ATR-FTIR characterization of transport properties of benzoic acid ion-pairs in silicone membranes

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Abstract

A novel technique based on Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy was used to study the transport of benzoic acid ion-pairs/salts in silicone membranes. The benzoic acid ion-pairs were prepared using various counter-ions with different degrees of lipophilicity, e.g. triethylamine (TA), diethylamine (DE), *tert*-butylamine (*t*-BA), 2-amino-2-methyl-propanol (AMP), and 2-amino-2-methyl-propanediol (AMPD). Silicone membrane, treated or untreated with propylene glycol (PG), was placed on the surface of a ZnSe crystal and the transport solution was applied to the upper surface of the membrane. A mathematical model, based on Fick's second law describing the build up of permeant concentration at the membrane/crystal interface with time was applied to determine diffusion coefficients. Absorption due to the acid ($1700\,cm^{-1}$) or benzoate anion ($1555\,cm^{-1}$) was observed at different regions without the interference from PG or silicone membrane. Benzoate anion, a charged species, was observed to permeate the membrane. The permeation of benzoate anion from sodium benzoate and polar ion-pairs of AMP and AMPD was very low in contrast to their high-saturated concentrations in PG as compared to the *t*-BA ion-pair. This indicated that benzoate anion preferentially permeates the membrane as an ion-pair rather than a single anion; otherwise its permeation should correspond to its concentration in PG instead of the lipophilicity of the ion-pairs. Additionally, the diffusion coefficient values of benzoic acid and benzoate anions through the treated and untreated membranes were not statistically different.
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1. Introduction

Many studies have pointed out the important role of ion-pair formation for transport of the ionic forms of drugs and peptides through biomembranes (Neubert, 1989; Neubert and Fischer, 1991; Quintanar-Guerrero et al., 1997; Gelder et al., 1999; Valenta et al., 2000; Hatanaka et al., 2000). Kinetic studies of transdermal penetration of physostigmine salicylate salt using flow-through diffusion cells supported the existence of the ion-pairs as an undissociated form in the donor compartment and its partitioning into the stratum corneum as one entity (Pardo et al., 1992). Results from diffusion studies using Franz diffusion cells indicated the possibility of flux enhancement of lignocaine salts by ion pairing (Valenta et al., 2000). These studies suggest the transport of ion-pairs without direct analytical measurements of ions and counter-ions in the membrane.

Attenuated Total Reflectant Fourier Transform Infrared (ATR-FTIR) spectroscopy has increasingly been used to measure the permeation of compounds across membranes and to determine their diffusion coefficient (Sammon et al., 2000; Hanh et al., 2000; Elabd et al., s2000; Pellet et al., 1997b; Dias et al., 2001). This technique offers the potential to evaluate and quantify penetrating species according to their specific absorption bands in real time. In this study, the transport of ion-pairs through silicone membrane was characterized using ATR-FTIR spectroscopy. Although there are differences between the silicone membrane and stratum corneum, which is a complex molecular structure, the trends observed are similar (Valenta et al., 2000). A simple membrane like silicone membrane has therefore been used. Benzoic acid was employed as a model compound. As an organic acid, its FTIR spectrum can exhibit both charged (benzoate anion) and uncharged (benzoic acid) bands at different regions. The benzoate anion bands are used for monitoring the permeation of the ion-pair form of benzoic acid. Organic bases such as tri-ethlyamine (TA), diethylamine (DE), *tert*-butylamine (*t*-BA), 2-amino-2-methyl-propanol (AMP) and 2-amino-2-methyl-propanediol (AMPD) with varying degrees of lipophilicity were used to form ion-pairs with benzoic acid and their diffusion coefficients were compared to that of inorganic ion-pair, sodium benzoate.

2. Materials and methods

2.1. Materials

Benzoic acid, TA, DE, *t*-BA, AMP, AMPD, sodium benzoate and propylene glycol (PG) were purchased from Fluka Chemie (Buchs, Switzerland) and used as received. Acetone, methanol, and other organic solvent (analytical grade) were obtained from Mallinckrodt Baker (USA). Silicone membranes (thickness 300 μm) produced by Samco (St. Albans, UK) were used as supplied.

