root of time ($t^{1/2}$) (Costa & Lobo, 2001:126, Higuchi, 1962:803).

3.3 Stability testing of semi-solid formulation

3.3.1 Visual appearance

No significant change in colour was observed for the gel and emulgel formulations at pH 5 and 7.4 over the three months at all the storage conditions. However, it was observed that the formulations containing Pheroid™ showed a radical change in colour. This might have been due to oxidation of certain components such as vitamin E present in the Pheroid™ system. Consequently, further investigation is necessary to find the cause of the discolouration as well as a method to prevent it.

3.3.2 Light microscopy

It was observed that the gel formulated at pH 5 depicted the formation of crystals. This might have been due to the fact that the solubility of ibuprofen was exceeded, leading to it precipitating from the formulation. No significant change was observed for the emulgel and Pheroid™ emulgel formulated at pH 5 and 7.4.

3.3.3 Mass variation

No significant changes were observed for any of the semi-solid formulations exposed to the different stability conditions (< 0.7 g; %RSD < 0.9) during the time of exposure with regards to mass variation.

3.3.4 Assay

To determine whether the ibuprofen concentration in the different formulations changed in relation to its initial or previous value during the course of storage, an assay was performed by means of HPLC analysis (section 2.2.3).

According to the ICH (2005:9) a change in concentration of more than 5% indicates instability of the product. Considering all of the formulations, the gel at pH 5 showed the most pronounced change in ibuprofen content at all of the different stability conditions. This may be due to the fact that the solubility of ibuprofen was exceeded, leading to ibuprofen precipitation from the formulation, thereby causing the formation of crystals. A possible contributing factor to the varying assay values obtained during the study might have been due to non-homogenous sample withdrawal. On the other hand, the emulgel and Pheroid™ emulgel at pH 5 depicted

relative instability (according to the ICH) only at 40 °C/75% RH with a change in ibuprofen content of more than 5% (6.78 and 6.46%, respectively. The gel, emulgel and Pheroid™ emulgel at pH 7.4 exhibited the least variation in ibuprofen concentration at all of the storage conditions. This might indicate that the pH at which a semi-solid formulation is produced will have a direct influence on the stability of the product.

3.3.5 pH

The pH of the various semi-solid formulations was measured each month for three months. No significant change was observed over the three months for any of the different formulations stored at the different stability conditions. Thus, considering pH-change, all of the semi-solid formulations were considered stable (< 5%). However, differences between the formulations could still be established. The gel, emulgel and Pheroid™ emulgel at pH 7.4 were the most stable formulations at all of the different stability conditions, with a maximum change (for all three stability conditions) in the pH-values of 0.04, 0.38 and 1.13, respectively. Again, these results indicated that the pH at which a semi-solid formulation is formulated will have an influence on the stability of the product, though in this case, it was not significant. Furthermore, the formulations at pH 5 were less stable at the various stability conditions, when considering pH. However, all of these formulations at all of the conditions were still considered stable with a change in pH-values of less than 5%. A trend could be depicted between the various formulations formulated at the different pH values, which could be placed in the following rank order: Gel > Emulgel > Pheroid™ emulgel. Interestingly, an increase in temperature and humidity did not play a significant role in changing the pH of the semi-solid formulations.

3.3.6 Viscosity

No significant changes in viscosity (%RSD < 5) was observed for the gel and emulgel formulated at pH 7.4 and stored at 25 °C/60% RH. The remaining formulations at all of the specified storage conditions exhibited a significant change in viscosity (%RSD > 5) with a decrease in viscosity being more pronounced at the higher temperature and humidity storage conditions. A possible contributing factor to the change in viscosity over three months at the specified storage conditions might have been due to the use of Pluronic® F-127 (viscosity enhancer). This viscosity enhancer possesses a melting point of approximately 56 °C (BAST Corporation, s.a). The problem with this might have been the temperature (70 °C) at which the formulations were prepared. The higher preparation temperature might have caused

the Pluronic® F-127 to degrade, thereby losing its ability to function appropriately as a viscosity enhancer.

3.3.7 Zeta potential

The stability of particle suspensions, colloidal dispersions, emulsions and other related systems is strongly influenced by electrical charges that exist at the particle-liquid interface of these systems (Malvern® Instruments, 2000:1.12). In order to determine the electrical charges of the oil droplets, zeta potential was measured. Zeta potential was not conducted on the gel formulations due to the gels being clear solutions whereas the emulgels contained oil droplets. Results obtained for the variation in zeta potential is seen in table C.5, annexure C. No significant changes were observed for any of the semi-solid formulations exposed to the different storage conditions (%RSD < 2.5) during the time of exposure. Therefore, it could be concluded that the different storage conditions and pH of the formulations provided no significant effect on the zeta potential of the semi-solid formulations.

3.3.8 Droplet size

Droplet size was not conducted on the gel formulations due to the gels being clear solutions whereas the emulgels contained oil droplets. Results obtained for the variation in droplet size ranged from 0.01 – 0.74 µm. This indicates a small change in the droplet size over the three months exposure to different temperature and humidity conditions.

4 Conclusions

A balance must be maintained between optimum solubility and maximum stability (Pefile & Smith, 1997:148). Despite the lower skin permeation of the gel formulated at pH 7.4, this formulation performed the best, as it was considered stable (least variation during the 3 month stability test) and the obtained tape stripping results showed that this formulation depicted the highest ibuprofen concentrations in the stratum corneum and epidermis. Thus, topical as well as transdermal delivery were obtained.

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FIGURE LEGENDS

Figure 1: *pH-solubility profile of ibuprofen*

Figure 2: *Log D profile of ibuprofen (Higgins et al., 2001:280)*

Figure 3: *Average cumulative amount of ibuprofen released from the formulations and that permeated the membrane over 6 h*

Figure 4: *Average cumulative amount of ibuprofen diffusion through the skin over 12 h*

Figure 5: *Nature of the relationship between flux and release rate obtained for membrane permeation studies*

Figure 6: *Nature of the relationship between flux and release rate obtained for skin permeation studies*

Figure 7: *Higuchi plot obtained for membrane permeation studies*

Figure 8: *Higuchi plot obtained for skin permeation studies*

TABLES:

Table 1: Viscosity parameters for the different semi-solid formulations

Formulation	Spindle	Entry code	rpm	Temperature
Emulgel	T-F	96	0.3	25°C, 30°C, 40°C
Pheroid™ emulgel	T-F	96	0.3	25°C, 30°C, 40°C
Gel	LV	62	30	25°C, 30°C, 40°C

rpm = revolutions per minute

Table 2: *Number of cells used (n), the average lag time values, standard deviations and p-values obtained for membrane permeation studies for the various formulations tested*

Formulation	n*	Average lag time (h)	Standard deviation	p-value	
				Kruskal-Wallis	Bonferoni on Dunn
E (pH 5)	8	0.799	0.050		0.286
E (pH 7.4)	8	0.943	0.068		0.002*
G (pH 5)	7	0.961	0.066		0.0008*
G (pH 7.4)	7	0.573	0.108	<0.001*	0.286
PE (pH 5)	9	0.920	0.034		0.005*
PE (pH 7.4)	10	0.833	0.064		0.286
Nurofen® gel	7	0.556	0.401		

(E = emulgel, G = gel, PE = Pheroid™ emulgel)
n* = data from leaking cells was discarded

Table 3: *Number of cells used (n), the average lag time values, standard deviations and p-values obtained for skin permeation studies for the various formulations tested*

Formulation	n*	Average lag time (h)	Standard deviation	p-value	
				Welch	Dunnet
E (pH 5)	9	1.609	0.006		0.000009*
E (pH 7.4)	10	1.630	0.007		0.000008*
G (pH 5)	8	1.563	0.005		0.923507
G (pH 7.4)	7	1.582	0.008	<0.001*	0.034251*
PE (pH 5)	9	1.617	0.008		0.000008*
PE (pH 7.4)	9	1.620	0.007		0.000008*
Nurofen® gel	10	1.558	0.004		

