International Journal of Pharmaceutics, 74 (1991) 105–116
Elsevier Science Publishers B.V.
ADONIS 037851739100280Z

IJP 02473

Determination of the acid dissociation constants in ethanol–water mixtures and partition coefficients for diclofenac

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(Received 21 January 1991)
(Modified version received 1 March 1991)
(Accepted 25 March 1991)

Key words: Acid dissociation constant; Partition coefficient; Ion pair formation; Solubility

Summary

The dissociation constants of diclofenac in ethanol–water mixtures, in connection with percutaneous absorption, were determined using the titration method. The acid dissociation constant of sodium diclofenac was decreased by the increase in the concentration of ethanol in the aqueous solution. The results were interpreted in terms of solvent polarity. It is suggested that ethanol, which is used as an enhancer for percutaneous absorption, assumes another role, increasing the proportion of the unionized form of the drug and forming ion pairs in low dielectric media. Also the partition coefficients for diclofenac were measured in an n-octanol/water or buffer system over the pH range from 3 to 8. The distribution behavior of diclofenac is dramatically affected in the presence of added cations. Above pH 7, ion pair formation promotes the distribution of the drug into lipophilic environment.

Introduction

The percutaneous absorption of sodium diclofenac, which is an anti-inflammatory drug, has recently been reported (Nishihata et al., 1988; Obata et al., 1990). The transfer of acidic drugs across membranes has usually been ascribed to the unionized form. However, sodium diclofenac was significantly absorbed from the skin although the drug is water-soluble and hydrophilic. Therefore, we are interested in the molecular form of the drug in the donor solution and into the skin.

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Ethanol–water mixtures enhance permeation of ionic solutes through the stratum corneum (Kurihara-Bergstorm et al., 1990). Ethanol is known to be an enhancer in percutaneous absorption. The role of ethanol is described to increase the solubility of a drug in an aqueous phase and to alter the skin's barrier properties, as reported in many cases (Good et al., 1985; Gale and Berggren, 1986; Berner et al., 1989a,b,c). Furthermore, it is suggested that ethanol increases the proportion of the unionized form of a drug through the solvent effect (Rubino, 1987) and then enhances the permeation of the drug through the skin as a result of the high partition coefficient of the unionized form.

In addition, sodium diclofenac in ethanol–

water mixtures may form ion pairs (Lee et al., 1987). Therefore, partition coefficients of the drug are determined to estimate the permeability in percutaneous absorption. A buffer solution or aqueous solution with added ions is used as a donor solution to maintain a pH of around 7. Under these conditions, the partition coefficients of the drug will be changed (Pandit et al., 1989).

Experimentally, we have determined the dissociation constants of sodium diclofenac in ethanol–water mixtures using the titration method in order to examine the proportion of the unionized drug present at physiological pH. We then measured the partition coefficient for diclofenac in an n-octanol/water or buffer system in which ions are added. We also investigated whether diclofenac might move to octanol in the form of an ion pair to analyze ion pair partition into the skin.

Materials and Methods

Materials

Sodium diclofenac (mp. 283–285 °C) was generously supplied by SS Pharmaceutical Co., Ltd (Tokyo, Japan) in 98.5% purity by the quantitative method described in '*Standards for Ingredients of Drugs not in the Japanese Pharmacopoeia*' (1983, Japan). Diclofenac was obtained by recrystallizing twice (mp. 166–170 °C) from an ethanol–water mixture in an acidic state by adding hydrochloric acid. Ethanol of guaranteed reagent grade (Wako Pure Chem. Ind. Ltd) was dried with molecular sieves (4A, Nakarai Chemicals Ind.) and purified by distillation. Fractionally distilled ethanol showed a water content of 0.05 wt% in the Karl Fischer test. Other chemicals used were all of analytical grade and used as received. Distilled water was used throughout the experiments.

