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Synthesis and transdermal penetration of NSAID glycoside esters

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Abstract

The glucoside and mannoside derivatives of the NSAIDs flurbiprofen, ibuprofen, ketoprofen and naproxen were synthesised and their penetration through human skin was determined. Experimental transdermal flux data showed that the parent NSAIDs penetrated human skin to a much higher extent than the glycosides.

Keywords: Flurbiprofen; Ibuprofen; Ketoprofen; Naproxen; Glycoside; Transdermal penetration

1. Introduction

The benefits associated with the topical route of drug administration have been well documented. The greatest advantages include a non-invasive treatment regimen, bypassing of first pass metabolism and quick interruption of treatment (Naik et al., 2000; Flynn, 1993; Beckett, 1982). These reasons have led to the recent upsurge in research in the transdermal delivery of drugs. The skin is an efficient barrier between the internal plasma and the harsh exterior, providing a great challenge to the scientist in the quest for transdermal drug delivery (Pefile and Smith, 1997). Much research has been conducted to improve the flux of drugs through the skin, concentrating primarily on decreasing the barrier function of this complex membrane system. Another feasible option is the synthesis of derivatives with improved transdermal flux, but which do not compromise the drug's efficacy (Bonina et al., 2001). A further approach is to use enhancers with optimum permeation properties that can be delivered concurrently with the drug.

NSAIDs possess anti-pyretic, analgesic and anti-inflammatory activity. Their primary use is as anti-inflammatory agents for the treatment of musculoskeletal disorders, including rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Their use is limited to the relief from pain and inflammation, as they do not halt the progression of the pathological injury caused to the tissue. They are also used in the treatment of inflammation, fever, primary dysmenorrhoea and in the management of mild pain (Insel, 1996).

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Transdermal drug delivery offers a number of advantages over the traditional NSAID formulations, namely oral delivery and injection. It includes the elimination of first pass metabolism, minimisation of pain and discomfort, possible sustained drug release over a specific period of time and vastly improved patient compliance (Mitragotri, 2000). There is much concern regarding the scale of morbidity associated with gastric complications resulting from NSAIDs used by the elderly in the treatment musculoskeletal and joint pain associated with rheumatoid and osteoarthritis (Grahame, 1995).

Optimal transport through the skin requires a drug to possess lipophilic as well as hydrophilic properties (Sloan and Wasdo, 2003; Guy and Hadgraft, 1992). Previous research indicated the ideal log *P* value for optimal transdermal permeation of non-steroidal anti-inflammatory drugs (NSAIDs) to be between 2 and 3 (Hadgraft and Wolff, 1993; Hadgraft et al., 2000). In this investigation NSAIDs, all having log *P* values higher than 2, were selected for the preparation of their glycosides with the objective of determining the skin penetration properties of these compounds as a function of their structure and stereochemistry. This information will enhance our database on the relationship between physical properties and the transdermal penetration of compounds.

In this study, the glucose and mannose esters of flurbiprofen (**1**), ibuprofen (**2**), ketoprofen (**3**) and naproxen (**4**) were successfully prepared from glucose and mannose, respectively, in an enzyme catalysed reaction.

2. Materials and methods

2.1. Chemicals

Ibuprofen, ketoprofen and naproxen were generously donated by Lennon (South Africa) and flurbiprofen was generously donated by Abbott Laboratories (South Africa) and BASF (Germany). The *Candida antarctica* lipase B (SP 435) was generously donated by the Department of Microbiology and Biochemistry, University of the Free State, South Africa. Reagent grade glucose, mannose, *t*-butanol, *o*-phosphoric acid and 4 Å molecular sieves were purchased from Sigma–Aldrich (South Africa). Reagent grade chloroform and ethanol were obtained from Merck (Midrand, South Africa) and HPLC grade acetonitrile was obtained from Saarchem (South Africa). Double distilled deionised water was obtained from a Milli-Q water purification system (Millipore, USA).

2.2. Synthesis

The method employed for synthesis was adapted from that described by Stamatis et al. (2001) and Tsitsimpikou et al. (1997). In this enzymatic reaction, *C. antarctica* Lipase B (SP 435) is used for the esterification of the NSAID with the sugar. The reaction is regiospecific, forming an ester bond between the carboxyl group of the acid and the hydroxyl group on C-6 of glucose or mannose.