(E = emulgel, G = gel, PE = Pheroid™ emulgel)
n* = data from leaking cells was discarded

FIGURES:

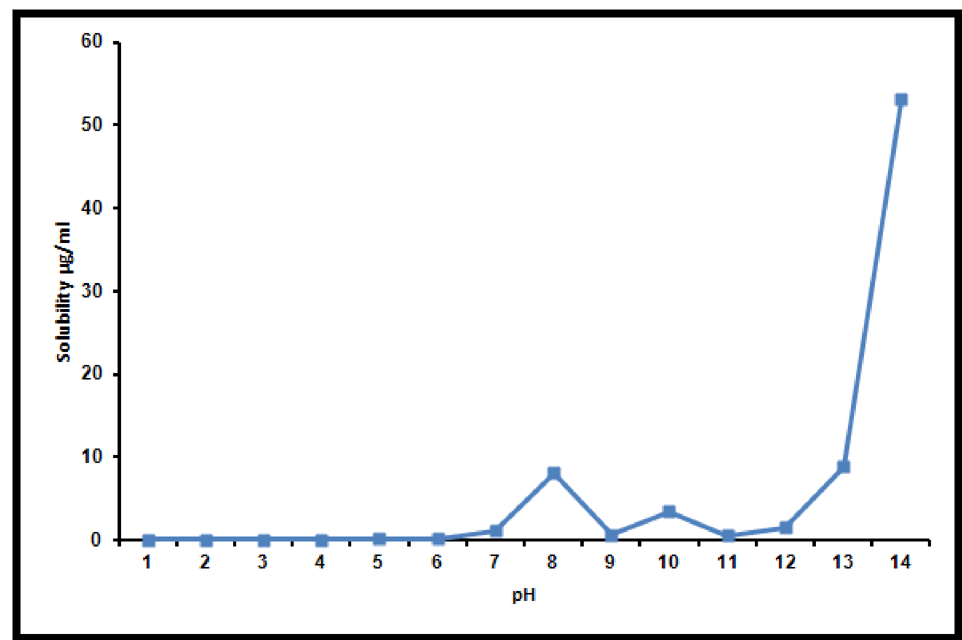

Figure 1: *pH-solubility profile of ibuprofen*

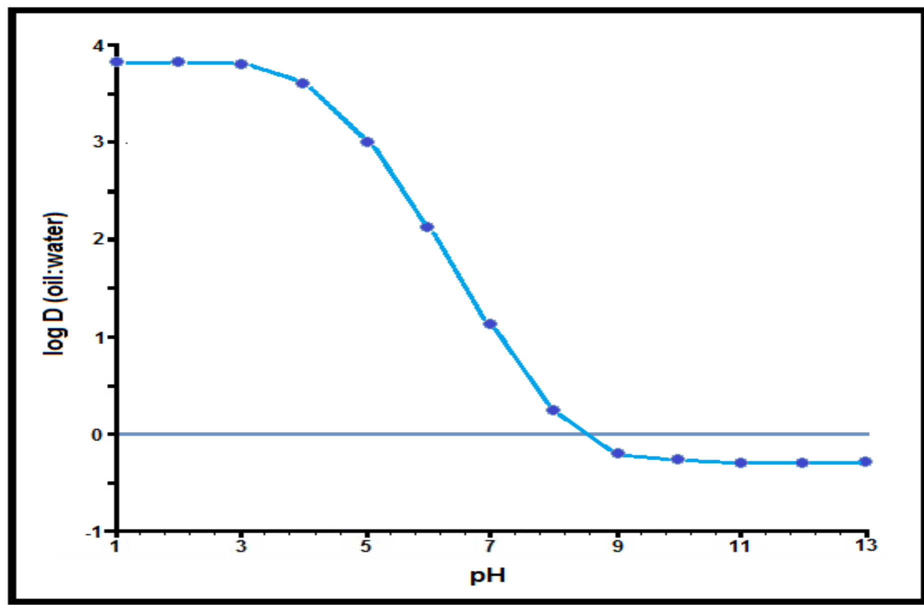

Figure 2: *Log D profile of ibuprofen (Higgins et al., 2001:280)*

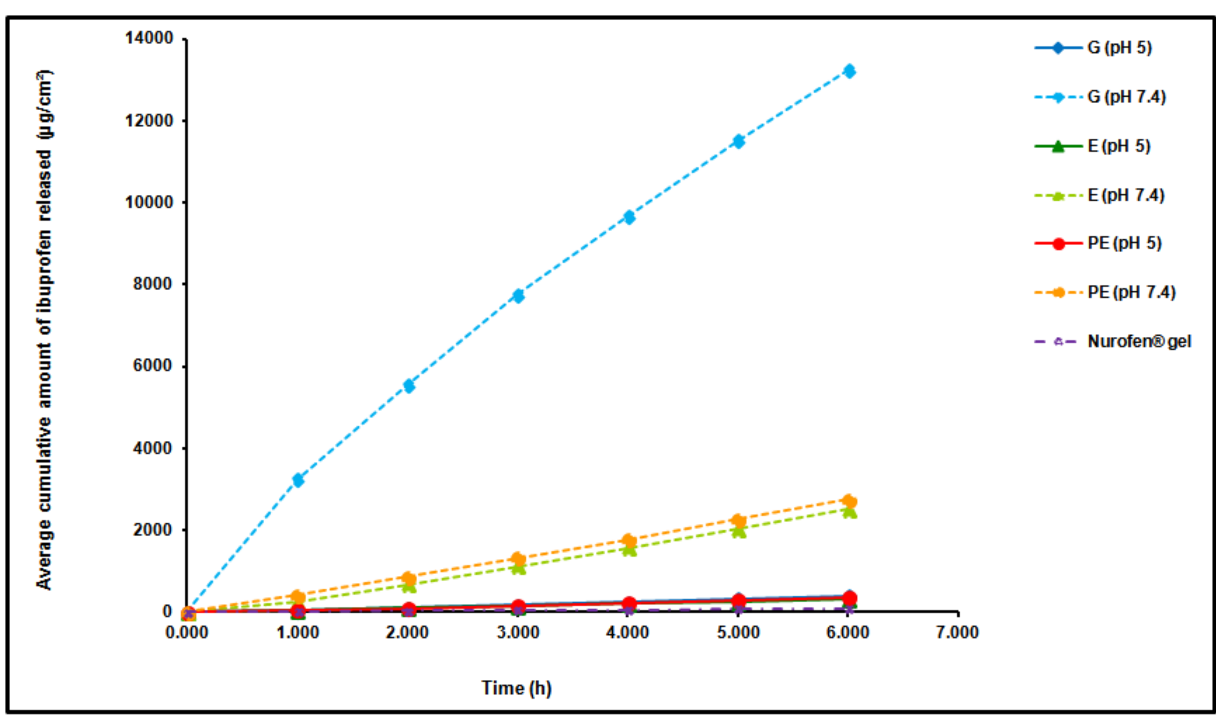

Figure 3: *Average cumulative amount of ibuprofen released from the formulations that permeated the membrane over 6 h (E = emulgel, G = gel, PE = Pheroid™ emulgel)*