Equipment

The pH values of all solutions were measured on a digital pH meter (model IM-40S, Toa Ion Meter, Toa Electronics Ltd, Japan). The partitioning of all samples to equilibrium was accomplished using an incubator (model, M-100 I.D, Taiyo Kagaku Kogyo K.K., Japan). Ultraviolet absorption spectra and absorbance were measured using a spectrophotometer (Ubest-30, Japan Spectroscopic Co. Ltd, Tokyo, Japan). The Karl Fischer test was carried out using a Karl Fischer moisture automatic titration apparatus (C-SA, KFA-1, Tsutsui Rikagakukikai Co., Ltd, Tokyo, Japan). The concentration of sodium ion was measured by flame atomic absorption spectrometry (model Z-8100, Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer, Hitachi, Ltd, Tokyo, Japan).

Determination of the pKa values

The ionization constants of diclofenac were determined by the titration method at 25 °C. A 50 ml solution of diclofenac (2.67×10^{-3} mol/l) in ethanol–water solution was titrated with 0.01 N NaOH at more than 50% v/v of ethanol. At less than 50% v/v of ethanol, diclofenac is not dissolved. In this case, the solution of sodium diclofenac was titrated with 0.01 N HCl. At 50% v/v of ethanol, the pKa values of diclofenac and sodium salt determined by both methods were in good agreement. The pKa values were obtained from the half-neutralization point of the titration curve. At 40 v/v % ethanol, diclofenac was precipitated during the titration after the half-neutralization point. However, at 20 and 30 v/v % of ethanol, the pKa values were obtained from the titration curve before precipitation, by calculation based on the concentration ratio of acid ions that combined with protons and that which lost protons. The change of the ethanol concentration at pKa was corrected by adding the amount of the 0.01 N NaOH or 0.01 N HCl at the half-neutralization point. This experiment was performed in triplicate.

Solubility determination

The aqueous solubility of sodium diclofenac was measured in buffer solutions at pH ranging from 3 to 8 (McIlvaine buffer). Successive aliquots (45.5 mg) of the compound were added to the buffer (10 ml) at 25 °C until saturation was indicated by the presence of undissolved material over a period of up to 18 h. Then the pH was checked, and the sample was filtered (Ekicrodisc,

0.2 μm, Gelman Sciences Japan, Ltd, Japan) and assayed spectrophotometrically for sodium diclofenac at 284.5 nm.

Determination of apparent partition coefficients at different pH values — preparation of samples

n-Octanol was chosen as the oil phase because of its widespread use in partitioning work and the belief that it may mimic the properties of biological membranes more accurately than other solvents. Two methods of adjusting the pH of the aqueous phase were used. (1) The pH of the aqueous phase was adjusted to the required value using hydrochloric acid or sodium hydroxide solution. These solutions were unbuffered and at uncontrolled ionic strength because any added salt might affect the solubility. (2) McIlvaine buffers were used, which vary in pH values from 3 to 8, prepared with 0.1 M citric acid and 0.2 M disodium phosphate. Each buffer was adjusted to a constant ionic strength of 0.5 with KCl as described by Elving et al., (1956). The water or buffer solution and octanol phases were saturated prior to the commencement of the partitioning experiment by adding 25 ml of water or buffer solution to 25 ml of octanol in a 100-ml flask with a ground stopper. The flask contents were covered with parafilm and shaken in an incubator at 17 rpm for 22 h. The shaking time to equilibration was decided by pre-experiment. After shaking, the aqueous and octanol phases were separated. The upper saturated octanol phase was removed using a Pasteur micropipette. Approximately 4 ml at the interface was discarded and the other saturated aqueous phase was saved.

Procedure for measurement of partition coefficients

Saturated octanol (5 ml) was added to water or buffer (5 ml) in a 12-ml test tube. Two or three test tubes were prepared for each water or buffer solution at each pH. The tubes were then placed in the incubator and shaken to allow them to reach equilibrium. After that, the tubes were removed from the incubator and centrifuged (model SCR 20B, Himac centrifuge, Hitachi Koki Co., Ltd, Japan) at 1600 × *g* for 10 min to facilitate phase separation. The concentration of drug in the oil phase (C_o) was obtained by diluting the sample with ethanol and then measuring using a U.V. spectrometer at 279 nm. The concentrations of drug in the aqueous phase (C_w) were obtained by dilution with water and measured at 284.5 nm. After equilibrium, the pH of the aqueous phase was checked. The partition coefficient (P) was calculated from the ratio of the overall concentration of compound in the ionized and unionized states between the two phases.

