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Evaluation of the pH effect of formulations on the skin permeability of drugs by biopartitioning micellar chromatography☆

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Abstract

Dermal absorption of chemicals is an area of increasing interest for the pharmaceutical and cosmetic industries, as well as in dermal exposure and risk assessment processes. Biopartitioning micellar chromatography (BMC) is a mode of reversed phase micellar chromatography that has proved to be useful in the description and prediction of several pharmacological properties of xenobiotics including oral drug absorption, ocular and skin drug permeability. The present paper deals with the application of biopartitionig micellar chromatography to evaluate the pH effect on the skin permeability of twelve non-steroidal anti-inflammatory drugs and lidocaine. For this purpose the BMC retention of the whole set of compounds at several pHs between 3.5 and 8 was obtained. Using the BMC retention–permeability model previously reported, the permeability of the compounds at different pH values was estimated. The predicted permeability values at different pH values for ketoprofen, lidocaine, salicylic acid and ibuprofen agree with those experimental reported in literature for these compounds using excised human and rat skin.

Keywords: pH effects; Pharmaceutical analysis; Biopartitioning micellar chromatography; Micellar liquid chromatography; Retention–permeability relationship; Non-steroidal anti-inflammatory drugs

1. Introduction

A research priority in pharmaceutical technology is alternative routes of administration to oral delivery that overcome potential disadvantages, such as the first-pass effect or adverse side effects. In this context, transdermal administration of drugs has assumed an important place in modern drug therapy. There are several categories of pharmaceutical products which are targeted to the skin or utilize the skin as a port of entry into the body and these include transdermal drug delivery systems, gels, creams, ointments and lotions. However, measurement of the penetration of chemicals through skin is laborious and can involve ethical difficulties with either human or animal experiments. Hence, there is a need for methods capable of predicting dermal absorption in a simple, fast and ethical way.

The rate and amount of percutaneous absorption of a compound strongly depends on both the physiologic characteristics of the skin (e.g., skin thickness, hydratation and temperature) and the physico-chemical nature of the compound (e.g., hydrophobicity, polarity, physical state, water solubility and molecular mass or size) [1]. In general, substances with greater hydrophobicity are absorbed more readily by the skin than less hydrophobic. Dermal absorption generally increases as $\log P$ does from -1 to 3.5. Highly lipophilic substances ($\log P > 5$) can pass easily through the stratum corneum but are generally too water insoluble to pass through the remaining sub-layers and enter the bloodstream. On the

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other hand, the rate of absorption of substances through the skin is inversely proportional to molecular mass and size. In general, small molecules (< 150 daltons) that are both lipid- and water-soluble are the most readily absorbed.

In addition, the physico-chemical properties of the vehicle have a direct effect on the drug release from topical formulations. One of the factors that is very often ignored in dermal formulation design and in the prediction of skin permeability is that many potential permeants are weak acids or bases and may therefore be ionized. The skin surface pH is around 5.5 and often a pH between 4 and 7 is chosen for the aqueous phase of a dermal formulation. According to the pH-partition theory, only the unionised forms of drugs are able to permeate through phospholipid membranes [2,3]. However, there has been increasing evidence that the ionised species can contribute to transdermal absorption of drugs [4–7]. When the penetrating species exist in both ionised and unionised forms, it is the unionised ones that permeates faster through the lipid regions while the ionised penetrate slower through the aqueous regions. However, some contribution of the ionised forms to the overall permeability is generally expected.

The drug transport rate across membranes is quantified by the membrane permeability coefficient, K_p that is defined as the linear rate of drug movement through the membrane, in this case the whole skin. In the current literature, it is possible to find several alternative approaches to in vitro estimate the drug permeability. All of them provide a useful first rough estimation of the drugs permeability coefficients. These alternative approaches may be classified in three general categories: ex vivo methodologies that employ excised skin from human or animal sources, in vitro models that evaluate the permeability coefficient through synthetic model membranes such as silicone rubber and polydimethylsiloxane membranes (PDMS) and those based on mathematical models. The latter category includes the so-called structure–permeability relationships (QSPRs), which develop mathematical expressions relating the logarithm of the permeability coefficient ($\log K_P$) with several physico-chemical parameters such as the octanol–water partition coefficient [8–11], molecular size descriptors (i.e. molecular mass, molecular volume, molar refractivity and molecular connectivity indexes) [1,12–13] and hydrogen bond descriptors [13].