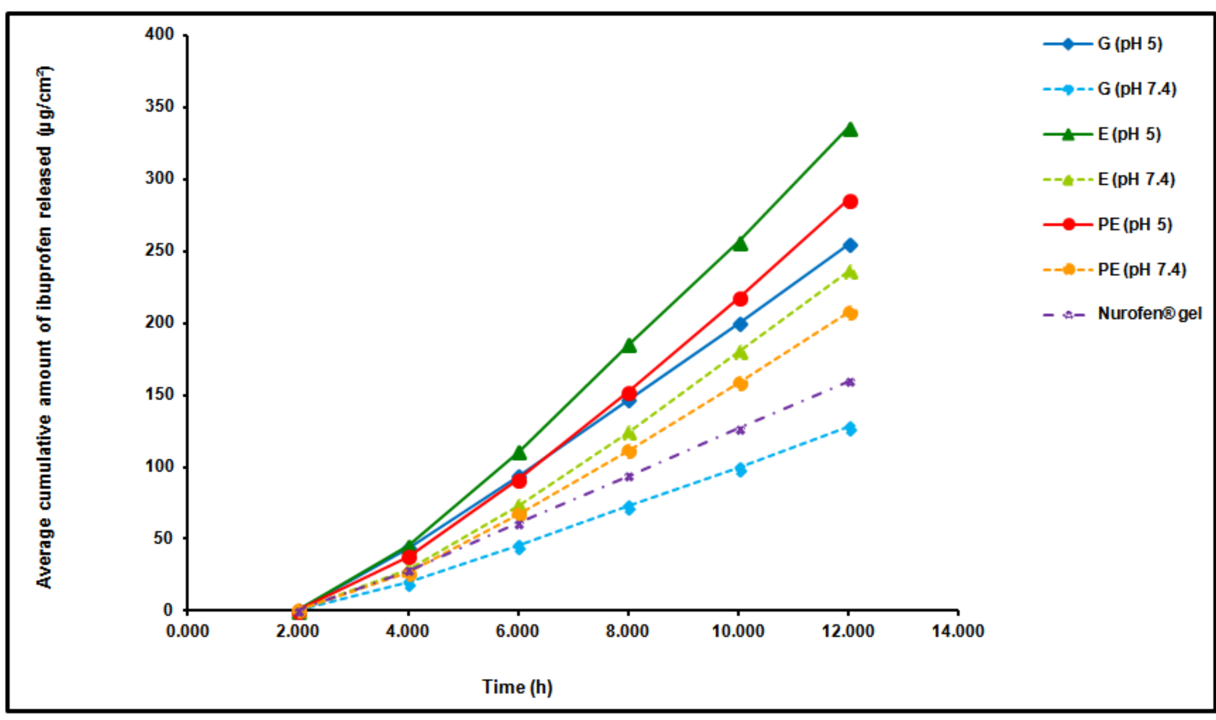

Figure 4: *Average cumulative amount of ibuprofen that permeated the skin over 12 h (E = emulgel, G = gel, PE = Pheroid™ emulgel)*

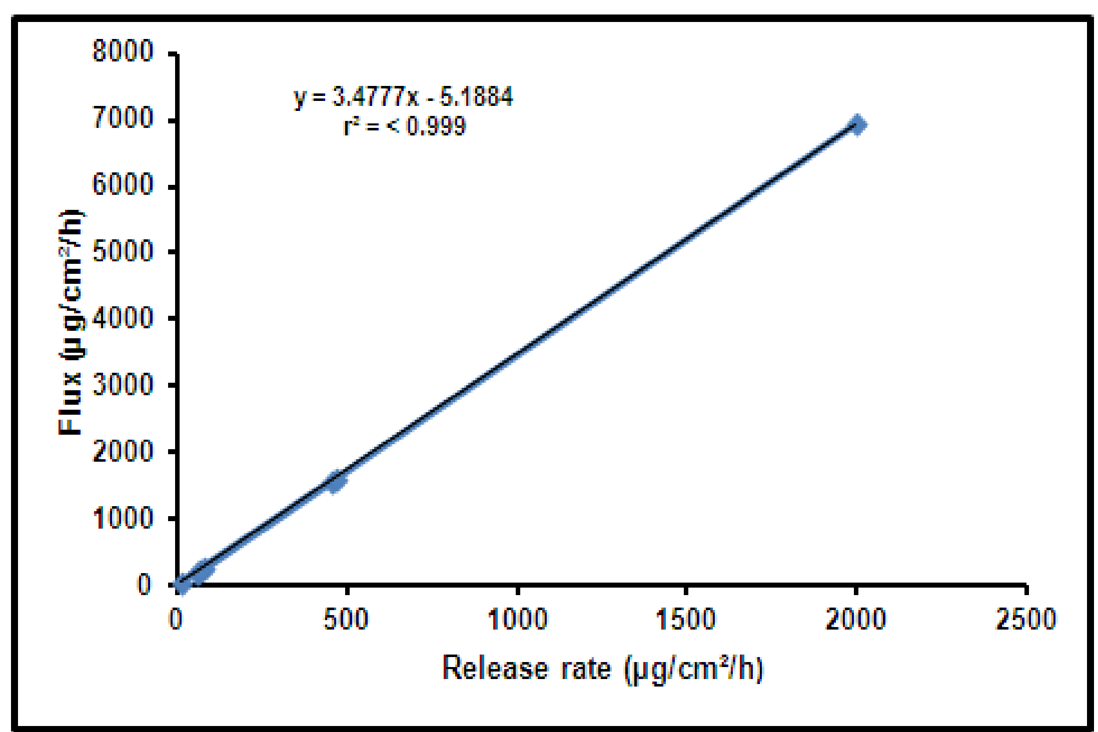

Figure 5: *Nature of the relationship between flux and release rate obtained for membrane permeation studies*

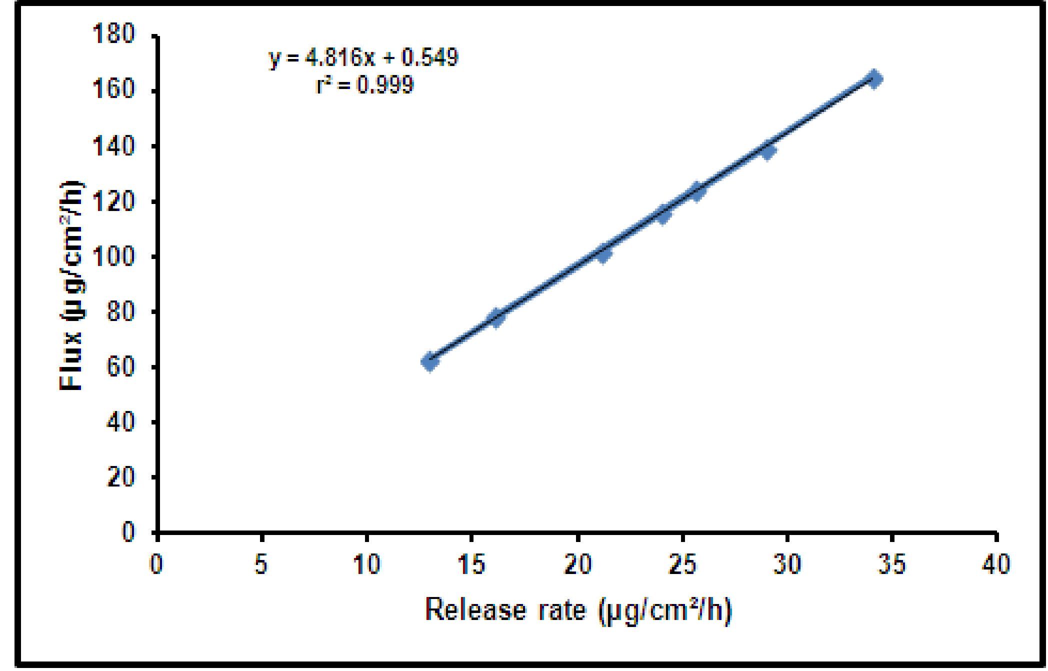

Figure 6: *Nature of the relationship between flux and release rate obtained for skin permeation studies*