After the oil phases were separated, 3 ml of the oil phase was extracted 4 times with 3–5 ml of 0.1 N hydrochloric acid solution to measure the sodium content in the oil phase. The sodium content in the hydrochloric acid solution was then determined by flame atomic absorption spectrometry. Control experiments were carried out under the same conditions without the drug to estimate background sodium levels in the octanol. This experiment was carried out in a Pyrex glass test tube treated with 0.1 N HNO_3.

Results and Discussion

Acid dissociation constant

The titration curve of diclofenac in ethanol–water solution with 0.01 N NaOH is shown in Fig. 1. The pKa values were obtained from the half-neutralization point of the titration curve.

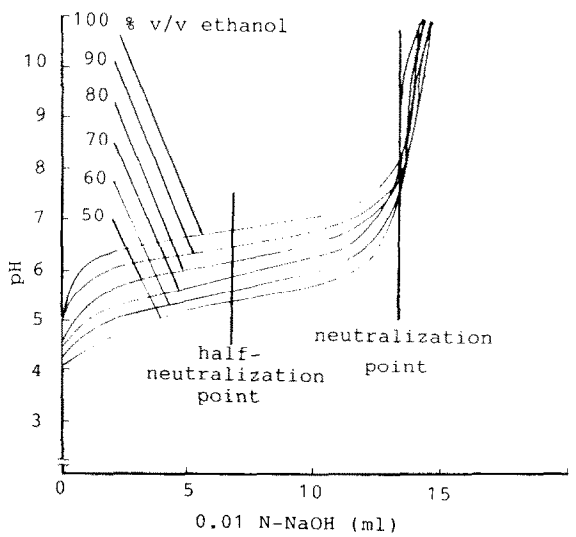

Fig. 1. Titration curve of diclofenac in water–ethanol mixtures with 0.01 N NaOH.

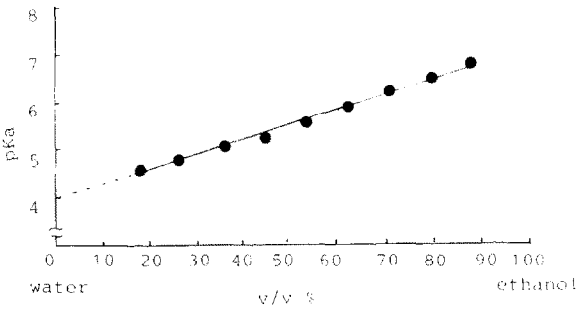

Fig. 2. Relationship between pKa and ethanol concentration in the water–ethanol mixtures.

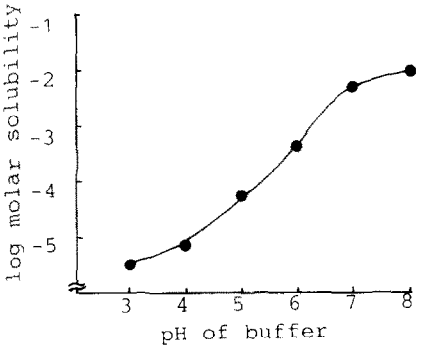

Fig. 4. Aqueous solubility of sodium diclofenac with respect to buffer pH.

The results of the pKa values with ethanol content are summarized in Fig. 2. The pKa values linearly increase with the ethanol content (correlation coefficient, $r = 0.999$).

As shown in Fig. 3, the variation in pKa with reciprocal of dielectric constant is linear ($r = 0.968$). The increase in pKa value with the addition of ethanol is due to the decrease in the dielectric constant of the solvent. The change in the pKa values is expressed by the Born equation (Robinson and Stokes, 1968):

$$\Delta pKa = Ne^2/4.3RT \cdot \left(1/r_H + z_A^2/r_A - z_{HA}^2/r_{HA}\right) \cdot (1/\epsilon - 1/\epsilon_w) \quad (1)$$