The use of chromatographic parameters in QSPRs instead of molecular descriptors gives rise to the retention–permeability relationships (QRPRs). One advantage of the QRPRs models is that it is easy to predict the effect of variables such as pH, ionic strength, temperature and addition of modifiers and/or enhancers on the permeability of drugs. This kind of studies are of great interest for pharmaceutical industry in order to predict in a rapid and accurate way the effect of these variables on the resulting permeability of compounds in order to optimize the vehicle features.

Several QRPR have been reported in the literature for predicting skin permeability, including the use of immobilized artificial membranes (IAM) columns [14] and immobilized keratin stationary phases [15]. In a previous paper, the usefulness of biopartitioning micellar chromatography (BMC) to obtain a skin permeability model using the retention and the melting point as predictive variables for the $\log K_P$ values of 42 structurally unrelated compounds was demonstrated [16]. Biopartitioning micellar chromatography is a mode of reversed phase micellar chromatography that has proved to be useful in the description and prediction of several pharmacological properties of xenobiotics including oral drug absorption, penetration across the blood–brain barrier and ocular tissue permeability [17–21]. The success of BMC in constructing these models could be attributed to the similarities between the BMC system and the biological barriers–extracellular fluids interphases [17]. So, drug retention in BMC, which depends on its hydrophobic, electronic and steric properties reflects adequately the extension of biopartitioning process.

The aim of the present work is to study the pH effect on the skin permeability of a set of 12 non-steroidal anti-inflammatory drugs (NSAIDs) and lidocaine by means of BMC. The permeability coefficients at each pH value were estimated using the model proposed previously [16] and compared with experimental values taken from literature obtained using excised human and rat skin.

2. Experimental

2.1. Instruments and measurements

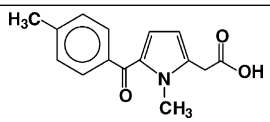
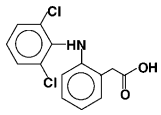
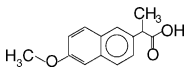
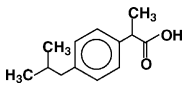
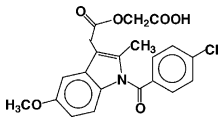
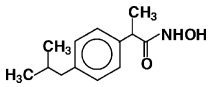
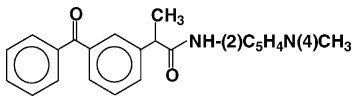
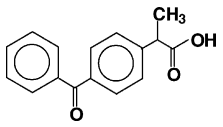
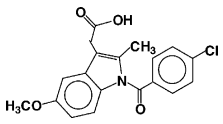
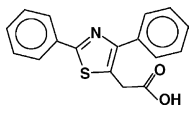
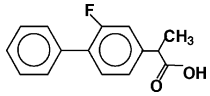
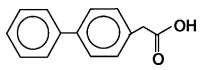
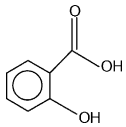
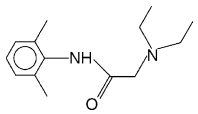
A Hewlett Packard HP 1100 chromatograph with an isocratic pump, an autosampler an UV–vis detector, a column thermostat and an HP Vectra computer (Amsterdam, The Netherlands) equipped with HP-Chemstation software (A.07.01 [682], 1999) was used. The solutions were injected into the chromatograph by the autosampler with a 20 μl loop. Kromasil octadecylsilane C_{18} columns of 5 μm particle size (150 mm × 4.6 mm i.d.) and a guard column of similar characteristics (35 mm × 4.6 mm i.d.) (Scharlau, Barcelona, Spain) were used. The mobile phase flow rate was 1.0 ml min^{-1}. The detection was performed in UV at 230 nm. The column was thermostated at 36.5 °C. The retention factors (k) values were averages of triplicate measurements and were calculated taking as void volume the first perturbation in the chromatogram after injection. This value was always ranged between 0.93 and 0.96 ml.

A Crison Micro pH 2000 pH meter from Crison Instruments (Alella, Barcelona, Spain) was employed to adjust the pH of the mobile phases.

2.2. Reagents and standards

Mobile phases were prepared by aqueous solutions of polyoxyethylene(23) lauryl ether (Brij 35, Acros Chimica, Geel, Belgium) 0.04 M. The pH was adjusted to the desired value (3.5–8.0) with 0.05 M citrate buffer prepared with sodium citrate (analytical reagent, Guinama, Valencia, Spain)

Table 1
Structure, logarithm of protonation constant (log *K*) octanol–water partition coefficient (log *P*) and melting point for the non-ionic forms of the compounds studied