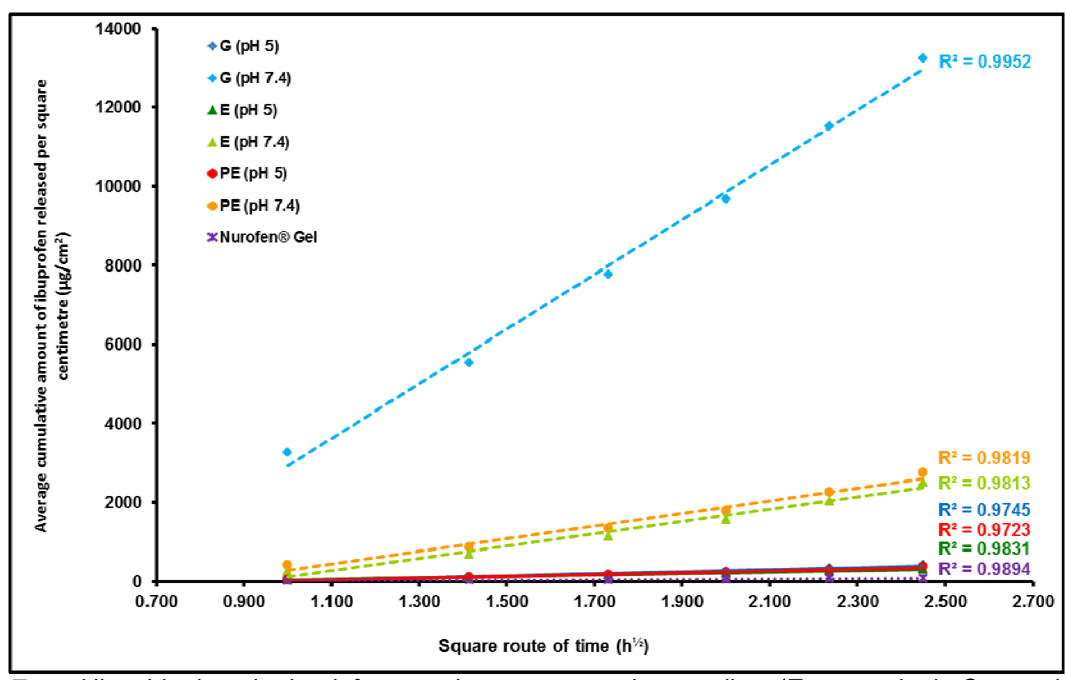

Figure7: *Higuchi plot obtained for membrane permeation studies (E = emulgel, G = gel, PE = Pheroid™ emulgel)*

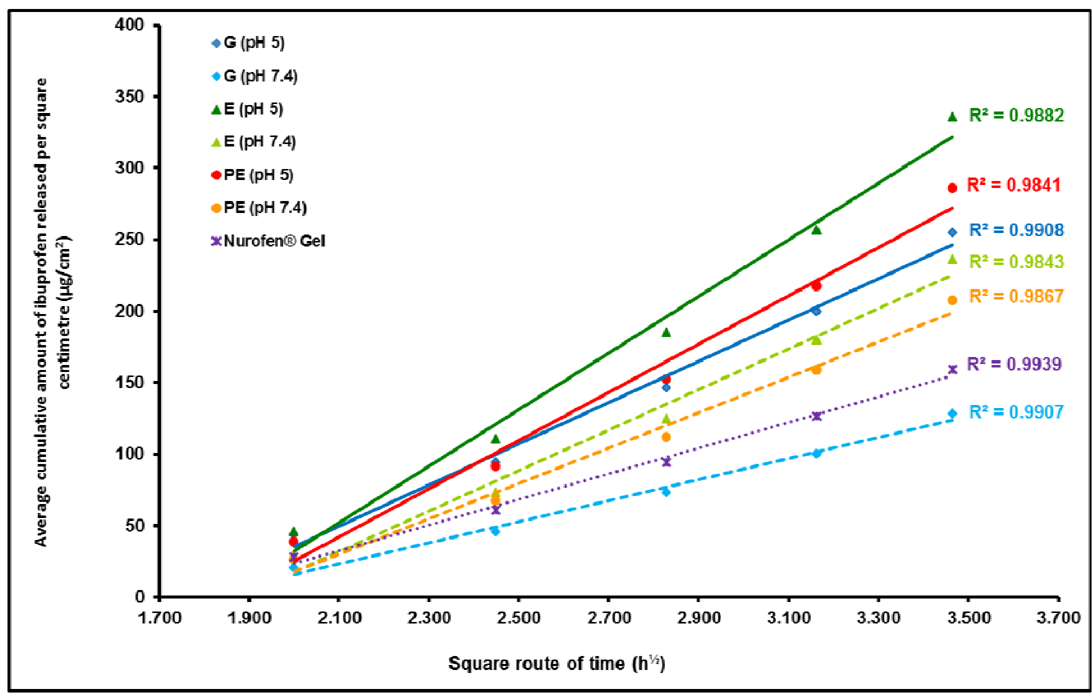

Figure 8: *Higuchi plot obtained for skin permeation studies (E = emulgel, G = gel, PE = Pheroid™ emulgel)*

CHAPTER 5
CONCLUSIONS AND FUTURE PROSPECTS

In order to develop new or improved dosage forms, a thorough understanding of the physiological factors affecting percutaneous penetration and the physicochemical properties of the API, as well as the compatibility of the formulation ingredients is essential. This eliminates problems associated with stability and poor *in vivo* dissolution, leading to the formation of a stable, efficacious, easy to administer and safe pharmaceutical dosage form (Mahato, 2007:11, Wells & Aulton, 2002:337). Therefore, the aim of this study was to determine the influence of selected formulation factors on the transdermal delivery of ibuprofen.

The aqueous solubility, pH-solubility profile, log P-value and log D-values (pH 5 and 7.4) of ibuprofen were determined. According to Naik *et al.,* (2000:319) the ideal aqueous solubility of APIs for transdermal delivery should be more than 1 $mg.ml^{-1}$. However, results showed that ibuprofen depicted an aqueous solubility of 0.096 $mg.ml^{-1} \pm 25.483$, which indicated poor water solubility and would therefore be rendered unsuitable for transdermal delivery if only considering the aqueous solubility. The pH-solubility profile depicted that ibuprofen was less soluble at low pH-values and more soluble at higher pH-values. Previous research indicated that the ideal log P-values for transdermal API permeation of NSAIDs are between 2 and 3 (Swart *et al.,* 2005:72). However, results obtained during this study indicated a log P-value of 4.238 for ibuprofen. This value is not included in the ideal range, which is an indication that the lipophilic/hydrophilic properties are not ideal, and this will therefore, contribute to poor ibuprofen penetration through the skin. Furthermore, the obtained log D-values at pH 5 and 7.4 were 3.105 and 0.386, respectively. Therefore, it would be expected that ibuprofen incorporated into a formulation prepared at a pH of 5 would more readily permeate the skin compared to ibuprofen incorporated into a formulation prepared at a pH of 7.4.

An artificial membrane was used to conduct the membrane permeation studies over a period of 6 h, in order to determine whether ibuprofen was in fact released from the formulations through the membrane. Skin permeation studies were conducted using Franz diffusion cells over a period of 12 h where samples were withdrawn at specified time intervals. The following observations could be made when all the formulations were compared to Nurofen® gel:

All the formulations exhibited an increase in the average cumulative amount of ibuprofen released from the formulations and that permeated the membrane when compared to Nurofen® gel. This increase was statistically significant ($p < 0.05$) for the gel, emulgel and Pheroid™ emulgel at pH 7.4. The gel at pH 7.4 exhibited the highest cumulative amount of ibuprofen that permeated the

membrane. Preparations formulated at a pH of 5, did not differ significantly from Nurofen® when the average cumulative amounts of ibuprofen that permeated the membrane were compared. The following rank order for the average cumulative amount released from the formulations could be established: Gel (pH 7.4) >>>> Pheroid™ emulgel (pH 7.4) > Emulgel (pH 7.4) >>> Gel (pH 5)> Pheroid™ emulgel (pH 5) ≈ Emulgel (pH 5) > Nurofen® gel.

