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Use of ATR-FTIR to Characterize the Permeation of Benzoic acid Ion-pairs in Silicone Membranes

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Abstract

Benzoic acid ion-pairs/salts were prepared using triethylamine (TA), diethylamine (DE) and *tert*-butylamine (*t*-BA), 2-amino-2-methyl-propanol (AMP), 2-amino-2-methyl-propanediol (AMPD) with different in lipophilicity as counter ions. For Attenuated Total-Reflectant Fourier Transform Infrared (ATR-FTIR) studies, silicone membrane either untreated or treated with PG overnight was placed on the surface of a ZnSe crystal and the studied solution was applied on 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 coefficient. Absorptions due to the acid ($1700 \ cm^{-1}$) and benzoate anion ($1555 \ cm^{-1}$) were observed at different regions without the interferences from PG or silicone membrane. Benzoate, a charged species, was observed to permeate the membrane. The permeation of benzoate from sodium benzoate and polar ion-pairs of AMP and AMPD was very low although their saturated concentrations in PG were considerably very high compared to *t*-BA ion-pair. Thus benzoate would rather permeate through the membrane as an ion-pair than as a single anion, otherwise its permeation should correspond to its concentration in PG instead of the lipophilicity of ion-pairs. Additionally, the diffusion coefficient values of benzoic acid and benzoate anion through the treated and untreated membranes are not statistically different.

Keywords: Benzoic acid; ATR-FTIR; Ion-pair, Permeation, Silicone membrane.

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1. Introduction

Many studies have pointed out the important role of ion-pair formation for transport the ionic forms of hydrophilic 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 diffusion cells of the flow-through type 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 of diffusion studies by Franz-type glass diffusion cells indicated the possibility to enhance the flux of lignocaine salts by using ion-pair (Valenta et al., 2000). All of these studies suggest the transport of ion-pairs without direct analytical measurement of ion and counter ions in the membrane.

Attenuate Total-Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy has been increasingly used to monitor the permeation of compounds across membranes and to determine the diffusion coefficient (Sammon et al., 2000; Hanh et al., 2000; Elabd et al., 2000; Pellet et al., 1997b; Dias et al., 2001). This technique offers the potential to evaluate and to 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 silicone membrane and stratum corneum which is a complex molecular structure but the trends observed are similar (Valenta et al., 2000), a simple membrane like silicone membrane has therefore been used in this study. Benzoic acid was employed as a model compound. Organic bases such as triethlyamine (TA), diethylamine (DE), *tert*-butylamine (*t*-BA), 2-amino-2-methyl-propanol (AMP), 2-amino-2-methyl-propanediol (AMPD) with different in 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 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 mm) produced by Samco (St. Albans, UK) were used as supplied.

2.2 Preparation of ion pairs/salts

Ion-pairs/salt 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 AMPD ion-pair. Since AMDP cannot dissolve in acetone, methanol was used as the solvent. After mixing, for TA and DE ion-pairs, the mixtures were stirred for approximately 10 hours and the solutions were concentrated under vacuum. Using the general procedure to prepare TA ion-pair (B-TA) of benzoic acid resulted in the formation of viscous liquid that did not crystallize. According to the IR spectrum, this liquid comprises both the acid and salt form. 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 dried under vacuum. Solution 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 coefficient was performed as described by 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 CH_2Cl_2/water partition coefficient was then calculated.

2.4 ATR-FTIR studies

IR spectra were obtained by using an EQUINOX 55 spectrometer (Bruker, Germany) equipped with a MCT detector. An out-of-compartment II-ATR cell was mounted on the optical bench. 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. A ll experiment were performed in triplicate at ambient temperature, 25±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, during the experiment 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 (used as received) or treated by presaturated with PG overnight.

The m ean t hickness w as c alculated f rom m easurement t he m embrane a t ten different points using a micrometer. The thickness was determined before and after presaturated membrane with PG overnight.

2.5 Data Analysis

In t he ATR-FTIR experiments, a diffusion model using a solution of Fick's second l aw t hat s atisfies b oth i nitial and subsequent boundary conditions, has been employed to describe the build up of permeant concentration at the membrane/crystal interface with time. Therefore, diffusion can be defined by Eq. (1).

$$\frac{C}{C_0} = 1 - \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} \times \exp\left(\frac{-D(2n+1)^2 \pi^2 t}{4h^2}\right)$$ (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 the Beer-Lambert law is obeyed, 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 relating to the permeant and A_0 the area under the curve of the penetrant peak corresponding to the situation where the membrane is saturated (in the plateau region of the curve).