Compound	Structure	log *K*	log *P*[a]	MP[b] (°C)
Tolmetin		3.5[c]	2.56	168.4
Diclofenac		4.5[d]	4.02	174.6
Naproxen		4.15[c]	3.10	137.6
Ibuprofen		5.2[c]	3.79	94.2
Acemetacin		4[d]	4.13	242.4
Ibuproxam		–	2.79	152.4
Piketoprofen		–	4.24	–
Ketoprofen		4.6[e]	3.00	149.2
Indomethacin		4.5[c]	4.23	219.4
Fentiazac		3.6[d]	4.60	215.8
Flurbiprofen		4.27[e]	3.81	133.9
Fenbufen		4.51[c]	3.18	162.0
Salicilic acid		2.97[c]	2.26	93.8
Lidocaine		7.86[c]	2.44	144.2

[a] From EPIWIN v. 3.05 program of EPI Suite software of Syracuse Reseach Corporation (SRC) (US Environmental Protection Agency Version), 2000.
[b] From DERMWIN SRC v.1.42 program of EPI Suite software of Syracuse Reseach Corporation (SRC) (US Environmental Protection Agency Version), 2000.
[c] From ref. [23].
[d] From ref. [24].
[e] From ref. [25].

and the appropriate amount of 2 M solution of hydrochloric acid (for analysis, Merck, Darmstadt, Germany).

Some of the NSAIDs were kindly donated by several pharmaceutical laboratories: acemetacin was from Laboratorios Fher (Barcelona, Spain), diclofenac from Novartis (Barcelona, Spain), indomethacin from Laboratorios Llorens (Barcelona, Spain), ketoprofen from Rhône-Poulenc Rorer (Madrid, Spain), naproxen from Syntex Latino (Madrid, Spain), piketoprofen from Laboratorios Farmacéuticos Almirall (Barcelona, Spain), ibuproxam from Laboratorios Fher (Barcelona, Spain), tolmetin from Laboratorio Estedi (Barcelona, Spain) and lidocaine from Seid (Barcelona, Spain). The following compounds were acquired from different pharmaceuticals: ibuprofen from Nurofen 400 (Boots Healthcare, Madrid, Spain), fentiazac from Donorest 100 (Wyeth-Orfi, Barcelona, Spain), flurbiprofen from Froben 50 (Laboratorios Knoll, Madrid, Spain) and fenbufen from Cincopal (Cyanamid Ibérica, Madrid, Spain). Salicylic acid was from Panreac (Purissimum, Barcelona, Spain).

Stock standard solutions of compounds of 1000 mg l^{-1} were prepared using methanol (HPLC grade, Labscan, Dublin, Ireland) as solvent. Working solutions were obtained by dilution of the stock standard solutions in the mobile phase. Solutions were stored at 4 °C.

Water used to prepare solutions was purified through a Barnstead E-Pure (Sybron, Boston, MA, USA). Mobile phases were vacuum-filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA, USA) and degassed in an ultrasonic bath. All solutions injected into the chromatograph were filtered through 0.45 μm pore size disposable nylon filters (Micron Separations, Westboro, MA, USA).

2.3. Data sources, software and data processing

Melting point data was obtained from the Dermwin SRC v.1.42 program of EPI Suite software of Syracuse Research Corporation (SRC) (US Environmental Protection Agency Version). This software was kindly donated by SRC. EPI integrates SRC's suite of 10 programs based on QSAR models.

Microsoft Excel 2000 and Statgraphics version 2.1 were used to perform the statistical analysis of the regressions.

3. Results and discussion

3.1. Estimation of compounds permeability at different pH values

Martínez-Pla et al. [16] reported a retention-skin permeability model (QRPR) using the logarithm of the BMC retention factor ($\log k_{BMC}$) and melting point (MP) of 42 unrelated drugs as predictive variables (Eq. (1)):

$$\log K_p = (-3.3 \pm 0.3) + (1.3 \pm 0.2)\log k_{BMC} - (0.0080 \pm 0.0014)\mathrm{MP},$$
$$r^2 = 0.83;\ \text{S.E.} = 0.51,\ F = 93,$$
$$N = 42;\ P < 0.0001 \quad (1)$$

The model explained up to 83% of the variability in the permeability data what is considered adequate for skin permeability studies taking into account the intrinsic variability of the $\log K_P$ data (being K_P expressed in cm h^{-1}) reported in the literature due to differences in the experimental