On the other hand, all the formulations exhibited an increase in the average cumulative amount of ibuprofen that permeated the skin when compared to Nurofen® gel. This increase was statistically significant ($p<0.05$) for the gel, emulgel and Pheroid™ emulgel at pH 5, as well as the emulgel and Pheroid™ emulgel at pH 7.4. The emulgel at pH 5 exhibited the highest cumulative amount of ibuprofen that permeated the skin. The following rank order for the average cumulative amount released from the formulations and that permeated the skin could be established: Emulgel (pH 5) >> Pheroid™ emulgel (pH 5) > Gel (pH 5) > Emulgel (pH 7.4)> Pheroid™ emulgel (pH 7.4) ≈ Emulgel (pH 7.4) >> Nurofen® gel > Gel (pH 7.4). From this rank order it was clear that a trend was followed where the pH of formulation also played a role in ibuprofen permeation.

All the formulations exhibited a higher release rate and flux when compared to Nurofen® gel. This was statistically significant for the emulgel, gel and Pheroid™ emulgel at pH 7.4. The gel at pH 7.4 exhibited the highest release rate and flux. This was observed for the membrane and skin permeation studies.

All the formulations (including Nurofen® gel) presented a correlation coefficient (r^2) of 0.972 – 0.995 for membrane permeation studies, and 0.950 – 0.978 for skin permeation studies; indicating that the release of ibuprofen from each of the formulations could be described by the Higuchi model.

All the formulations exhibited a prolonged lag time compared to Nurofen® gel which indicated that the ibuprofen was retained for a longer time by the base. This was statistically significant ($p < 0.05$) for the emulgel at pH 7.4, the gel and Pheroid™ emulgel at pH 5. The gel at pH 7.4 exhibited a lag time closest to that of Nurofen® gel ($p>0.286$). The gel at pH 7.4 exhibited a lag time closest to that of Nurofen® gel and this difference could not be classified as statistically significant ($p > 0.2857$). This was observed for the membrane and skin permeation studies.

Nurofen® gel exhibited the highest ibuprofen concentration in the stratum corneum as well as in the epidermis followed by the gel at pH 7.4. This indicated that topical and transdermal delivery of ibuprofen was achieved.

The pH of a formulation plays an important role with respect to API permeation. Ibuprofen is reported to have a pK_a value 4.4 (Dollery, 1999:I1); and by application of the Henderson-Hasselbach equation, at pH 5, 20.08% of ibuprofen will be present in its unionised form and at

pH 7.4, 0.1% ibuprofen will exist in its unionised form. Since the unionised form of APIs is more lipid soluble than the ionised form, unionised forms of APIs permeate more readily across the lipid membranes (Surber & Smith, 2000:27). Therefore, it would be expected that ibuprofen formulated at pH 5 would be more permeable than formulations at pH 7.4. However, this did not correspond to the results (membrane studies) obtained in this study. This may be attributed to the solubility of ibuprofen in the different formulations. According to the pH-solubility profile of ibuprofen obtained in this study, it is more soluble at pH 7.4 than at pH 5. This is due to the fact that ibuprofen is a weak acidic compound, and for every 3 units away from the *pK_a-value*, the solubility changes 10-fold (Mahato, 2007:14). However, with regard to skin permeation studies, enhanced permeation was obtained with the formulations prepared at pH 5. This was in accordance with Corrigan *et al.,* (2003:148) who stated that NSAIDs are less soluble and more permeable at low pH values, and more soluble and less permeable at high pH values. This was most probably due to the fact that unionised species, although possessing a lower aqueous solubility than the ionised species, resulted in enhanced skin permeation due to being more lipid-soluble. As the unionised species is then absorbed, equilibrium will be re-established, resulting in unionised molecules available for absorption.

The stability of a pharmaceutical dosage form relates to the various changes (change in colour, texture, assay, viscosity, pH, mass, zeta potential and droplet size) that may occur during preparation, as well as the impact of these changes on its suitability for use (Shaikh & Sial, 1996:83).

No significant change in colour was observed for the gel and emulgel formulations at pH 5 and 7.4 over the three months at all the storage conditions. However, it was observed that the formulations containing Pheroid™ showed a radical change in colour at all the storage conditions. This may be due to oxidation of certain components present in the Pheroid™ system. Consequently, further investigation is necessary to find the cause of the discolouration and a method to prevent it.

The gel formulated at pH 5 depicted the formation of crystals. This may be due to the fact that the solubility of ibuprofen was exceeded, leading to ibuprofen precipitating from the formulation. No significant change was observed for the emulgel and Pheroid™ emulgel formulated at pH 5 and 7.4.

The gel at pH 5 showed the most pronounced change in ibuprofen content at all of the storage conditions. This may be due to the fact that the solubility of ibuprofen was exceeded, leading to ibuprofen precipitation from the formulation, thereby causing the formation of crystals. On the other hand, the emulgel and Pheroid™ emulgel at pH 5 depicted relative instability (according to the ICH) only at 40 °C/75% RH with a change in ibuprofen content of more than 5% (6.78 and 6.46%, respectively). The gel, emulgel and Pheroid™ emulgel at pH 7.4 exhibited the least variation in the change of ibuprofen content at all of the storage conditions. It might indicate that the pH at which a semi-solid formulation is produced will have a direct influence on the stability of the product.

No significant changes ($p > 0.05$) in viscosity (%RSD < 5) was observed for the gel and emulgel formulated at pH 7.4 and stored at 25 °C/60% RH. The remaining formulations at all the specified storage conditions exhibited a significant change in viscosity (%RSD > 5) with a decrease in viscosity being more pronounced at the higher temperature and humidity storage conditions. A possible contributing factor to the change in viscosity over three months at the specified storage conditions might have been the use of Pluronic® F-127 (viscosity enhancer). This viscosity enhancer possesses a melting point of approximately 56 °C (BAST Corporation. s.a). The problem with this might have been the temperature (70 °C) at which the formulations were prepared. This might have caused the Pluronic® F-127 to degrade, thereby losing its ability to function appropriately as a viscosity enhancer.

No significant change ($p > 0.05$) was observed for mass variation, pH, zeta potential and droplet size over the three months for any of the different formulations stored at the different storage conditions.

A balance must be maintained between optimum solubility and maximum stability (Pefile & Smith, 1997:148). Despite the lower skin permeation of the gel formulated at pH 7.4, this formulation performed the best, as it was considered stable (least variation during the 3 month stability test) and the obtained tape stripping results showed that this formulation depicted the highest ibuprofen concentrations in the stratum corneum and epidermis. Thus, topical as well as transdermal delivery were obtained.

Future prospects for further investigation include the following:

- The cause for discolouration of the Pheroid™ formulations need to be investigated and a method to prevent it needs to be found.
- Preparation of different semi-solid formulations containing different surfactants should be formulated to investigate the effect of the change in viscosity and to maintain it.
- Different co-solvents in the formulation of the different semi-solid formulations prepared should be included, in order to investigate which co-solvent system delivers improved skin permeation.
- Formulations containing the pure S-(+) enantiomer of ibuprofen should be prepared and the obtained results compared with racemic formulations to prove that the anti-inflammatory effect of ibuprofen resides in the pure S-(+) enantiomer.
- More lipid-based excipients should be included in formulations in order to investigate improved solubility of ibuprofen, since it is a more lipid-soluble active pharmaceutical ingredient (API).