A typical reaction mixture consisted of 3 mM NSAID, 1 mM of glucose or mannose, 300 mg of *C. antarctica* Lipase B enzyme (SP 435) and 100 mg of 4 Å molecular sieves (20 mg/ml of organic solvent) in 5 ml of *t*-butanol. The reaction was carried out in a stoppered 100 ml round bottom flask and was stirred at 200 rpm and 60 °C for 120 h. The reaction mixture was then filtered and washed with chloroform under suction. All reactions were followed on Alugram® Silica G/UV_{254} thin layer chromatography (TLC) plates using chloroform:methanol 10:1 as eluent. Developed TLC plates were examined under UV light (254 nm) and sprayed with a 1% vanilin/H_2SO_4 solution, which was heated for 10 min at 150 °C. Glycosides were deep blue coloured. Reactions of flufenamic acid and mefenamic acid, with the carboxylic acid group directly attached to the aromatic ring and of indomethacin and diclofenac, where the acid group is in a crowded environment, proved unsuccessful.

The NSAID glucosides and mannosides were isolated using column chromatography with a chloroform:methanol 10:1 eluent on silica gel. The esters were analysed by ^{1}H and ^{13}C NMR on a Varian Gemini-300 spectrometer in dimethyl sulfoxide (DMSO). Infrared (IR) spectra were recorded on a Nicolet Magna-IR 550 spectrometer with KBr pellets and the melting points were determined by DSC on a Shimadzu DSC 50. Mass spectrometry (MS) was performed on an analytical VG 7070E mass spectrometer.

Table 1
HPLC data

NSAID	Flow rate (ml/min)	Wave length (nm)	Mobile phase (acetonitrile:H_2O)	Retention time (min)
Flurbiprofen (**1**)	1.2	214	60:40	6.30
Flurbiprofen glucoside (**5**)	0.9	214	50:50	3.81
Flurbiprofen mannoside (**6**)	0.9	214	50:50	3.91
Ibuprofen (**2**)	1.2	214	60:40	10.57
Ibuprofen glucoside (**7**)	0.9	214	50:50	4.37
Ibuprofen mannoside (**8**)	0.9	214	50:50	4.35
Ketoprofen (**3**)	1.2	233	60:40	4.86
Ketoprofen glucoside (**9**)	1.4	233	40:60	4.89
Ketoprofen mannoside (**10**)	1.2	233	50:50	3.35
Naproxen (**4**)	1.2	214	60:40	5.83
Naproxen glucoside (**11**)	1.2	214	40:60	4.23
Naproxen mannoside (**12**)	0.9	214	50:50	5.22

2.3. Chromatographic procedure

The NSAIDs and NSAID glycosides were assayed by HPLC. The HPLC system consisted of a Hewlet Packard (HP) Agilent 1100 series auto sampler, HP Agilent 1100 series variable wave detector (vwd) and HP Agilent 1100 series isocratic pump. A Phenomenex (Luna C-18, 5 μ, 250 × 4.60 mM) column was used together with a Securityguard pre-column (C-18, 4 × 3 mM) insert (Phenomenex) and the Agilent Chemstation for LC Systems software package was used for data analysis. The flow rate, UV wavelengths, mobile phase compositions and retention times for each of the compounds are presented in Table 1. *o*-Phosphoric acid was used to set the pH of the mobile phases at 2.25. A volume of 100 μl was injected for each of the samples. Calibration curves were constructed ranging from concentrations of 0.125 μg/ml to 5.0 μg/ml. Excellent linearity ($0.95 \leq r^2 \leq 0.99$) was obtained for the NSAIDs and their glycosides.

2.4. Aqueous solubility

The aqueous solubility of the NSAIDs and their glycosides was determined whereby excess amounts of these solutes were equilibrated at 32 °C in phosphate buffer at pH values of 4.0, 5.5 and 7.0 (British Pharmacopoeia, 2004). After 24 h, the samples were filtered (PTFE filter media with polypropylene housing, 0.45 μm pore size, Whatman Inc., Haverhill, MA) and assayed by HPLC. The results are presented in Table 3.