2.2. Preparation of ion-pairs/salts

Ion-pairs/salts were prepared by direct acid base reaction. The solution at equimolar amount of each base was added to the solution of benzoic acid. Acetone was used as a solvent for preparing all ion-pairs except the AMPD ion-pair. Since AMDP does not dissolve in acetone, methanol was used as the solvent. For preparation of TA and DE ion-pairs, the mixing solutions were stirred for approximately 10 h and the solutions were concentrated under vacuum. The general procedure for preparing TA ion-pair (B-TA) of benzoic acid resulted in the formation of a viscous liquid that did not crystallize. According to the IR spectrum, this liquid comprises both the acid and salt form as discussed in Section 3. This mixture was dried under vacuum overnight and further used for ATR-FTIR studies as a liquid. DE ion-pair (B-DE) gave a yellow solid when the mixture was allowed to dry under vacuum; it also contained both the acid and salt species. Both B-TA and B-DE were used for comparing the diffusion coefficients of benzoic acid and benzoate ion-pairs of each mixture. AMP (B-AMP) and *t*-BA (B-*t*-BA) ion-pairs were precipitated during the mixing of benzoic acid with each base. The white precipitates were collected by filtration, thoroughly washed with acetone, and vacuum dried. Solutions of benzoic acid and AMPD were concentrated under vacuum and recrystallized from acetone to give a white solid of AMPD benzoate (B-AMPD).

2.3. Determination of apparent partition coefficient

Determination of dichloromethane (CH_2Cl_2)/water partition coefficients of benzoic acid ion-pairs/salts

was performed according to the method previously described (Zhou et al., 2002). Briefly, 2 ml of CH_2Cl_2 containing 5 mM of ion-pair was mixed with 2 ml of water by vortexing for 5 min. The mixture was then centrifuged at 2000 rpm for 10 min. Benzoic acid content in CH_2Cl_2 was analyzed by UV spectrometry at 230 nm. The content in the aqueous phase was determined by subtracting its content in the organic phase from the total benzoic acid content. The content in the two phases was used to calculate the CH_2Cl_2/water partition coefficient.

2.4. ATR-FTIR studies

IR spectra were obtained by using an EQUINOX 55 spectrometer (Bruker, Germany) equipped with a MCT detector. The Horizontal Attenuated Total Reflectance Accessory (HATR) was mounted into the sample compartment. The internal reflection crystal (Pike Technologies, USA), which was made of zinc selenide (ZnSe), had a 45° angle of incidence to the IR beam. Spectra were acquired at a resolution of $4\,cm^{-1}$ and the measurement range was $4000–700\,cm^{-1}$. All spectra (25 scans) were collected every 2 min for 30 min and every 5 min thereafter. The spectrometer was linked to a PC equipped with Bruker OPUS software to allow the automated collection of IR spectra. The IR spectra were imported to GRAMS/AI 7 (Thermo Galactic, USA) for peak area integration. The integrated absorbance was plotted against the corrected midpoint time of the measurement. All experiments were performed in triplicate at ambient temperature, $25 \pm 2\,°C$.

ATR-FTIR studies were conducted as described by Watkinson et al. (1995). Briefly, a membrane was placed between the surface of ZnSe crystal and a saturated reservoir of permeant in the upper surface of the membrane. Saturated permeant which provided a constant concentration at all times, with an excess of solid in case of solid ion-pairs, was applied in the PVC trough above the membrane. The B-TA ion-pair and the saturated solutions of benzoic acid, sodium benzoate and B-DE, B-t-BA, B-AMP, B-AMPD ion-pairs in PG, which were prepared at room temperature, were applied to the membranes, either untreated or treated with PG overnight.

The mean thickness was determined by measuring the membrane at 10 different points using a micrometer. The thickness was determined before and after presaturating the membrane with PG overnight.