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ANNEXURE A

Table A.1: *Linearity results of ibuprofen*

Standard (µg/ml)	Peak area
0.10	0.16
0.20	0.33
0.30	0.58
0.40	0.64
0.50	0.92
1.01	1.78
2.02	3.38
3.02	5.42
4.03	7.38
5.04	9.22
1.08	18.16
20.16	34.91
30.24	54.27
40.32	74.38
50.4	92.54
100.8	182.7
201.6	369.0
302.4	540.9
403.2	725.8
504	898.1
Slope	1.7878
y-intercept	1.6718
r^2	0.9998

r^2 = Correlation coefficient

Table A.2: Accuracy results of ibuprofen

Concentration spiked (µg/ml)	Peak area 1 (mAU)	Peak area 2 (mAU)	Mean peak area (mAU)	Recovery	
				µg/ml	%
10.032	18.7	18.5	18.6	9.4	93.7
10.032	18.9	19.1	19.0	9.6	95.9
10.032	18.7	18.5	18.6	9.4	93.7
50.16	90.7	90.8	90.8	49.8	99.2
50.16	90.7	90.8	90.7	49.8	99.2
50.16	90.8	90.9	90.9	49.9	99.4
250.8	458.2	458.1	458.1	255.3	101.8
250.8	458.2	458.7	458.4	255.5	101.9
250.8	457.9	457.9	457.9	255.2	101.7
Mean	98.50				
SD*	3.12				
%RSD**	3.17				

***Standard deviation**
****Relative standard deviation**

Table A.3: Repeatability results of ibuprofen

Concentration spiked (µg/ml)	Peak area 1 (mAU)	Peak area 2 (mAU)	Mean peak area (mAU)	Concentration recovered	
				(µg/ml)	%
50.4	100.3	100.8	100.6	48.6	97.1
50.4	100.2	100.3	100.3	48.4	96.8
50.4	100.0	100.2	100.1	48.3	96.7
				Mean	96.85
				SD*	0.19
				%RSD**	0.20

***Standard deviation**
****Relative standard deviation**

Table A.4: Interday precision results of ibuprofen

Day	%			Mean (%)	SD*	%RSD**
1	99.2	99.2	99.4	99.3	0.08	0.08
2	97.1	96.8	96.7	96.8	0.19	0.2
3	100.0	98.8	99.2	99.3	0.51	0.51
Between days				98.5	1.20	1.21

***Standard deviation**
****Relative standard deviation**

Table A.5: *Reproducibility results of ibuprofen*

Concentration spiked (µg/ml)	Peak area 1 (mAU)	Peak area 2 (mAU)	Mean peak area (mAU)	Concentration recovered	
				(µg/ml)	%
50.8	94.7	95.7	95.2	50.8	100.0
50.8	93.6	94.5	94.1	50.2	98.8
50.8	94.5	94.4	94.5	50.4	99.2
				Mean	99.3
				SD*	0.51
				%RSD**	0.51

***Standard deviation**
****Relative standard deviation**

Table A.6: *Sample stability results of ibuprofen*

Time (h)	Peak area (mAU)	% Recovery
0	457.8	100
1	457.0	99.8
2	457.1	99.9
3	456.6	99.8
4	457.1	99.8
5	456.9	99.8
6	457.1	99.8
7	456.9	99.8
8	457.6	100.0
9	457.6	100.0
10	457.8	100.0
11	457.8	100.0
12	457.6	100.0
13	457.9	100.0
14	458.0	100.1
15	458.2	100.1
16	458.5	100.2
17	458.4	100.1
18	458.8	100.2
19	458.6	100.2
20	458.9	100.3
21	458.9	100.2
22	458.5	100.2
23	459.8	100.4
24	459.6	100.4
Mean	458.0	100.0
SD*	0.8	0.2
%RSD**	0.2	0.2

***Standard deviation**
****Relative standard deviation**

Table A.7: *Sample repeatability results of ibuprofen*

Injection	Peak area (mAU)	Retention time (min)
1	460.0	5.22
2	459.2	5.22
3	460.6	5.21
4	461.0	5.2
5	459.9	5.19
6	460.0	5.16
Mean	460.1	5.2
SD*	0.6	0.02
%RSD**	0.1	0.41

***Standard deviation**
****Relative standard deviation**

Table A.8: *Results of pH- and solubility values*

pH	Solubility (mg/ml)	pH	Solubility (mg/ml)
1	0.023	8	8.119
2	0.026	9	0.633
3	0.028	10	3.444
4	0.018	11	0.576
5	0.188	12	1.458
6	0.182	13	8.890
7	1.121	14	53.141

ANNEXURE B

Table B.1: *Average cumulative amount of ibuprofen released from the formulations that permeated the membrane over 6 h.*

Time (h)	Average cumulative amount released µg.cm^{-2}						
	E (pH 5)	**E (pH 7.4)**	**G (pH 5)**	**G (pH 7.4)**	**PE (pH 5)**	**PE (7.4)**	**Nurofen® gel**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	51.80	242.54	36.93	3252.40	42.24	410.87	23.04
2	103.42	678.14	103.67	5544.35	96.71	868.53	37.11
3	156.40	1131.19	173.75	7762.11	158.68	1327.61	49.97
4	209.17	1565.99	248.68	9671.33	224.27	1781.58	62.02
5	261.74	2035.72	325.48	11530.30	291.42	2266.79	74.35
6	316.35	2505.27	407.81	13248.40	362.78	2745.25	87.00

(E = emulgel, G = gel, PE = Pheroid™ emulgel)

Table B.2: *Relationship between flux (apparent release constant) and release rate obtained for membrane permeation studies*

Formulation	n	Mean	
		Release rate (µg.cm^{-2}.h)	**Apparent release constant (µg.cm^{-2}.h)**
E (pH 5)	8	52.87	182.42
E (pH 7.4)	8	452.02	1558.35
G (pH 5)	7	74.14	254.84
G (pH 7.4)	7	1995.63	6938.58
PE (pH 5)	9	64.35	221.02
PE (pH 7.4)	10	466.30	1607.98
Nurofen® gel	7	12.67	43.88

(E = emulgel, G = gel, PE = Pheroid™ emulgel, n = number of cells used)

Table B.3: *Values obtained to fit the Higuchi model for membrane permeation studies*

Time (h)	Cumulative amount released per square centimeter (µg.cm^{-2}.h)						
	E (pH 5)	**E (pH 7.4)**	**G (pH 5)**	**G (pH 7.4)**	**PE (pH 5)**	**PE (7.4)**	**Nurofen® gel**
1.414214	51.797	242.540	36.9309	3252.4035	42.24356	410.871	23.0432
2.000000	103.420	678.144	103.666	5544.3523	96.71374	868.531	37.1076
2.449490	156.397	1131.191	173.749	7762.1107	158.6787	1327.61	49.9722
2.828427	209.174	1565.992	248.68	9671.3283	224.2686	1781.58	62.0201
3.162278	261.743	2035.715	325.484	11530.298	291.4212	2266.79	74.3532
3.464102	316.346	2505.217	407.813	13248.398	362.7824	2745.25	87.0013

(E = emulgel, G = gel, PE = Pheroid™ emulgel, n = number of cells used)

Table B.4: *Average cumulative amount of ibuprofen that permeated the skin over 12 h.*

Time (h)	Average cumulative amount released µg.cm^{-2}						
	E (pH 5)	**E (pH 7.4)**	**G (pH 5)**	**G (pH 7.4)**	**PE (pH 5)**	**PE (7.4)**	**Nurofen® gel**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	45.2425	28.818	43.8654	20.4282	38.1893	26.447	27.9958
6	110.796	73.3326	94.0804	45.6949	91.5089	67.0751	60.9159
8	185.033	124.354	146.45	72.9287	152.304	111.477	93.9617
10	256.196	179.516	199.917	99.9608	217.399	158.463	126.578
12	335.547	235.884	254.781	127.94	285.564	207.462	159.469