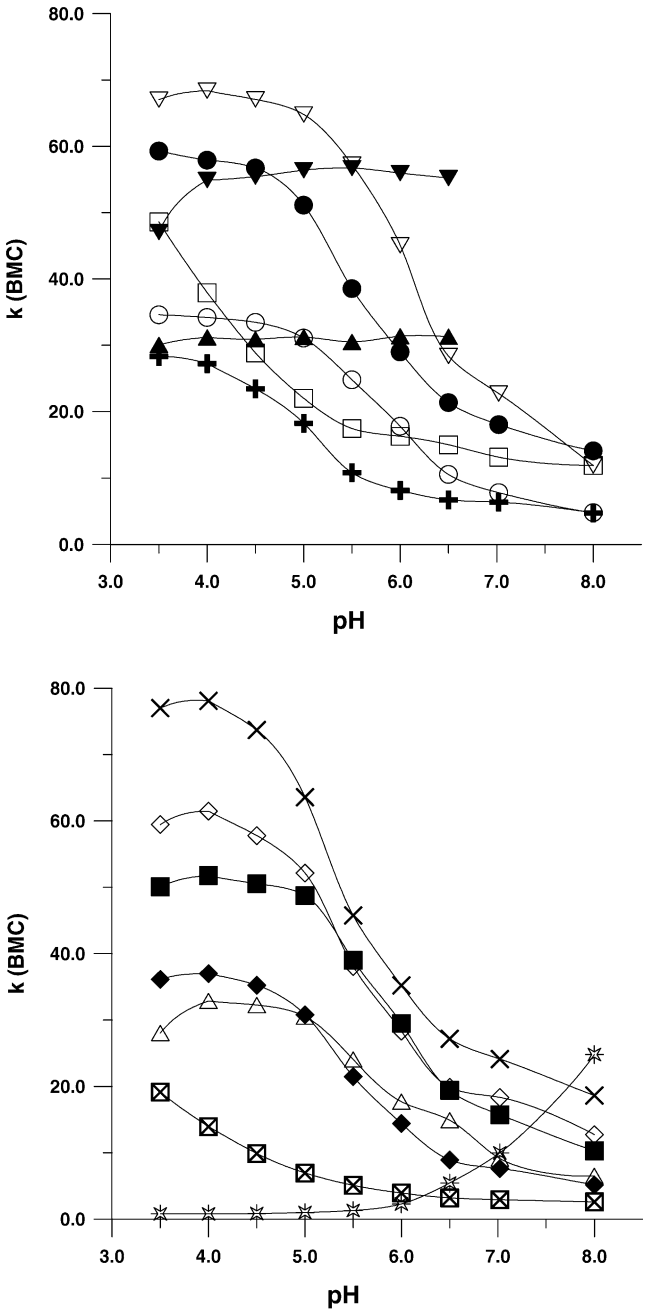


Fig. 1. Effect of the mobile phase pH on the chromatographic retention for the set of compounds studied: (+) tolmetin; (●) diclofenac; (◯) naproxen; (▽) ibuprofen; (□) acemetacin; (▲) ibuproxam; (▼) piketoprofen; (◆) ketoprofen; (◊) indomethacine; (×) fentiazac; (■) flurbiprofen; (△) fenbufen; (⊠) salicylic acid; and (✳) lidocaine.

conditions (i.e. variability may be as high as 25% of the permeability data, [22]). As commented above, one interesting advantage of the QRPRs is that since chromatographic retention depends on the ionisation degree of the solutes the proposed model may predict in a simple and fast way the permeability values of a given solute at any pH.

Hence, we have focused our study on several drugs widely employed for topical administration: the NSAIDs and the local anesthetic lidocaine. Table 1 shows the structure, the protonation constant in aqueous media and the logarithm of octanol–water partition coefficient (log *P*) values for the non-ionic forms of the compounds studied.