2.5. n-Octanol/water partition coefficient (log P)

Equal volumes of *n*-octanol and phosphate buffer (pH 7.0) were saturated for a period of 24 h. Solutions of the NSAIDs and their glycosides (30 μg/ml) were prepared in the pre-saturated *n*-octanol phase, where after 5 ml of these solutions were transferred to test tubes containing 5 ml pre-saturated buffer. The tubes were stoppered and agitated for 1 h, followed by centrifugation. The buffer phase was assayed directly by HPLC. The *n*-octanol phase was diluted 1000 times with acetonitrile prior to assay by HPLC. Partition coefficients were calculated as the ratio of drug concentration in the *n*-octanol phase to that in the buffer phase.

ACD Labs and K_{ow}Win (http://esc.syrres.com/interkow/kowdemo.htm) prediction softwares were used to predict the partition coefficients for the NSAIDs and glycosides. The predicted and experimental values are presented in Table 4.

2.6. Skin preparation

The skin used in the transdermal permeation studies was obtained from the abdomen of female patients who underwent cosmetic surgery and was prepared following standard procedures (Blank and Mc Auliffe, 1985). The full-thickness skin was frozen at −20 °C within 24 h after removal. Prior to preparation, the skin was thawed to room temperature, where after the excess fat was carefully removed. After immersing the skin in water at 60 °C for 1 min the epidermis was gently separated from the remaining tissue with forceps, floated on filter paper in distilled water and left to dry. The pre-

pared samples were kept frozen at −20 °C until used. The epidermis was thawed prior to experimental use and was carefully inspected visually for any defects.

2.7. Skin permeation

Vertical Franz diffusion cells with 1.8 ml receptor compartments and 1.075 cm^2 diffusion areas were used during the permeation studies. The epidermal skin layer was mounted carefully on the lower half of the Franz cell with the epidermis facing upwards. The upper and lower parts of the Franz cell were fastened together by means of a clamp, with the epidermis acting as a seal between the donor and receptor compartments. The receptor compartments were filled with isotonic phosphate buffer (pH 7.4). Special care was taken that there were no air bubbles between the buffer and the epidermis in the receptor compartment. The prepared Franz cells, containing the buffer, were equilibrated for 1 h in a water bath at 37 °C, prior to the addition of the saturated solutions to the donor compartments. Only the receptor compartment was in contact with the water at 37 °C and each Franz cell was equipped with a stirring magnet. After an hour, 1 ml of freshly prepared saturated solution of the NSAID or NSAID glycoside was added to each donor compartment, which was immediately covered with Parafilm®, to avoid the evaporation of any of the constituents.

Donor solutions of NSAIDs and NSAID glycosides were prepared by equilibrating excess amounts of each with phosphate buffer (pH 7.0) in stoppered vials. These solutions were stirred in a water bath at 37 °C over a period of 24 h. After 24 h, 1 ml of saturated solution was withdrawn and added to each donor compartment. To ensure that sink conditions prevail, an excess amount of solute was present in the donor compartments for the duration of the transdermal experiment.

At predetermined intervals (2, 4, 6, 8, 10, 12 and 24 h), the entire receptor volumes were withdrawn and replaced with 37 °C fresh buffer solution in order to maintain sink conditions. The withdrawn samples were assayed directly by HPLC to determine the concentrations of the compounds that had permeated through the epidermis. Hydrolysis of the glycoside esters during the process was negligible and only in some instances traces of the hydrolysis products were observed. The permeation data were plotted as the cumulative amount of drug penetrated through the skin as a function of time. All values are the averages of six parallel experiments each on skin from the same source.

3. Results

3.1. Synthesis

The products obtained were 6′-glucopyranosyl-2-(3-fluoro-4-biphenyl) propanoate (flurbiprofen glucoside) (**5**), 6′-glucopyranosyl-2-(4-isobutyl-phenyl) propanoate (ibuprofen glucoside) (**6**), 6′-glucopyranosyl-2-(3-benzoylphenyl) propanoate (ketoprofen glucoside) (**7**), 6′-glucopyranosyl-2-(6-methoxy-2-naphthyl) propanoate (naproxen glucoside) (**8**), 6′-mannopyranosyl-2-(3-fluoro-4-biphenyl) propanoate (flurbiprofen mannoside) (**9**), 6′-mannopyranosyl-2-(4-isobutyl-phenyl) propanoate (ibuprofen mannoside) (**10**), 6′-mannopyranosyl-2-(3-benzoylphenyl) propanoate (ketoprofen mannoside) (**11**) and 6′-mannopyranosyl-2-(6-methoxy-2-naphthyl) propanoate (naproxen mannoside) (**12**). The structures of the products were determined by physical methods (^{1}H and ^{13}C NMR, MS, IR) and are presented in Table 2. The NSAID glycosides were obtained as mixtures of the α- and β-pyranosyl anomers (Furniss et al., 1981) in the ratios depicted in Table 2, as were determined by NMR.