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

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 appears at 1710 cm⁻¹ and the non-hydrogen bonded acid C=O stretch occurs at 1735 cm⁻¹. A strong asymmetric carboxylate stretching band is usually seen at 1650-1540 cm⁻¹, while a weaker symmetric stretching band occurs near 1400 cm⁻¹ (Silverstein et. al., 1991). Fig. 1 shows FTIR spectra of PG and the solution of benzoic acid and sodium benzoate in PG. The absorption bands from PG do not interfere with benzoic acid and benzoate anion in the regions of studies. Formation of the carboxylate anion requires the presence of a counter ion. Hence, an "ammonium" band in the 2700-2200 cm⁻¹ region will 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 spectrum obtained from the sodium salt shows only the absorption of anionic species.

For the preparations of B-TA and B-DE, besides the anionic absorbances, the acid peak at 1700 cm⁻¹ was also detected. 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. For the others, which are primary amines, ion-pair or salt formations were obtained and the acid carbonyl stretch of benzoic acid in PG at 1700 cm⁻¹ is lost (Fig. 2). Using sodium or different bases, TA, DE, t-BA, AMP, AMPD do not change the FTIR pattern of benzoate anion in the region of 1620-1500 cm⁻¹. Absorption due to the ring C=C and the asymmetric carboxylate anion C=O stretch were seen at 1598 and 1555 cm⁻¹, respectively. A

symmetric carboxylate C=O stretching band cannot be observed due to the interference from PG absorption in this region, 1459-1227 cm^{-1}.

The solvent with a high dielectric constant (ε) such as water ($\varepsilon = 80$) is unfavorable for ion-pair formation, however the interaction becomes increasingly important in solvents with $\varepsilon < 40$ (Kraus, 1956). In this study PG was chosen as a solvent since it does not change the properties and diffusion barrier of the silicone membrane (Dias et. al., 2001). Additionally, compounds studied are soluble in PG and ion-pair formation would be able to exist in PG with ε of 32.1.

As the diffusion experiment through membrane progress there is an increase in the IR peak areas associated with the penetrating species. This build up of permeant at the membrane/crystal interface will continue until a plateau is reached which represents saturation of the membrane. The rate at which the plateau is attained is related to diffusion coefficient and the plateau level (A_0) itself is related to the solubility of the permeant in the membrane. 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 treatment with PG is not statistically different. Hence, the increase in the membrane swelling by PG is not significant in the context of diffusional pathlenght. The thickness of membrane was assumed as the diffusional pathlength.

The results of CH_2Cl_2/water partition coefficient determination of sodium benzoate, B-AMPD, B-AMP, and B-t-BA were -1.14, -1.25, -1.12 and -0.85, respectively. In contrast to their substantially high benzoate concentrations of sodium benzoate, B-AMPD, B-AMP in PG (Fig 2), due to the high hydrophilicity, permeations of benzoate anion from these sources through silicone membrane is very low compare to that from B-t-BA. Because of the very small amount of these observable bands, diffusion coefficients of benzoate anion from these sources cannot be obtained correctly under the condition of this study. However, it is interesting to point out that if benzoate anion permeates through the membrane as a single species not as an ion-pair, the permeation of benzoate from B-AMP, B-AMPD and sodium benzoate should be higher than that from B-t-BA. According to these results, it is hypothesized that benzoate anion permeates through the membrane as an ion-pair.

The A_0 value represents the saturation of membrane with a permeant. These values for benzoic acid and benzoate anion from the same sources obtained from the untreated and treated membranes are not statistically different except for the benzoate anion from B-DE that is lower in untreated membrane (Table 1 and 2). Although the diffusions of benzoic acid and benzoate from different sources were investigated in different environment, their A_0 values are in the same order of their concentrations in solutions (Fig.2).

Table 1 and 2 also show the results of diffusion coefficients of benzoic acid and benzoate anion, respectively, from various compounds. The diffusion coefficients of benzoic acid and benzoate anion from the same compound through treated membranes are slightly less than those in untreated membranes but the values are not statistically different. These results demonstrate that pre-treatment of the membrane with PG does not significantly affect the diffusion of compounds. The similarity in diffusion coefficients for treated and untreated membranes was also reported by Pellette et al. (1997a) using water as vehicle.