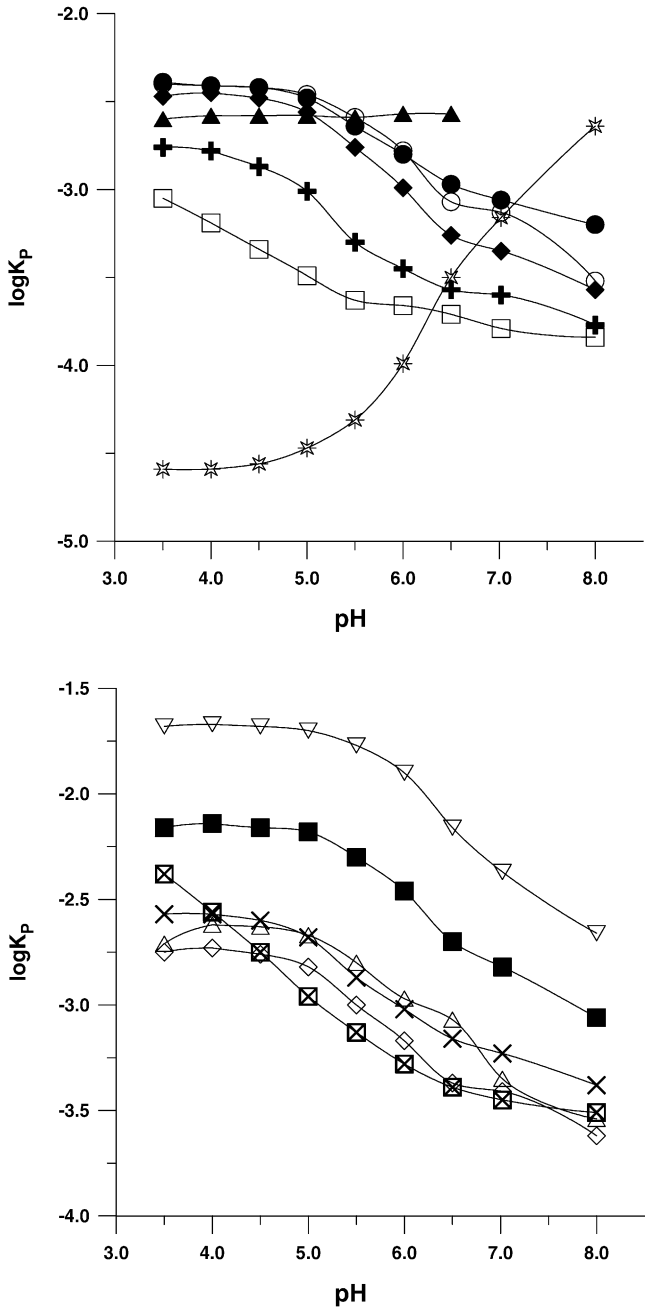


Fig. 2. Predicted logarithm of permeability coefficients (cm h^{-1}) by the BMC retention model at different pH values for: (+) tolmetin; (●) diclofenac; (◯) naproxen; (▽) ibuprofen; (□) acemetacin; (▲) ibuproxam; (▼) piketoprofen; (◆) ketoprofen; (◊) indomethacine; (×) fentiazac; (■) flurbiprofen; (△) fenbufen; (⊠) salicylic acid; and (✲) lidocaine.

Table 2
Fitting parameters for Eq. (2)

Compound	$k_{HA}\pm$ ts	$K_A \pm$ ts	$(K \pm$ ts$)\ 10^5$	r^2	S.E.
Tolmetin	29.1 ± 1.2	5.5 ± 0.8	1.1 ± 0.3	0.997	0.61
Diclofenac	59.5 ± 1.8	15.6 ± 1.9	4.0 ± 1.0	0.997	1.15
Naproxen	34.6 ± 1.2	5.2 ± 1.0	7.0 ± 1.2	0.998	0.52
Ibuprofen	68 ± 2	13 ± 3	13.8 ± 0.1	0.995	1.74
Acemetacin	53 ± 7	14.3 ± 1.9	0.18 ± 0.08	0.990	1.70
Ketoprofen	37.4 ± 1.3	5.8 ± 1.3	3.5 ± 0.9	0.997	0.79
Indomethacine	61 ± 3	15 ± 3	3.7 ± 1.4	0.994	1.71
Fentiazac	79 ± 3	21 ± 3	2.7 ± 0.9	0.995	1.94
Flurbiprofen	51.7 ± 1.9	11 ± 2	8 ± 3	0.995	1.33
Fenbufen	31 ± 2	7 ± 3	9.40 ± 0.01	0.97	2.12
Salicylic acid	21 ± 3	3.3 ± 0.9	0.18 ± 0.10	0.990	0.76
Lidocaine	0.9 ± 0.4	29.8 ± 1.1	214 ± 1	0.998	0.38

k_{HA}, BMC retention of the protonated form of each drug; k_A, retention of the deprotonated form; *K*, protonation constant found under BMC conditions, r^2, correlation coefficient; S.E., standard error of the estimate.

The retention of compounds in a mobile phase of 0.04 M Brij 35 was measured at several pH values in the range 3.5–8. As can be observed in Fig. 1, for all the NSAIDs except for piketoprofen and ibuproxam, retention decrease as pH increase, according to the acidic character of compounds and their protonation constants. For piketoprofen and ibuproxam, retention remains constant in the range of pH values studied. On the other hand, lidocaine is scarcely retained at pH values below six due to its basic character. Over this pH value, retention of compound increases.