(E = emulgel, G = gel, PE = Pheroid™ emulgel, n = number of cells used)

Table B.5: *Relationship between flux (apparent release constant) and release rate obtained for skin permeation studies*

Formulation	n	Mean	
		Release rate (µg.cm^{-2}.h)	**Apparent release constant (µg.cm^{-2}.h)**
E (pH 5)	9	34.0691	164.7471
E (pH 7.4)	10	24.0362	115.8808
G (pH 5)	8	25.6347	124.6178
G (pH 7.4)	7	12.93614	62.79049
PE (pH 5)	9	28.9463	139.7401
PE (pH 7.4)	9	21.1108	101.9372
Nurofen® gel	10	16.08765	78.34717

(E = emulgel, G = gel, PE = Pheroid™ emulgel, n = number of cells used)

Table B.6: *Values obtained to fit the Higuchi model for skin permeation studies*

Time (h)	Cumulative amount released per square centimeter (µg.cm^{-2}.h)						
	E (pH 5)	**E (pH 7.4)**	**G (pH 5)**	**G (pH 7.4)**	**PE (pH 5)**	**PE (7.4)**	**Nurofen® gel**
1.414214	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.000000	45.242	28.818	43.865	20.428	38.189	26.447	27.996
2.449490	110.796	73.333	94.080	45.695	91.509	67.075	60.916
2.828427	185.033	124.354	146.450	72.9289	152.304	111.477	93.962
3.162278	256.196	179.516	199.917	99.961	217.399	158.464	126.578
3.464102	335.547	235.884	254.781	127.940	285.564	207.462	159.469

(E = emulgel, G = gel, PE = Pheroid™ emulgel, n = number of cells used)

Table B.7: *Number of cells used (n), the average ibuprofen concentration obtained in the stratum corneum, standard deviations and p-values for the various formulations tested*

Formulation	n*	Average ibuprofen concentration (µg/ml)	Standard deviation	p-value	
				Kruskal-Wallis	Bonferoni on Dunn
E (pH 5)	9	12.777	11.046		0.016791*
E (pH 7.4)	10	2.546	2.105		2.86^{-7}
G (pH 5)	8	26.199	15.987		0.286
G (pH 7.4)	7	23.392	5.628	<0.001*	0.286
PE (pH 5)	9	6.548	5.236		0.000145*
PE (pH 7.4)	9	8.300	5.198		0.001685*
Nurofen® gel	10	45.851	20.004		

*(E = emulgel, G = gel, PE = Pheroid™ emulgel, * = statistically significant)*

n* = data from leaking cells was discarded

Table B.8: *Number of cells used (n), the average ibuprofen concentration obtained in the epidermis, standard deviations and p-values for the various formulations tested*

Formulation	n*	Average ibuprofen concentration (µg/ml)	Standard deviation	p-value	
				Kruskal-Wallis	Bonferoni on Dunn
E (pH 5)	9	14.1347	16.4871		0.285714
E (pH 7.4)	10	6.4412	4.55578		0.034274*
G (pH 5)	8	5.0211	1.85004		0.016823*
G (pH 7.4)	7	10.58514	4.43365	0.0010	0.285714
PE (pH 5)	9	4.4532	1.89079		0.002698*
PE (pH 7.4)	9	4.5624	2.48652		0.002837*
Nurofen® gel	10	18.5725	10.11402		

*(E = emulgel, G = gel, PE = Pheroid™ emulgel, * = statistically significant)*

n* = data from leaking cells was discarded

ANNEXURE C

Table C.1: Mass variation (g) values obtained for all the semi-solid formulations after storage at the different conditions

Formulation	Storage condition (°C/% RH)	MONTH				Average	SD*	%RSD**
		0	1	2	3			
E (pH 5)	25/60	50.30	50.29	50.29	50.27	50.29	0.01	0.02
	30 /60	50.40	50.36	50.35	50.33	50.36	0.03	0.06
	40 /75	50.28	50.43	50.24	50.20	50.29	0.09	0.17
E (pH 7.4)	25/60	50.34	50.34	50.33	50.32	50.34	0.01	0.02
	30 /60	50.35	50.47	50.44	50.39	50.41	0.05	0.09
	40 /75	50.56	50.57	50.53	50.48	50.53	0.03	0.06
G (pH 5)	25/60	50.00	50.36	50.35	50.40	50.28	0.16	0.32
	30 /60	50.02	50.39	50.38	50.37	50.29	0.16	0.32
	40 /75	50.08	50.49	50.47	50.43	50.37	0.17	0.33
G (pH 7.4)	25/60	50.03	50.39	50.60	50.69	50.43	0.25	0.51
	30 /60	50.02	50.38	50.36	50.33	50.27	0.14	0.29
	40 /75	50.02	50.43	50.39	50.34	50.30	0.16	0.32
PE (pH 5)	25/60	50.10	50.10	50.09	50.07	50.09	0.01	0.02
	30 /60	50.01	50.00	49.98	49.96	49.99	0.02	0.04
	40 /75	50.21	50.20	50.17	50.13	50.18	0.03	0.06
PE (pH 7.4)	25/60	50.17	50.17	50.16	50.15	50.16	0.01	0.02
	30 /60	50.06	50.06	50.04	50.02	50.04	0.02	0.04
	40 /75	50.99	50.98	51.89	50.84	51.17	0.42	0.81

(E = emulgel, G = gel, PE = Pheroid™ emulgel)

RH = Relative Humidity

***Standard deviation**

****Relative standard deviation**

Table C.2: *Assay (%) values obtained for all the semi-solid formulations after storage at the different conditions*

Formulation	Storage condition (°C/% RH)	MONTH				Average	SD*	%RSD**
		0	1	2	3			
E (pH 5)	25/60	107.41	106.92	109.89	108.54	108.19	1.321	1.221
	30 /60	107.41	105.56	99.09	104.00	104.02	3.567	3.429
	40 /75	107.41	99.10	105.43	100.63	103.14	3.920	3.801
E (pH 7.4)	25/60	103.41	102.51	102.26	106.03	103.55	1.724	1.665
	30 /60	103.41	97.74	106.62	107.98	103.94	4.554	4.382
	40 /75	103.41	101.17	108.83	105.85	104.82	3.289	3.138
G (pH 5)	25/60	101.71	106.76	106.28	111.86	106.65	4.151	3.892
	30 /60	101.71	99.70	103.23	109.90	103.64	4.420	4.265
	40 /75	101.71	102.11	105.67	107.08	104.14	2.646	2.541
G (pH 7.4)	25/60	114.02	104.53	102.63	109.03	107.55	5.079	4.722
	30 /60	114.02	102.64	103.82	109.44	107.48	5.274	4.907
	40 /75	114.02	102.51	104.79	109.51	107.71	5.119	4.753
PE (pH 5)	25/60	105.03	105.68	104.45	105.85	105.25	0.641	0.609
	30 /60	105.03	94.90	102.88	106.72	102.38	5.230	5.108
	40 /75	105.03	94.93	102.98	98.57	100.38	4.523	4.506
PE (pH 7.4)	25/60	103.43	93.95	105.17	102.26	101.20	4.981	4.921
	30 /60	103.43	90.94	106.27	104.33	101.24	6.970	6.884
	40 /75	103.43	98.84	107.24	102.74	103.06	3.441	3.339

(E = emulgel, G = gel, PE = Pheroid™ emulgel)

RH = Relative Humidity

***Standard deviation**

****Relative standard deviation**

Table C.3: pH values obtained for all the semi-solid formulations after storage at the different conditions