2.5. Data analysis

In the ATR-FTIR experiments, a diffusion model using a solution of Fick's second law that satisfies both the initial and subsequent boundary conditions was used to describe the build up of permeant concentration at the membrane/crystal interface with time. Therefore, diffusion can be defined by Eq. (1).

$$\frac{C}{C_0} = 1 - \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} \times \exp\left(\frac{-D(2n+1)^2\pi^2 t}{4h^2}\right) \tag{1}$$

where C is the permeant concentration at the interface at time t, C_0 the solubility of penetrant in membrane, D the diffusion coefficient of penetrant, and h the diffusional pathlength (Wurster et al., 1993). Assuming that Beer–Lambert law is obeyed, the concentration terms can be replaced with experimental absorbance values to give Eq. (2) where A is the area under the curve of the penetrant peak (at time t) of the IR absorbance associated with the permeant, and A_0 the area under the curve of the penetrant peak corresponding to the situation where the membrane is saturated.

$$\frac{A}{A_0} = 1 - \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} \times \exp\left(\frac{-D(2n+1)^2\pi^2 t}{4h^2}\right) \tag{2}$$

The values of D/h^2 and A_0 were calculated using a program developed in-house to fit the experimental data to Eq. (2). Values of the diffusion coefficient were subsequently calculated using the diffusional pathlength.

2.6. Statistics

Statistical analysis was carried out using one factor ANOVA and the LSD's multiple comparison procedure. The P-value of 0.05 was considered significant.

3. Results and discussion

A solvent with a high-dielectric constant (ε) such as water ($\varepsilon = 80$) is unfavorable for ion-pair formation. However, the interaction between opposite ions
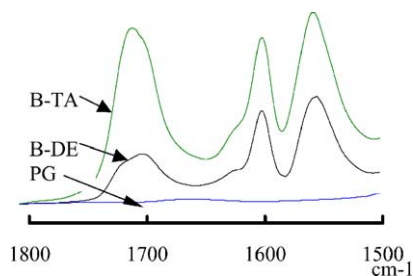
Fig. 1. FTIR spectra of PG and saturated solutions of B-TA and B-DE in PG.

Fig. 2. FTIR spectra of saturated solutions of B-AMPD, B-AMP, B-*t*-BA and sodium benzoate (Na-BEN) in PG.

becomes increasingly important in solvents with $\varepsilon <$ 40 (Kraus, 1956). In this study, PG was chosen as a solvent because it does not change the properties and diffusion barrier of the silicone membrane (Dias et al., 2001). Importantly, the absorption bands from PG do not interfere in the regions of study (Fig. 1). Additionally, the compounds studied are soluble in PG and also ion-pair formation would be able to exist in PG with ε of 32.1.

ATR-FTIR has been successfully used to measure the permeation of compounds. However, this technique is limited to IR-active permeants. Benzoic acid and its salt or carboxylate anion are IR-active species and their strong absorption bands occur in different regions. In general, the H-bonded carboxylic cyclic dimer peak of aryl conjugated acids appears at $1710–1680 \, cm^{-1}$, and the non-hydrogen bonded acid C=O stretch occurs between 1760 and $1730 \, cm^{-1}$ (Silverstein et al., 1991; Pavia et al., 1979). The frequency for carboxylate salt $[\!-\!C\overset{\displaystyle O}{\underset{\displaystyle O}{\lessgtr}}\,]$ absorption is lower than that for the carboxylic acid because of resonance. The carboxylate anion shows two absorption bands; a strong asymmetric carboxylate stretching band is usually seen at $1650–1550 \, cm^{-1}$, while a weaker symmetric stretching band occurs near $1400 \, cm^{-1}$ (Silverstein et al., 1991). In our studies, only an asymmetric stretching can be observed at $1555 \, cm^{-1}$ for benzoate anion (Figs. 1 and 2); a symmetric stretching band was obscured by the strong PG absorption in the same region, $1459–1227 \, cm^{-1}$. The formation of carboxylate anion requires the presence of a counter-ion. Hence, an "ammonium" band in the $2700–2200 \, cm^{-1}$ region should appear in addition to the two characteristic carbonyl absorption bands of asymmetric and symmetric stretches (Silverstein et al.,
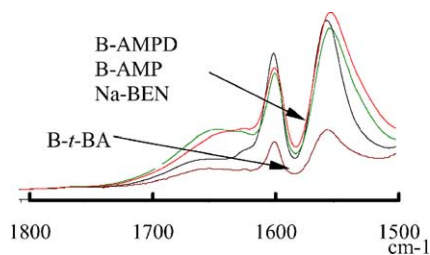
1991). However, due to the interference from PG, only anionic absorption bands were observed in the spectra of all organic salts. Alkali metal salt itself has no vibrational spectrum, so the spectrum obtained from sodium salt shows only the absorption of the anionic species. For benzoic acid and benzoic ion-pairs, the ring C=C stretching bands were observed at about $1598 \, cm^{-1}$ (Figs. 1 and 2). These bands were not used for analysis, since they cannot distinguish between the acid and carboxylate anion.