where N is the Avogadro constant, e is the elementary charge, z is the charge number of the ion, r is the radius of the ion, R is the gas constant, ϵ is the dielectric constant of the ethanol–water solvent, ϵ_w is the dielectric constant of water, A is anion and H is proton. The values of ϵ at 25 °C used in Fig. 3 are from the literature (Kagakubinran, 1984). Nishihata et al. (1988) have reported that ethanol added in a donor solution affects the skin penetration of diclofenac by increasing the solubility of diclofenac in the donor solution rather than by altering the skin barrier properties in percutaneous absorption of diclofenac in vivo. However, there is another possible way in which ethanol affects the drug. If the aqueous solution was adjusted to pH 7, the pKa value of the drug increases in the ethanol–water solution. Therefore, sodium diclofenac cannot ionize completely at physiological pH and a significant proportion of the unionized drug may permeate through the skin.

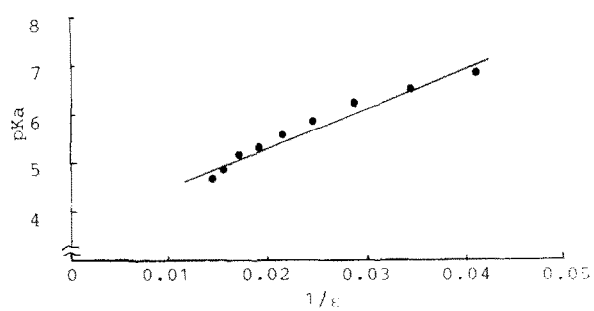

Fig. 3. Relationship between pKa and the reciprocal of the dielectric constant in the water–ethanol mixtures.

Solubility

The aqueous solubility of sodium diclofenac is strongly dependent on pH (Fig. 4). The insolubility at low pH reflects the low water solubility of the drug when its carboxyl group is undissociated (HA). When pH values are above the pKa, solubility in water increases rapidly and reflects the abundant water solubility of the ionized compound (A^-).

Partition coefficient

The relationship between pH and partition coefficient was studied in water or buffer solution (aqueous phase). The dissociation of the acid

drug in water and the partition of the undissociated drug into the oil phase are expressed as follows:

$$H^+_w + A^-_w \overset{K}{\rightleftharpoons} HA_w \overset{P}{\rightleftharpoons} HA_o \quad (2)$$

where HA is the unionized form, A^- is the monoanion, and the subscripts w and o denote the aqueous and oil phases, respectively. P is the partition coefficient of the unionized form. If we assume that ion pair formation between buffer cations (Na^+) can occur in the aqueous phase and move to the oil phase as follows:

$$A^-_w + Na^+_w \overset{K_s}{\rightleftharpoons} NaA_w \overset{P_s}{\rightleftharpoons} NaA_o \quad (3)$$

The ionization constants of HA, K, and of sodium salt, K_s, are defined as:

$$K = [H^+]_w[A^-]_w/[HA]_w \quad (4)$$

$$K_s = [Na^+]_w[A^-]_w/[NaA]_w \quad (5)$$

where the square brackets indicate molar concentrations of each species. The partition coefficient refers to the ratio of concentrations of aqueous and oil phases as follows:

$$P = [HA]_o/[HA]_w \quad (6)$$

The partition coefficient of sodium salt of drug is expressed by:

$$P_s = [NaA]_o/[NaA]_w \quad (7)$$

The experimentally obtained, apparent partition coefficient (P') is defined by considering that $[NaA]_w$ is always smaller than $[HA]_w$ or $[A^-]_w$, as follows:

$$\begin{aligned} P' = C_o/C_w &= \{[HA]_o + [NaA]_o\} \\ &\quad /\{[HA]_w + [A^-]_w\} \\ &= \{P + P_s[NaA]_w/[HA]_w\}/\{1 + K/[H^+]_w\} \\ &= \{P + (P_s/K_s)[Na^+]_w \cdot K/[H^+]_w\} \\ &\quad /\{1 + (K/[H^+]_w)\} \end{aligned} \quad (8)$$

In Eqn 8, when the concentration of protons is high in the acidic state, or is low in the alkaline state, the partition coefficient is shown as:

$P' = P$ when $[H^+]$ is high; and (9a)

$P' = (P_s/K_s)[Na^+]_w$ when $[H^+]$ is low (9b)

At an intermediate $[H^+]_w$ value where pH < 6, $[NaA]_w/[HA]_w$ is low. In this case, Eqn 8 is described as:

$$P' = P/(1 + K/[H^+]_w) \quad (10)$$

Therefore, the pH versus log P' are plotted according to the equation:

$$\log P' = \log P - \log(1 + 10^{pH - pKa}) \quad (10')$$

If the pKa value and linearity of Eqn 10′ are obtained, the value of P is determined from Eqn 10′.