3.2. Physicochemical properties and transdermal flux

The aqueous solubility (mg/ml) and octanol/water partition coefficients (log *P*) of the NSAIDs and their glycosides are presented in Tables 3 and 4 respectively. The experimental flux values (*J*), predicted flux values, lag times (TL) and permeability coefficients (k_p) of the NSAIDs and their glycosides are presented in Table 5. The steady state flux was calculated from the slope of the linear portion of the cumulative transdermal concentration versus time plot. The lag time was calculated by the extrapolation of the linear portion of the curve to its intersection with the *x*-axis.

The permeability coefficients (k_p) were calculated using Fick's first law of diffusion (Rieger, 1993).

$$k_p = \frac{V_R(dC/dt)}{A(\Delta C)} \quad (1)$$

Table 2
NSAIDs and glycosides

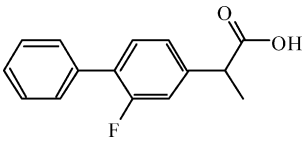
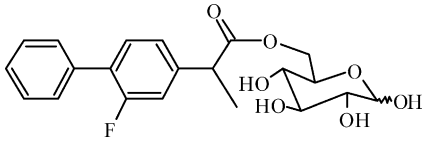
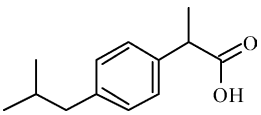
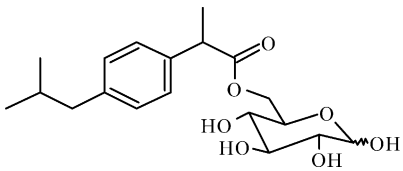
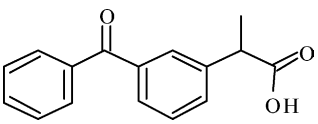
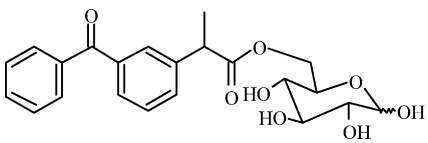
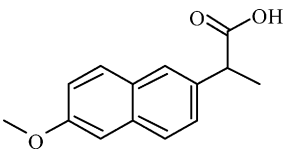
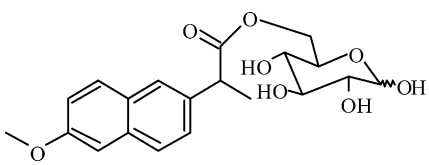
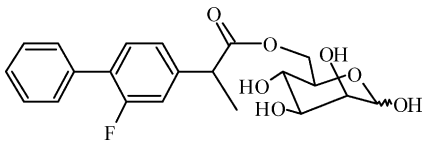
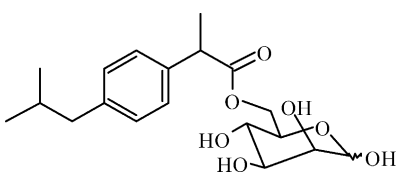
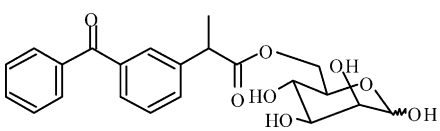
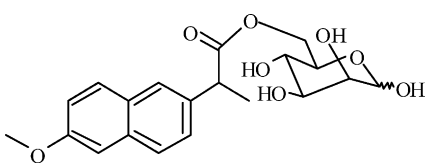
NSAID	No.	Glycoside	No.	Yield %	α:β ratio
	**1**		**5**	37	1:0.15
	**2**		**6**	32	1:0.72
	**3**		**7**	45	1:0.95
	**4**		**8**	21	1:0.36
			**9**	39	1:0.43
			**10**	56	1:0.30
			**11**	30	1:0.29
			**12**	25	1:0.44

Table 3
Aqueous solubility (in phosphate buffer at 32 °C) of the NSAIDs and their glycosides