The treatment of the *k*–pH curves allows the estimation of protonation constants of compounds in Brij 35 micellar medium at 36.5 °C. For this purpose, the retention factors in BMC at different pH values were adjusted to the following

Table 3
Fitting parameters for Eqs. (5) and (6)

Compound	$(K_{P,ion} \pm$ ts$)\ 10^4$	$(K_{P,neutral} \pm$ ts$)\ 10^3$	r^2	S.E. 10^4
Tolmetin	1.8 ± 0.6	1.75 ± 0.07	0.994	0.50
Diclofenac	6.5 ± 1.4	3.99 ± 0.13	0.995	1.10
Naproxen	3 ± 2	3.9 ± 0.3	0.992	1.00
Ibuprofen	15 ± 7	21.1 ± 0.5	0.997	4.40
Acemetacin	1.8 ± 0.3	0.98 ± 0.07	0.990	0.31
Ketoprofen	2.2 ± 1.5	3.5 ± 0.14	0.994	1.15
Indomethacine	2.6 ± 0.9	1.84 ± 0.08	0.992	0.61
Fentiazac	4.6 ± 0.9	2.72 ± 0.10	0.995	0.74
Flurbiprofen	0.08 ± 0.03	7 ± 2	0.994	2.10
Fenbufen	0.03 ± 0.03	2.2 ± 0.2	0.96	1.74
Salicylic acid	3.8 ± 1.7	4.5 ± 0.4	0.98	1.70
Lidocaine	–*	2.6 ± 0.2	0.990	0.81

$K_{P,ion}$, permeability coefficient (cm h^{-1}) of the completely ionsionsed form of the drugs; $K_{P,neutral}$, permeability coefficient (cm h^{-1}) of the neutral fraction of the drugs; r^2, correlation coefficient; S.E., standard error of the estimate.

* Non-statistically significant at the 95% confidence level.

expression by using the Marquardt iterative strategy:

$$k_{BMC} = \frac{k_{HA}Kh + k_A}{1 + Kh} \quad (2)$$

where k_{BMC} and h are the retention and the proton concentrations at each pH values and k_{HA} and k_A are fitting parameters representing the BMC retention of the protonated and deprotonated forms of compound, respectively. K is the fitting parameter corresponding to the protonation constant in this experimental condition. For acid compounds k_{HA} and k_A correspond to the retention of the neutral and anionic form, respectively, while for basic compounds these parameters represent the retention of the cationic protonated and neutral deprotonated base.

Table 2 shows the fitting parameters and the statistical analysis of the models obtained for the different compounds studied. As can be observed in all cases, the adequacy of the models to the data was satisfactory ($0.993 < r^2 < 0.998$). As can be observed for acidic compounds the protonation constants under BMC conditions were higher than those reported in literature in pure aqueous medium [23–25], while the opposite behaviour was observed for lidocaine. These displacements in the protonation constants are due to the preferential estabilization of the more hydrophobic form of the acid/base