Besides the carboxylate anion peak at $1555 \, cm^{-1}$, the acid peak at $1700 \, cm^{-1}$ was also detected for B-TA and B-DE (Fig. 1). Probably due to the steric hindrance of TA and DE structures, a tertiary and secondary amine respectively, a simple mixing cannot afford a complete ion-pair or salt formation. These mixtures were also employed in the study since it is interesting to characterize the diffusion of both acid and carboxylate anion simultaneously. When the primary amines including AMPD, AMP and *t*-BA were used as counter-ions for benzoic acid, ion-pairs or salts were, however, completely formed, as demonstrated by the disappearance of the acid peak at $1700 \, cm^{-1}$ (Fig. 2).

Fig. 3 shows a typical set of data and the non-linear fit to Eq. (2). Similar diffusion profiles were obtained for the others, either in untreated or treated membranes. The membrane thickness before and after PG treatment is not statistically different. Hence, the increase in membrane swelling by PG is not significant in the context of diffusional pathlength. The thickness of the membrane was assumed as the diffusional pathlength.

The areas of benzoate anion bands at $1555 \, cm^{-1}$ for saturated solutions of B-AMPD, B-AMP and sodium benzoate are much higher than that of B-*t*-BA (Fig. 2). This suggests that the concentration of these species is significantly higher than B-*t*-BA. In contrast to the
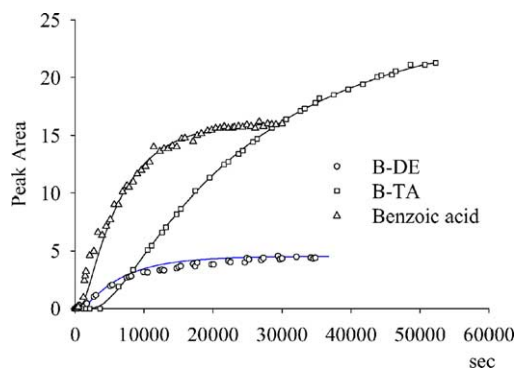
Fig. 3. Diffusion profiles of benzoic acid from B-TA liquid and saturated solutions of benzoic acid and B-DE in PG, through treated silicone membrane.

Table 1
Values of diffusion coefficients of benzoic acid and benzoate anion from different compounds

Membrane/compounds	$D \times 10^{-8}$ (cm^2/s)	
	Benzoic acid	Benzoate anion
Untreated		
B-DE	8.94 ± 1.12	3.49 ± 1.02
B-TA	3.61 ± 1.83	2.99 ± 0.50
Benzoic acid	11.88 ± 4.17	–
B-t-BA	–	6.97 ± 0.81
Treated		
B-DE	8.81 ± 1.48	2.28 ± 0.31
B-TA	2.88 ± 0.08	2.23 ± 0.05
Benzoic acid	11.64 ± 3.60	–
B-t-BA	–	5.66 ± 0.78