Compound	Melting point (°C)	Solubility (mM)		
		pH 4.0	pH 5.5	pH 7.0
(**1**)	110–111[a]	0.070	1.094	6.943
(**5**)	144	1.702	1.443	2.512
(**6**)	113	2.685	2.559	2.825
(**2**)	75–77[a]	0.233	2.155	12.883
(**7**)	128	4.179	4.794	12.242
(**8**)	99	4.114	3.984	7.870
(**3**)	94[a]	0.370	2.728	13.854
(**9**)	[b]	5.954	9.267	12.274
(**10**)	[b]	3.529	4.339	8.313
(**4**)	152–154[a]	0.543	6.678	81.174
(**11**)	76	3.727	5.097	7.306
(**12**)	[c]	2.145	3.969	6.503

[a] The Merck Index, 2001.
[b] Oil.
[c] Amorphous solid.

where

- dC/dt is the steady-state slope of a plot of the cumulative amount of substance which had penetrated the skin as a function of time (μg/h);
- k_p is the permeability coefficient (cm/h);

Table 4
Partition coefficients (log *P*) of NSAIDs and glycosides

Compound	Literature	Predicted		Experimental[e]
		ACD[c]	K_{ow}Win[d]	
(**1**)	4.16[a]	4.12	4.16	3.34
(**5**)		3.73	1.99	1.92
(**6**)		3.73	1.99	1.56
(**2**)	3.51[a]	3.72	3.79	3.39
(**7**)		3.33	1.98	1.57
(**8**)		3.33	1.98	1.79
(**3**)	3.12[a]/5.2[b]	2.81	3.00	2.44
(**9**)		2.43	1.18	0.98
(**10**)		2.43	1.18	1.11
(**4**)	3.34[a]	3.00	3.10	2.72
(**11**)		2.61	1.29	1.32
(**12**)		2.61	1.29	1.31

[a] From in-built database of ACD software (Hadgraft et al., 2000).
[b] Cordero et al. (1997).
[c] Calculated using ACD software.
[d] Calculated using K_{ow}Win (http://esc.syrres.com/interkow/kowdemo.htm).
[e] At pH 7.0 in phosphate buffer.

- A is the diffusional area (1.075 cm^2);
- ΔC is the concentration differential existing across the membrane. This was effectively equal to the saturation concentration in the donor phase (μg/ml);
- V_R is the volume of the receptor compartment (1.8 ml).

It is possible to use the experimental values obtained for log *P*, aqueous solubility and molecular weight (M_W) to estimate the flux values (J_{max}) for the NSAIDs and glycosides. By using the Potts and Guy equation (Eq. (2)) (Potts and Guy, 1992), the log k_p may be calculated, from where the permeability coefficient (k_p) can be obtained. The estimated flux (μg/(cm^2 h)) may be obtained from the product of the permeability coefficient and the aqueous solubility at the same pH (Eq. (3)) (Hadgraft et al., 2000).

$$\log k_p = -2.7 + 0.71 \log P - 0.0061 M_W \quad (2)$$

$$J_{max} = k_p \times \text{aqueous solubility} \quad (3)$$

4. Discussion

4.1. Aqueous solubility

The aqueous solubility (in phosphate buffer, Table 3) of all compounds increased with increasing pH from 4.0 to 7.0 (although some had a lower solubility at pH 5.5). This phenomenon may be explained by an increase in the ionisation of the compounds with an increase in pH to 7.0 and a subsequent higher aqueous solubility. Whereas the solubility of the parent NSAIDs increased substantially (up to 100 times) through the pH range, that of the glycosides only increased marginally (at most three times). The reason for this is that the parent NSAIDs are carboxylic acids experiencing a higher degree of ionisation with increased pH than the glycosides and mannosides do. The high solubility observed for naproxen (**4**) is in good agreement with the value (∼100 mM) found by Rautio et al. (2000). At pH 4.0 and 5.5, the glycosides generally have a higher solubility than the corresponding parent NSAIDs (except for naproxen) while at pH 7.0, this relationship does not exist. The solubility measured for ketoprofen (**3**) (13.854 mM or 3.5 mg/ml at pH 7.0) is comparable to that (8.9 mg/ml at pH 6.6) reported by Cordero et al. (1997).