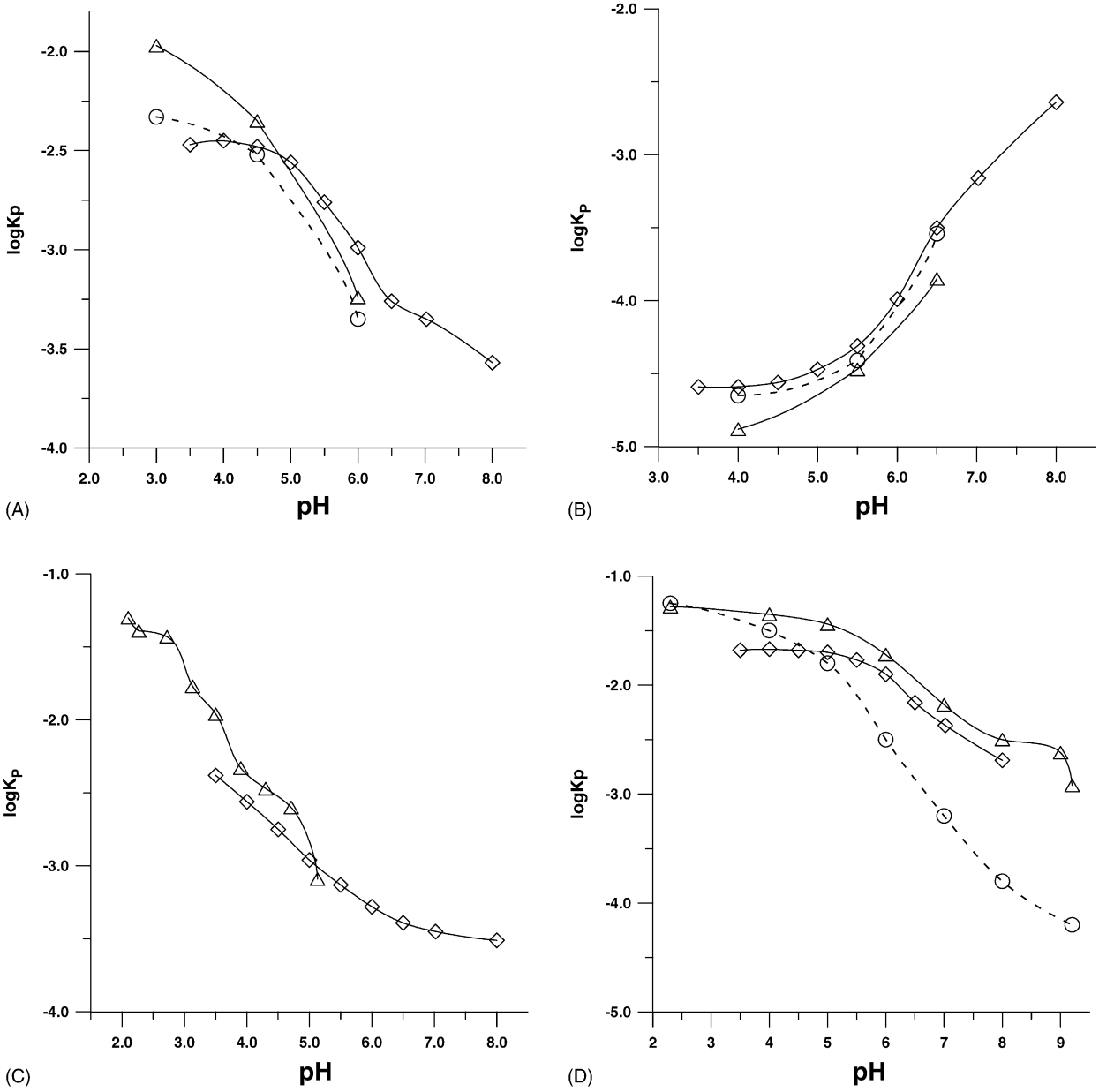


Fig. 3. Permeability coefficients (cm h^{-1}) at different pH values for: (A) ketoprofen; (B) lidocaine; (C) salicylic acid; and (D) ibuprofen. (△) Experimental permeability values obtained using rat skin (A) and human skin (B–D), predicted permeability values obtained with (◊) BMC and (◯) mathematical QSPR models.

pairs, the neutral form, which interact strongly with the neutral Brij 35 micelles and to the effect of the temperature, which decreases protonation constants.

It is important to point out that in the physiological fluids, several micellar aggregates of phospholipids and other endogenous compounds exist, therefore, similar displacements in the protonation constants could exist.

In Fig. 2, the predicted permeability coefficients by the BMC retention model at different pH values for the drugs studied are shown. As can be observed significant changes in the permeability values are predicted for all the compounds except for piketoprofen (whose melting point could not be found in the literature) and ibuproxam (as commented above). For the rest of NSAIDs, permeability decreased with pH, while for lidocaine the predicted log K_P values only become significant at pHs over 5.5.

These results may be interpreted considering that the total flux (J_{tot}) of a permeant through the skin is a composite term, which can be attributed to transport of both the ionized and unionized forms [26]:

$$J_{tot} = K_{P,ion} c_{ion} + K_{P,neutral} c_{neutral} \quad (3)$$

where $K_{P,ion}$, $K_{P,neutral}$, c_{ion} and $c_{neutral}$ are the permeability values and concentrations of the ionized and neutral species, respectively. Since $J_{tot} = K_P c_{total}$ where c_{total} is the total concentration of the compound in the membrane, Eq. (3) could be write as a function of the molar fractions $\delta_{neutral}$ and δ_{ion} of the neutral and ionized forms in the skin,

$$K_P = K_{P,ion}\delta_{ion} + K_{P,neutral}\delta_{neutral} \quad (4)$$

Taking into account, that is the neutral form that permeates faster through the skin, modifications on the pH that provoke an increase in the $\delta_{neutral}$ leads to an improvement in the drug skin permeability.

From Eq. (4) and using the values of K_P estimated and the molar fractions at several pH, it is possible to estimate $K_{P,ion}$ and $K_{P,neutral}$ of each molecule by means of non-linear curve fitting of Eq. (5) or Eq. (6) for acid and basic compounds, respectively.

$$K_P = \frac{K_{P,neutral} Kh + K_{P,ion}}{1 + Kh} \quad (5)$$

$$K_P = \frac{K_{P,ion} Kh + K_{P,neutral}}{1 + Kh} \quad (6)$$

Results are summarised in Table 3. As can be observed and even though, permeability of the ionised forms are always one order of magnitude lower than the corresponding to the neutral forms, their contribution to the overall permeability cannot be neglected. These results agree with those previously reported by several authors [26–29].