The diffusion coefficient of benzoic acid from different sources should be expected to be similar, the discrepancy however was observed as shown in Table 1.

The diffusion coefficients of benzoic acid from these sources increased in the following order, B-TA < B-DE < pure benzoic acid. The fact that not only a free acid but also ion-pair species presented in the system under study is demonstrated by considerably high diffusion coefficients of benzoic acid from pure saturated benzoic acid solution (Table 1). Generally, the diffusion coefficient is directly proportional to the product of average velocity and molecular mean free path which is the average distance a molecule travels between collisions (Castellan, 1983). For B-TA and B-DE, a benzoic acid molecule may travel in a shorter distance before collision due to crowded environment with ion-pair species. The diffusion coefficients from these species are thus lower than that from pure benzoic acid.

The diffusion coefficients of benzoate ion-pair from B-DE and B-TA are comparable but lower than that from B-t-BA. Unlike B-DE and B-TA, B-t-BA did not contain the free acid. The diffusion of B-t-BA was mainly due to only 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. Furthermore, another factor that influences diffusion coefficients of these ion-pairs is their molecular sizes. Diffusion coefficient is inversely proportional to the square root of molecular weight. Molecular weight of TA (101.19) is higher than those of DE (73.14) and t-BA (73.14). Moreover, t-BA, a primary amine, might be able to form stronger ionic bonding with benzoate ion than DE and TA that 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 attribute to the higher average velocity of this ion-pair when collisions take place yielding higher diffusion coefficient.

As shown in Fig.2, the concentration of benzoic acid in B-DE is lower than that in B-TA. The mean free path of B-DE ion-pair should attribute to the higher values of diffusion coefficient of B-DE compare to B-TA. The diffusion processes of these ion-pairs however were set in different conditions. Whereas the diffusion of B-DE took place in association with PG as a solvent, the diffusion of B-TA was purely a random walk of the ion-pair through the membrane. Without solvent, ion-pair of B-TA might be more tightly bound to each other than that of B-DE. The smaller size of B-TA might offset its high concentration of free acid, the diffusion coefficient of B-TA is thus not different from that of B-TA.

Acknowledgements

References

Castellan, G. W., 1983. Physical Chemistry, 3rd Ed., Addison-Wesley, California, pp. 745-757.

Dias, M., Raghavan, S. L., Hadgraft, J., 2001. ATR-FTIR spectroscopic investigations on the effect of solvents on the permeation of benzoic acid and salicylic acid through silicone membranes. Int. J. Pharm., 216, 51–59.

Elabd, Y. A., Sloan, J. M., Barbari, T. A., 2000. Diffusion of acetonitrile in conformational isomers of an H12MDI polyurethane. Polymer, 41, 2203-2212.

Gelder, J. V., Witvrouw, M., Pannecouque, C., Henson, G., Bridger, G., Naesens, L., Clercq, E. D., Annaert, P., Shafiee, M., Mooter, G. V. D., Kinget, R., Augustijns, P., 1999. Evaluation of the potential of ion pair formation to improve the oral absorption of two potent antiviral compounds, AMD3100 and PMPA. Int. J. Pharm., 186, 127-136.

Hanh, B. D., Neubert, R. H. H., Wartewig, S., 2000. Investigation of drug release from suspension using FTIR-ATR technique: part I. Determination of effective diffusion coefficient of drugs. Int. J. Pharm., 204, 145-150.

Hatanaka, T., Kamon, T., Morigaki. S., Katayama, K., Koizumi, T., 2000. Ion pair skin transport of a zwitterionic drug, cephalexin. J. Control. Release., 66, 63-71.

Kraus, C. A., 1956. The ion pair concept: Its evolution and some applications. J. Phys. Chem., 60, 129–141.

Neubert, R., 1989. Ion pair transport across membranes. Pharm. Res. 6, 743-747.

Neubert, R., Fischer, S., 1991. Influence of lipophilic counter ions on the transport of ionizable hydrophilic drugs. J. Pharm. Pharmacol., 43, 204-206.