The relationships between the concentrations of diclofenac and sodium salt in water and octanol at various concentrations are shown in Fig.

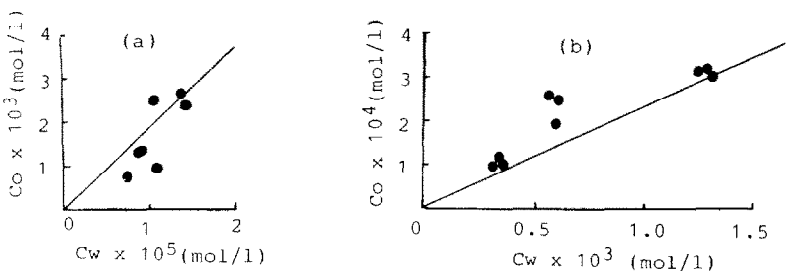

Fig. 5. Relationship between the concentration of diclofenac and sodium diclofenac in water and octanol at various values of pH. The values of pH were adjusted by HCl or NaOH. (a) Diclofenac, pH 2.97–4.82; (b) sodium diclofenac, pH 6.03–8.03.

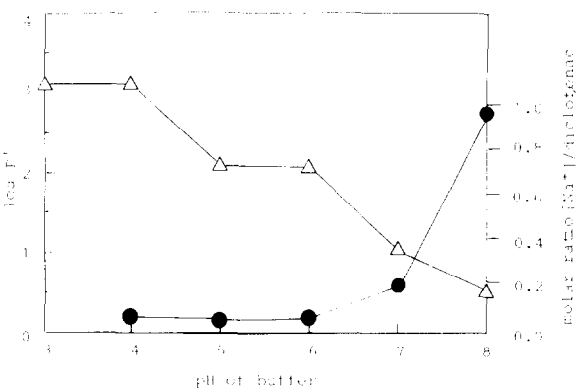

Fig. 6. Relationship between the partition coefficient (log P') of sodium diclofenac (△) and the molar ratio of Na^+ to diclofenac ions (●) present in the octanol phase with respect to pH.

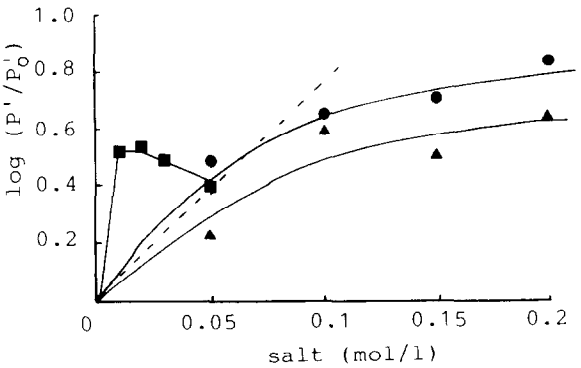

Fig. 7. Partition coefficient of diclofenac (1.15×10^{-3} mol/l) as a function of added salt concentration at non-adjusted pH. ●, NaCl; ▲, KCl; ■, $CaCl_2$.

5. This shows the linear relationship between C_o and C_w of diclofenac and sodium salt over a concentration range of $0.58 \times 10^{-3} - 2.30 \times 10^{-3}$ mol/l. The results of these experiments indicate that the slope in Fig. 5, P', was independent of the concentration of diclofenac and sodium salt over the pH range of 3.0–7.0, adjusted by HCl or NaOH. Since P' remained relatively constant, it did not appear that diclofenac and the sodium salt were associated in the n-octanol and water phases.