3.2. Validation of the proposed QRPRs procedure

In order to validate the results obtained, the estimated permeability coefficients at several pH values were compared with experimental data obtained using excised human or rat skin [26,30–32]. Several authors have used quantitative structure–permeability relationships (QSPRs) based on the Potts and Guy equation in order to study the effect of pH on the skin permeability using the distribution coefficient (log D) instead of the octanol–water partition coefficient (log P) [30,31]. The results obtained with this approach were also compared.

Fig. 3 shows the comparison between the permeability constants at different pH values obtained using the BMC and QSRPs models and the experimental data for ketoprofen, lidocaine, salicilyc acid and ibuprofen. As can be observed in Fig. 3, excellent agreement between skin permeability values predicted from retention in BMC and experimental data (human and rat skin) were obtained. Good agreement is also observed with predicted values from QSPRs except in the case of ibuprofen. As can be seen in Fig. 3D, the QSPR predicted a decreased in the skin permeability already at pH 4. However, this decrease in the permeability of ibuprofen does not occur in human skin until pH 6.

In contrast, the BMC retention–permeability model predicts a similar behavior to that found experimentaly. This fact may be explained taking into account that the QSRP model uses the protonation constants obtained in aqueous media, but as comented above displacements in the protonation constants of drugs in physiological environments are expected to occur.

This fact, in addition to the simplicity, automation, sample throughput, and reproducibility of BMC, make this technique a more convenient approach to predict the effect of pH on the skin permeability of drugs than the traditional QSPR

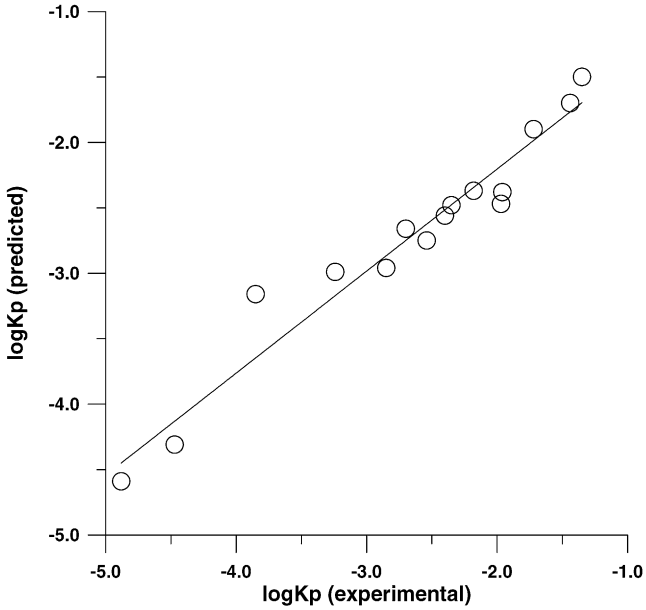


Fig. 4. Permeability coefficients (cm h^{-1}) predicted for ketoprofen, salicylic acid, ibuprofen and lidocaine using the BMC retention model compared to the experimentally obtained values with human or rat skin.

approaches using the shake-flask methodology and software estimations.

Fig. 4 showed the BMC predicted permeability values for ketoprofen, salicylic acid, ibuprofen and lidocaine at different pH values versus the actual experimental permeability values obtained with human or rat skin. The statistics of the fitted line showed a slope and intercept close to unity and zero (0.8 ± 0.1 and -0.6 ± 0.3, respectively, $r^2 = 0.95$, $n = 15$) suggesting a good agreement between the values predicted with the BMC based approach and the actual experimental permeability values observed. In addition, considering that these data had been obtained from different sources (human and rat skin) and by different research groups in not necessarily the same experimental conditions [26,30–32], the correlation between the predicted values and the actual ones can be considered adequate.

4. Conclusions

The results showed in this paper indicate that biopartitioning micellar chromatography is a very useful technique to predict the effect of pH on the skin permeability of drugs. Using this approach, it is possible to estimate the permeability constants of the ionized and neutral forms of drugs. The results indicate that the ionised forms of the compounds contribute to the overall permeability, although the contribution of the neutral forms is approximately one order of magnitude greater.

The proposed BMC methodology is fast, reproducible, simple and economical and provides similar results than the conventional in vivo approaches that use human and rat skin for compounds studied.

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