Pardo, A., Shiri, Y., Cohen, S., 1992. Kinetics of transdermal penetration of an organic ion pair: physostigmine salicylate. J. Pharm. Sci., 81, 990-995.

Pellett, M. A., Watkinson, A. C., Hadgraft, J., Brain, K. R., 1997a. Comaparison of permeability data from traditional diffusion cells and ATR-FTIR spectroscopy Part I. Synthetic membranes. Int. J. Pharm., 154, 205-215.

Pellet, M. A., Watkinson, A. C., Hadgraft, J., Brain, K. R., 1997b. Comparison of permeability data from traditional diffusion cells and ATR-FTIR spectroscopy. Part II. Determination of diffusional pathlengths in synthetic membranes and human stratum corenum, Int. J. Pharm. 154, 217-227.

Quintanar-Guerrero, D., Allemann, E., Fessi, H., Doelker, E., 1997. Applications of the ion-pair concept to hydrophilic substances with special emphasis on peptides, Pharm. Res. 14, 119-127.

Sammon, C., Yarwood, J., Everall, N., 2000. A FTIR-ATR study of liquid diffusion processes in PET films: comparison of water with simple alcohols, Polymer. 41, 2521-2523.

Silverstein, R. M., Bassler, G. C., Morrill, T. C., 1991. Spectrometric identification of organic compounds, 5th Ed., John Wiley & Sons, New York, p. 118.

Valenta, C., Siman, U., Kratzel, M., Hadgraft, J., 2000. The dermal delivery of lignocaine: influence of ion pairing. Int. J. Pharm., 197, 77-85.

Watkinson, A. C., Joubin, H., Green, D. M., Brain, K. R., Hadgraft, J., 1995. The influence of vehicle on permeation from saturated solutions. Int. J. Pharm., 12, 27-36.

Wurster, D. E., Buraphacheep, V., Patel, J. M., 1993. The determination of diffusion coefficients in semisolids by Fourier transform infrared (FTIR) spectroscopy. Pharm. Res., 10, 616 620.

Zhou, H., Lengsfeld, C., Claffey, D. J., Ruth, J. A., Hybertson, B., Randolph, T. W., NG, K., Manning, M. C., 2002. Hydrophobic ion pairing of isoniazid using a prodrug approach, J. Pharm. Sci., 90, 1502-1511.

Figure legends
Fig. 1. FTIR spectra of PG and solutions of sodium benzoate and benzoic acid in PG.

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

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 and plateau for benzoic acid

Membrane/Compounds	$D \times 10^8$ (cm^2/sec)	Plateau level (A_0)
Untreated		
Benzoic acid from B-DE	8.94 ± 1.12	2.67 ± 1.43
Benzoic acid from B-TA	3.61 ± 1.83	22.56 ± 3.84
Benzoic acid	11.88 ± 4.17	15.02 ± 1.33
Presaturated with PG		
Benzoic acid from B-DE	8.81 ± 1.48	4.37 ± 1.41
Benzoic acid from B-TA	2.88 ± 0.08	21.31 ± 3.40
Benzoic acid	11.64 ± 3.60	16.64 ± 3.12