The partition coefficients of sodium diclofenac were determined as a function of pH in the buffers described above. The results of partition coefficients of sodium diclofenac and the molar ratio of Na^+ to diclofenac ions in the octanol are shown in Fig. 6. From the data in Fig. 5, Eqn 9 yields a P value of 1243. From Eqn 10′, good linearity between log P' and $-\log(1 + 10^{pH-pKa})$ (data not shown, $r = 0.979$) was observed by using the pKa value, 4.7, reported by Herzfeldt et al. (1983). The P value is determined to be 871 from the intercept. This P value is similar to the value (1243) obtained by Eqn 9.

If the pH dependence of the distribution of diclofenac is expressed by Eqn 10′, it can be calculated that the value of log P' at pH 8.0 should fall to −0.36, but actually the observed value was 0.51 at pH 8.0. This result indicated the possibility of ion pair formation of diclofenac.

Effect of added ions on the partition coefficient

The results for the partition coefficient of diclofenac as a function of the concentration of sodium chloride, potassium chloride and calcium chloride are plotted in Fig. 7. The pH was not adjusted and not changed before and after the distribution (pH: 5.67–5.86). It is clear that a significant increase in the partition coefficient is obtained in the presence of relatively low concentrations of added salts. Especially calcium chloride affects the partition coefficient at low concentration. Sodium ion has a greater effect on the partition coefficient than potassium ion.

The partition coefficient of diclofenac as a function of sodium chloride and potassium chloride is shown in Fig. 8. The values of pH were adjusted by NaOH to 8 and 9, and they changed

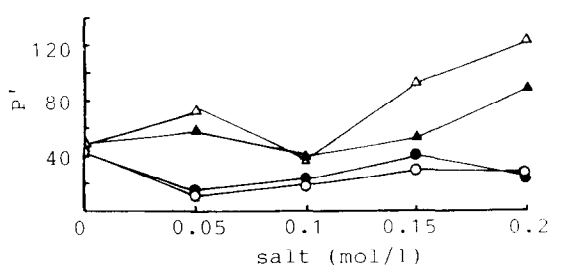

Fig. 8. Partition coefficient of diclofenac (1.15×10^{-3} mol/l) as a function of added salt concentration at pH 8 and 9 adjusted by NaOH. The following pH values were found after the distribution at pH 8 and 9, respectively: NaCl: ●, pH 5.6 and ○, pH 6.1; KCl: ▲, pH 5.6 and △, pH 6.1.

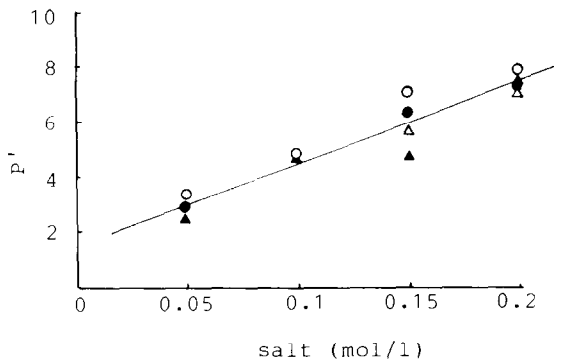

Fig. 9. Partition coefficient of sodium diclofenac ($1.08–1.12\times 10^{-3}$ mol/l) as a function of added salt concentration at pH 10.08 and 10.67, adjusted by NaOH. NaCl: ●, pH 10.08 and ○, pH 10.67, KCl: ▲, pH 10.08 and △, pH 10.67.

to 5.6–6.1 after the distribution. Some sodium ion from NaOH in the water moved to the oil phase for ion pairing with diclofenac. At this pH, diclofenac consists of diclofenac ion, sodium ion which was added, and a portion of diclofenac (unionized). In contrast to Fig. 7, however, it was found that the P' value is low and the increase in the P' value by adding salt is smaller than that of diclofenac. Also potassium ion caused a greater increase in the partition coefficient than sodium ion. These differences may be due to the fact that the portion of unionized diclofenac was low in Fig. 8 (with added NaOH), compared with Fig. 7 (water). Also the standard chemical potential of potassium ion relative to diclofenac ion may be higher than that of sodium ion.