Table 5
Experimental and predicted flux values, lag times and permeability coefficients

Compound	Experimental flux (μg/(cm^2 h))	Predicted flux (μg/(cm^2 h))	Lag time (h)	Permeability coefficient (cm/h)
(**1**)	28.377 ± 7.56	25.723	0.2	1.68×10^{-2}
(**5**)	1.225 ± 1.04	0.156	0.0	1.20×10^{-3}
(**6**)	0.479 ± 0.52	0.097	0.0	4.18×10^{-4}
(**2**)	29.890 ± 8.95	74.558	0.0	1.13×10^{-2}
(**7**)	1.840 ± 0.49	0.662	3.0	4.09×10^{-4}
(**8**)	2.900 ± 1.23	0.610	5.0	1.02×10^{-3}
(**3**)	8.951 ± 2.32	10.659	0.9	2.54×10^{-3}
(**9**)	0.342 ± 0.15	0.146	0.0	6.69×10^{-5}
(**10**)	0.329 ± 0.38	0.125	0.0	9.17×10^{-5}
(**4**)	4.076 ± 0.77	125.244	0.9	2.18×10^{-4}
(**11**)	0.134 ± 0.08	0.200	0.0	4.68×10^{-5}
(**12**)	0.183 ± 0.12	0.175	2.7	7.18×10^{-5}

4.2. Partition coefficient

The experimental partition coefficients (Table 4) indicate that glycosidation of the NSAIDs result in a lower partition coefficient. These results are as expected and validate the aqueous solubility data, whereby compounds with lower partition coefficients present with an increased hydrophilicity. The experimental partition coefficients and aqueous solubility data indicate that the NSAID glycosides are more hydrophilic than their parent NSAIDs in an unionised state.

The experimental partition coefficients of the parent NSAIDs are all lower than those reported in the literature, and the partition coefficient found for ketoprofen (**3**) (2.44) is significantly different from that (5.2) obtained by Cordero et al. (1997). This may be attributed to differences in the pH at which the partition coefficients were determined or the buffers used.

A Pearson correlation between the experimental partition coefficients and the predicted values, obtained from ACD and K_{ow}Win programs, found a correlation coefficient of 0.61 between the experimental values and those predicted by ACD and a correlation coefficient of 0.99 between the experimental values and those predicted by K_{ow}Win. This indicates a very good linear relationship ($r^2 = 0.96$) between the experimental log P values and those calculated by K_{ow}Win which makes this program a very useful prediction model.

Both the ACD and K_{ow}Win programs are unable to distinguish between stereoisomers, which the NSAID glucosides and mannosides are. The experimental aqueous solubility data and partition coefficients of the NSAID glycosides clearly show that stereochemistry plays an important role in the physicochemical properties of these compounds. The experimental transdermal permeability data also show that stereochemistry does influence the rate of transdermal permeation, as the transdermal flux values for the NSAID glucosides and mannosides differ from each other.

4.3. Transdermal permeation

The only experimental transdermal flux value found in the literature for any of these compounds was for ketoprofen (**3**) (Cordero et al., 1997). The flux measured in this study (8.95 μg/(cm^2 h)) is comparable to the literature value (16 μg/(cm^2 h)) while the permeability coefficient (2.54×10^{-3}) corresponds well with the reported value (2.7×10^{-3}).

In each case, the transdermal flux of the parent NSAIDs are at least a factor 10 higher than those of the glucosides or mannosides. By comparing the flux data of the glycosides, the compounds with the highest aqueous solubility (pH 7.0) and highest partition coefficient, presented with the highest transdermal flux. This is in agreement with previous findings (Hadgraft and Wolff, 1993; Hadgraft et al., 2000).

A possible explanation for the considerably lower transdermal fluxes of the NSAID glycosides in comparison to their parent NSAIDs is the formation of hydrogen bonds between the OH groups on the glycosides and the ceramides in the epidermis. Each glycoside

possesses four OH groups, presenting greater opportunity for hydrogen bonding to occur as well as for the formation of strong hydrogen bonds. This will be in agreement with previous findings (Du Plessis et al., 2002). Another possible explanation is the fact that the derivatives exhibit 100 times lower octanol solubility than the parent compounds. The short lag times may be indicative that this effect is involved.

The fairly large differences observed in most cases between the experimental and predicted flux values (3–30×) indicate the inaccuracy of the flux predictions.

5. Conclusion

These results confirm that transdermal flux is greatly determined by partition coefficient and aqueous solubility as the parent NSAIDS with log *P* values closer to 2–3 and higher aqueous solubility at pH 7 than that of the glycoside derivatives presented with higher transdermal flux.

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