Table 2
Values of diffusion coefficients and plateau for benzoate anion

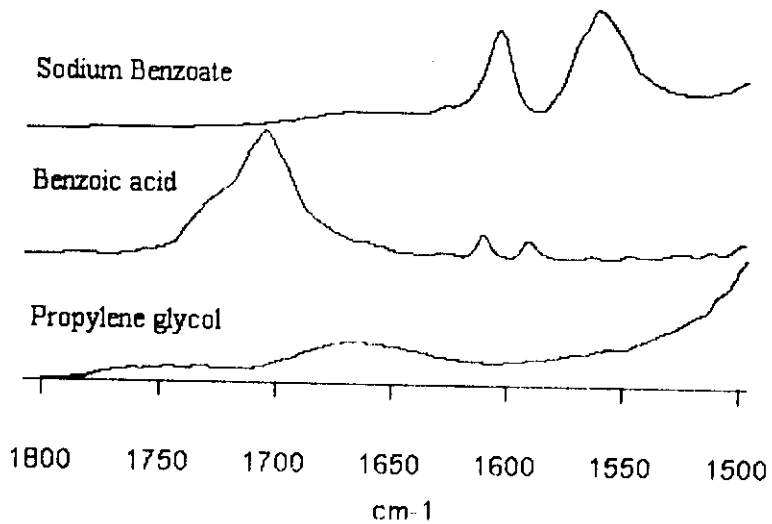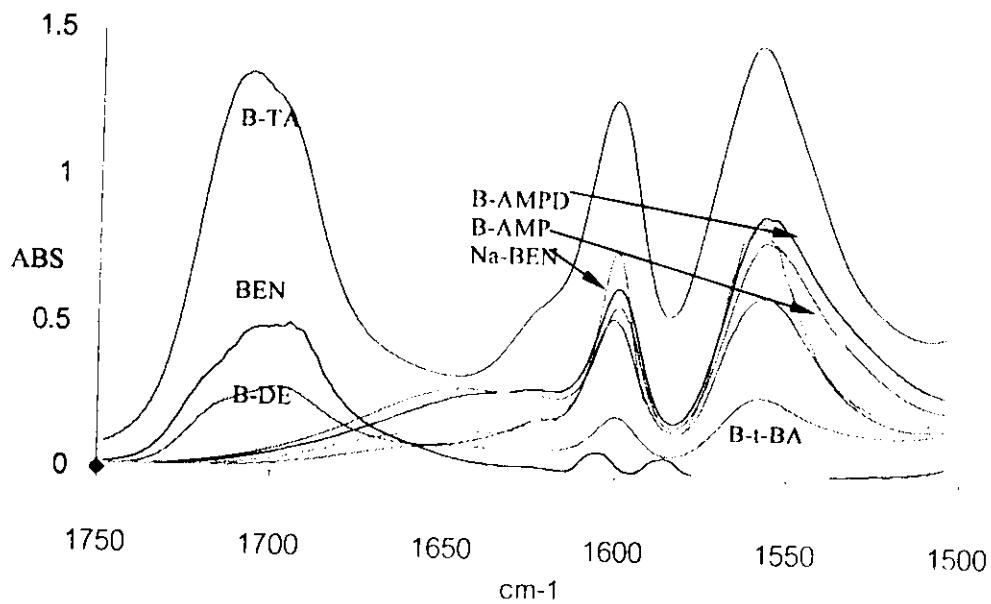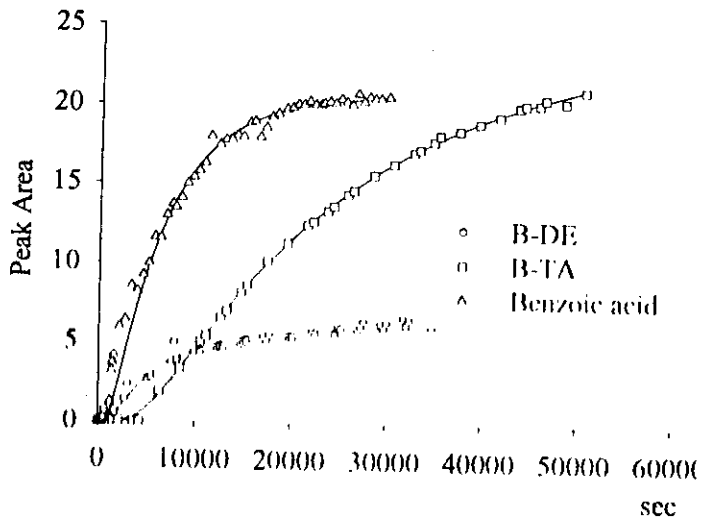| Membrane/Compounds	$D \times 10^{-8}$ (cm^2/sec)	Plateau level (A_0)
Untreated		
Benzoate from B-DE	2.99 ± 0.50	23.22 ± 2.28
Benzoate from B-TA	3.49 ± 1.02	43.56 ± 12.58
Benzoate from B-*t*-BA	6.97 ± 0.81	1.63 ± 0.07
Presaturated with PG		
Benzoate from B-DE	2.23 ± 0.05	33.70 ± 1.32
Benzoate from B-TA	2.28 ± 0.31	45.69 ± 4.44
Benzoate from B-*t*-BA	5.66 ± 0.78	2.19 ± 0.27

Sodium Benzoate

Benzoic acid

Propylene glycol

1800 1750 1700 1650 1600 1550 1500

cm-1

Fig 1

Fig. 2

Fig. 3