Fig. 9 shows the relationship between the partition coefficient of sodium diclofenac as a function of added salt concentration. The pH values were adjusted with NaOH to 10.08 and 10.67. As the concentration of NaCl or KCl increased, the values of P' increased. However, the increase in P' values with added salt is smaller than those in Figs 7 and 8. When $[H^+]$ is low in Eqn 9b, P' increases linearly with sodium ion in water. Potassium ion did not affect the partition coefficient more than sodium ion did. Since the diclofenac sodium salt was used, added potassium ion could not contribute to the formation of ion pairs.

In Figs 7 and 8, P' values were greatly increased by adding salts. Since the increase in P' value is due to the change in activity coefficients in the presence of salts, we need to identify the effect of salts on all the species present in the solution. Since the pH is below 6, $[NaA]_w$ and $[NaA]_o$ are almost zero. Diclofenac cannot move to the oil phase in ion pairing. The observed P' is given by the following equation derived from Eqn 8:

$$P' = [HA]_o/\{[HA]_w + [A^-]_w\} \tag{11}$$

If activities, activity coefficients and concentrations are represented by a, γ and C, respectively, then:

$$\begin{aligned} K' &= a_{H^+}a_{A^-}/a_{HA_w} \\ &= a_{H^+}\cdot\gamma_{A^-}\cdot C_{A^-}/(\gamma_{HA_w}\cdot C_{HA_w}) \\ &= K\cdot\gamma_{H^+}\cdot\gamma_{A^+}/r_{HA_w} \end{aligned} \tag{12}$$

where K is defined by Eqn 4.

The measured partition coefficient, P', can be written as:

$$\begin{aligned} P' &= C_{HA_o}/(C_{HA_w} + C_{A_w^-}) \\ &= \left[a_{HA_o}/\{a_{HA_w}(1 + K'\cdot\gamma_{HA_w}/a_{H^+}\cdot\gamma_{A^-})\}\right] \\ &\quad\cdot(\gamma_{HA_w}/\gamma_{HA_o}) \end{aligned} \tag{13}$$

The changes in P' resulted from the change in the activity coefficients γ_{HA} and γ_{A^-}, since we assume that γ_{HA_o} will remain constant, and a_{H^+} is constant because the pH value is not changed by adding salt. First, γ_{A^-} should decrease with increasing salt concentration and assuming that the effect on γ_{HA} is much smaller, P' will decrease. However, this result is not consistent with our data. Second, the increase in P' was ascribed to the salting out of diclofenac in the aqueous phase. The authors fitted their data (Fig. 7) to an equation analogous to the Setschenow formula, which has been used to describe the effect of

salts on the activity of non-electrolytes (Kojima and Davis, 1984):

$$\log(P'/P'_0) = kC$$

where P' is the partition coefficient in the presence of salt, P'_0 is the partition coefficient in the absence of salt, C is the concentration of salt, and k can be related to the salt-non-electrolyte interaction parameter. In our data, we obtained excessively high values for k. Examination of our results shows that the k value is 6 for the diclofenac and NaCl system at 0.1 M of sodium chloride concentration as shown by the broken line in Fig. 7.

The solubility and distribution behavior of diclofenac are dramatically affected by the presence of added cations, and this may be due to the salting out.

The effect of changing the activity coefficients (γ_A) in the presence of salts is expected to be negligible. Also the effect of changing the activity coefficient of the unionized form (γ_{HA}) cannot account for the increase in the partition coefficient.

Ion pairing with added ions has been proposed as a means of enhancing the partition coefficient (Tomlinson et al., 1982; Cools and Janssen, 1983; Davis et al., 1984). Many investigations reported that percutaneous absorption was enhanced by ion pairing (Gasco et al., 1984; Hadgraft et al., 1985; Green and Hadgraft, 1987; Green et al., 1988). Ion pairs are probably not present in water, but in the oil phases or at the interface. Sodium ion-selective electrodes were used to measure the concentration of the sodium ion. The concentration of sodium ion in the aqueous phase after the distribution of sodium diclofenac (1.078×10^{-3} mol/l) decreased from 0.078 mmol/l to 0.052 mmol/l. Considering the decrease in sodium ion by moving to the oil phase for ion-pairing, the partition coefficient (P'_{Na}) was 0.494 from the measurement of the concentration of sodium ion. On the other hand, the partition coefficient (P') was 0.621 from the measurement of the concentration of diclofenac with U.V. spectrometry. The P'_{Na} value was lower than the P' value.