substantially high concentrations of benzoate anions from B-AMPD, B-AMP and sodium benzoate, their permeation through the silicone membrane is very low as compared to that from B-t-BA. To clarify the permeation behavior of these ion-pairs, their lipophilicity was determined. The log CH_2Cl_2/water partition coefficients of B-AMPD, B-AMP, sodium benzoate and B-t-BA were -1.25, -1.12, -1.14, and -0.85, respectively. Thus, the low permeation of B-AMPD, B-AMP and sodium benzoate may probably be due to their high hydrophilicity. Because of their extremely small bands, the diffusion coefficient of benzoate anions cannot be determined reliably under the experimental conditions. However, it is important to note that if the benzoate anion permeates the membrane as a single species, and not as an ion-pair, then the permeation of benzoate anions from B-AMPD, B-AMP and sodium benzoate should be higher than that from B-t-BA. Accordingly, it is hypothesized that benzoate anion permeates the membrane as an ion-pair.

Table 1 shows the results of diffusion coefficient determination of benzoic acid and benzoate anion from various compounds. The diffusion coefficient of benzoic acid and benzoate anion from the same compound in treated membrane is slightly less than that in untreated membrane but the difference is not statistically significant. These results demonstrate that pretreatment of the membrane with PG does not significantly alter the diffusion of the compounds. The similarity in diffusion coefficient in treated and untreated membranes was also reported by Pellett et al. (1997a) using water as a vehicle.

The diffusion coefficient of benzoic acid from different sources should be similar, however, the discrepancies were observed as shown in Table 1. Furthermore, the presence of both acid and ion-pair species in B-TA and B-DE is demonstrated by a considerably low-diffusion coefficient of benzoic acid from these sources as compared to that from pure saturated benzoic acid solution. Generally, the diffusion coefficient is directly proportional to the product of average velocity and molecular-mean-free path, which is the average distance that a molecule travels between collisions (Castellan, 1983). For B-TA and B-DE, a benzoic acid molecule may travel a shorter distance before its collision due to a crowded environment caused by ion-pair species. The diffusion coefficient from these species is thus lower than that from pure benzoic acid.

The diffusion coefficient of benzoate ion-pairs from B-DE and B-TA is comparable to (but lower than) that from B-t-BA. Unlike B-DE and B-TA, B-t-BA does not contain free acid. The diffusion of B-t-BA was primarily due to ion-pair associated with PG, whereas those of B-TA and B-DE were disturbed by other diffusive species which might shorten their mean free paths, as reflected in their lower diffusion coefficient values. Another factor that influences the diffusion coefficient of these ion-pairs is their molecular size. Diffusion coefficient is inversely proportional to the square root of molecular weight (Castellan, 1983). The molecular weight of TA (101.19) is higher than that of DE (73.14) and t-BA (73.14). Moreover, t-BA, a primary amine, might be able to form a stronger ionic bond with benzoate anion than DE and TA, which are secondary

and tertiary amines, respectively. The small and compact size of B-*t*-BA ion-pair compared to B-TA and B-DE may also contribute to the higher average velocity of this ion-pair when collisions take place, thus yielding higher diffusion coefficient values.

The area ratios of benzoic acid to benzoate anion for saturated solution of B-TA and B-DE (Fig. 1) are 1.4 and 0.75, respectively. This suggests that the concentration of benzoic acid in B-DE is lower than that in B-TA. The higher mean-free path for benzoate anion of B-DE ion-pair should contribute to the higher diffusion coefficient of B-DE as compared to that of B-TA. The diffusion process of these ion-pairs, however, was set in different conditions. Whereas the diffusion of B-DE took place in association with PG, the diffusion of B-TA was purely a random walk of the ion-pair through the membrane. Without a solvent, the B-TA ion-pair might be more tightly bound to each other than the B-DE ion-pair. The smaller size of the B-TA ion-pair might offset its high concentration of free acid, thus the diffusion coefficient of the B-TA ion-pair is slightly lower than that of the B-DE ion-pair.

In conclusion, using the ATR-FTIR technique, the absorbance characteristics of permeants can be monitored with time. Furthermore, this technique provides evidence for the transport of a charged species as an ion-pair. The permeation of the ion-pair will be more important if the counter-ion is lipophilic. This information supports the use of ion-pairing agents for enhanced transport of ionic species through biomembranes.

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