Formulation	Storage condition (°C/% RH)	MONTH				Average	SD*	%RSD**
		0	1	2	3			
E (pH 5)	25/60	5.01	4.90	5.02	5.02	4.99	0.05	1.03
	30 /60	5.01	4.74	4.89	4.94	4.89	0.10	2.07
	40 /75	5.01	4.90	4.70	4.78	4.85	0.12	2.47
E (pH 7.4)	25/60	7.15	7.06	7.13	7.14	7.12	0.03	0.48
	30 /60	7.15	7.10	7.20	7.24	7.17	0.05	0.74
	40 /75	7.15	7.13	7.15	7.25	7.17	0.05	0.68
G (pH 5)	25/60	5.30	5.43	5.44	5.49	5.42	0.07	1.30
	30 /60	5.30	5.27	5.24	5.53	5.34	0.11	2.11
	40 /75	5.30	5.22	5.26	5.26	5.26	0.03	0.57
G (pH 7.4)	25/60	7.17	7.14	7.18	7.21	7.18	0.03	0.38
	30 /60	7.17	7.14	7.16	7.21	7.17	0.03	0.37
	40 /75	7.17	7.18	7.18	7.17	7.17	0.00	0.07
PE (pH 5)	25/60	5.25	5.08	5.04	4.99	5.09	0.10	1.89
	30 /60	5.25	4.92	4.99	4.99	5.04	0.13	2.52
	40 /75	5.25	4.88	4.90	4.89	4.98	0.15	3.11
PE (pH 7.4)	25/60	7.16	7.23	7.24	7.32	7.24	0.06	0.82
	30 /60	7.16	7.20	7.22	7.30	7.22	0.05	0.73
	40 /75	7.16	7.08	7.11	7.15	7.12	0.03	0.46

(E = emulgel, G = gel, PE = Pheroid™ emulgel)

RH = Relative Humidity

***Standard deviation**

****Relative standard deviation**

Table C.4: *Viscosity (cP) values obtained for all the semi-solid formulations after storage at the different conditions*

Formulation	Storage condition (°C/% RH)	MONTH				Average	SD*	%RSD**
		0	1	2	3			
E (pH 5)	25/60	838101.75	652692.74	637266.37	679444.87	701876.43	80084.34	11.41
	30 /60	838101.75	549296.85	685275.29	623597.41	674067.83	106240.05	15.76
	40 /75	838101.75	556033.70	752866.70	616079.48	690770.41	111012.60	16.07
E (pH 7.4)	25/60	933199.31	985141.35	865245.06	893949.87	919383.90	44980.03	4.89
	30 /60	933199.31	959658.51	784110.03	806175.64	870785.87	76617.50	8.80
	40 /75	933199.31	819844.59	664701.92	777861.36	798901.80	96081.65	12.03
E (pH 7.4)	25/60	2105019.57	2055713.70	2073873.89	2576403.38	2202752.63	216446.76	9.83
	30 /60	2105019.57	2129428.44	2429462.07	2109022.64	2193233.18	136700.58	6.23
	40 /75	2105019.57	1825587.02	1841989.77	1898520.67	1917779.26	111437.52	5.81
G (pH 7.4)	25/60	228.73	213.39	204.30	200.62	211.76	10.84	5.12
	30 /60	228.73	157.91	179.37	182.30	187.08	25.83	13.81
	40 /75	228.73	149.50	148.35	142.72	167.32	35.55	21.24
PE (pH 5)	25/60	1081800.42	826630.30	871298.46	851771.38	907875.14	101656.54	11.20
	30 /60	1081800.42	642245.77	1104256.56	889263.38	929391.53	185653.71	19.98
	40 /75	1081800.42	758920.09	974108.55	915136.76	932491.46	116676.21	12.51
PE (pH 7.4)	25/60	982883.74	1281953.02	1085992.07	1553477.11	1226076.49	217416.49	17.73
	30 /60	982883.74	1174749.33	977037.61	1068229.09	1050724.95	80188.37	7.63
	40 /75	982883.74	1044015.51	1301773.01	1009159.67	1084457.98	127326.92	11.74

(E = emulgel, G = gel, PE = Pheroid™ emulgel)

RH = Relative Humidity

***Standard deviation**

****Relative standard deviation**

Table C.5: *Zeta potential (mV) values obtained for all the semi-solid formulations after storage at the different conditions*

Formulation	**Storage condition (°C/% RH)**	**MONTH**				**Average**	***SD**	**%RSD****
		0	**1**	**2**	**3**			
E (pH 5)	25/60	19.79	20.31	20.07	20.19	20.09	0.19	0.96
	30 /60	19.79	20.56	19.80	20.60	20.19	0.39	1.94
	40 /75	19.79	20.36	19.96	20.32	20.11	0.24	1.20
E (pH 7.4)	25/60	19.58	19.58	19.61	19.66	19.61	0.03	0.16
	30 /60	19.58	19.52	19.82	20.37	19.82	0.33	1.68
	40 /75	19.58	19.88	19.78	20.16	19.85	0.21	1.05
PE (pH 5)	25/60	18.93	19.47	19.37	20.23	19.50	0.47	2.40
	30 /60	18.93	19.86	19.90	19.29	19.49	0.40	2.07
	40 /75	18.93	18.50	19.31	19.52	19.07	0.39	2.04
PE (pH 7.4)	25/60	19.88	19.52	19.77	19.93	19.78	0.16	0.80
	30 /60	19.88	19.52	19.48	19.57	19.61	0.16	0.80
	40 /75	19.88	19.63	20.13	20.10	19.94	0.20	1.01

(E = emulgel, G = gel, PE = Pheroid™ emulgel)

RH = Relative Humidity

***Standard deviation**

****Relative standard deviation**

Table C.6: *Droplet size (µm) values obtained for all the semi-solid formulations after storage at the different conditions*

Formulation	**Storage condition (°C/% RH)**	**MONTH**				**Average**	**SD***	**%RSD****
		0	**1**	**2**	**3**			
E (pH 5)	25/60	2.53	2.75	2.78	2.78	2.73	0.12	4.35
	30 /60	2.53	2.79	2.93	2.93	2.81	0.17	6.14
	40 /75	2.53	2.92	3.09	3.09	2.94	0.26	8.92
E (pH 7.4)	25/60	2.66	2.80	2.83	2.83	2.82	0.11	4.08
	30 /60	2.66	2.81	2.99	2.99	2.81	0.12	4.24
	40 /75	2.66	2.91	3.35	3.35	3.08	0.31	10.03
PE (pH 5)	25/60	0.26	0.28	0.26	0.26	0.26	0.01	3.92
	30 /60	0.26	0.27	0.25	0.25	0.26	0.01	2.96
	40 /75	0.26	0.26	0.26	0.26	0.26	0.00	0.80
PE (pH 7.4)	25/60	1.59	1.53	1.58	1.58	1.63	0.11	6.45
	30 /60	1.59	1.59	1.64	1.64	1.65	0.07	4.43
	40 /75	1.59	1.64	1.69	1.69	1.63	0.04	2.46

(E = emulgel, G = gel, PE = Pheroid™ emulgel)

RH = Relative Humidity

***Standard deviation**

****Relative standard deviation**

ANNEXURE D
AUTHOR'S GUIDE TO THE INTERNATIONAL JOURNAL OF PHARMACEUTICS

D.1 DESCRIPTION

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

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D.2 GUIDE FOR AUTHORS

D.2.1 INTRODUCTION

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

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- **Rapid Communications**

(a) These articles should not exceed 1500 words or equivalent space.
(b) Figures should not be included otherwise delay in publication will be incurred.
(c) Do not subdivide the text into sections. An Abstract should be included as well as a full reference list.

- **Notes**

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

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(b) Do not subdivide the text into sections. An Abstract and reference list should be included.

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