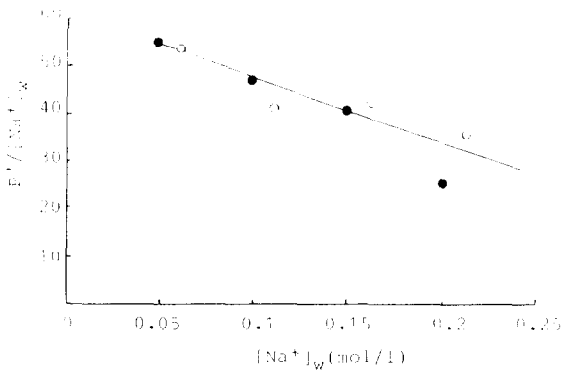

Fig. 10. Plot of $P'/[Na^+]_w$ vs $[Na^+]_w$, the latter being changed by addition of NaCl. The concentration of sodium diclofenac is $1.08–1.12 \times 10^{-3}$ mol/l. ●, pH 10.08; ○, pH 10.67.

Analysis of sodium ion in the octanol phase was carried out by flame atomic absorption, and analysis of diclofenac ion in the buffer and octanol phases by U.V. spectrophotometry at a series of pH values. It was shown that the penetration of sodium ion into the octanol phase is significant only at pH ≥ 7, as shown in Fig. 6. The molar ratio of sodium ion to diclofenac ion is almost 1 : 1 at pH 8. Below this pH, the ratio of sodium ion to diclofenac decreased, and it was shown that the partition of the undissociated compound (HA) became dominant. This evidence for ion pair partition was supplemented by the analysis of sodium ion in octanol equilibrated with phosphate buffer containing sodium ion but no diclofenac. The penetration of sodium ions into the octanol was insignificant.

The relationship between the $P'/[Na^+]_w$ and $[Na^+]_w$ is shown in Fig. 10. As the concentration of sodium ion increased by adding sodium chloride, the value of $P'/[Na^+]_w$ decreased and was not constant. The solubility product of the sodium salts was 1.06×10^{-4} $(mol/l)^2$. The ionic product of sodium and diclofenac is increased to the value of their solubility product by adding sodium chloride. Therefore, diclofenac cannot dissolve in the aqueous phase and move to the oil phase. Now in the alkaline state, (P_s/K_s) was obtained from the data of $P'/[Na^+]_w$ using Eqn 9b. At 0.05 mol/l of NaCl under the solubility product, (P_s/K_s) is 55.8 and 54.4 at pH 10.08 and 10.67, respectively.

Conclusions

The pKa value of the drug increased with an increase in the concentration of ethanol in the ethanol–water solution. Therefore, sodium diclofenac in the ethanol–water donor solution cannot ionize completely at physiological pH, and a significant proportion of the unionized drug may permeate through the skin, the surface of which is reported to be slightly acidic (pH 4.2–5.6) (Katz and Poulsen, 1971).

Above pH 7, ion pair formation operates to allow distribution of the drug into the oil phase. This process is likely to occur also in the skin. Since the donor phase is adjusted to almost pH 7, it is suggested that ion pair formation with naturally abundant cations plays a significant role in the absorption of diclofenac.

From these results, in the percutaneous absorption experiment where the donor solution contains ethanol, it has been concluded that sodium diclofenac may be absorbed through the skin partly in unionized form which resulted from the increasing pKa value of diclofenac. Partly it may be absorbed in ion pair form which ionized diclofenac forms, as a result of having a higher partition coefficient than the ionized form. Ethanol may accelerate the ion-pair formation of diclofenac and the unionized form in ethanol–water mixture.

Acknowledgements

The authors are grateful to Mr Touru Yamaguchi and Ms Satomi Takahashi for assistance with the experimental work. The skilled technical assistance of Ms Kiyoko Toyonaga in performing the flame atomic absorption spectrometric measurements is most appreciated. We also acknowledge the many fruitful discussions with Dr Kazuhide Komiya of the Department of Radiopharmacy, Hoshi University.

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