Quantitative Evaluation of Aqueous Isopropanol Enhancement of Skin Flux of Terbutaline (Sulfate). I. Ion Associations and Species Equilibria in the Formulation

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It has been reported previously that saturated terbutaline sulfate in aqueous isopropanol significantly enhances the terbutaline flux through human skin *in vitro*. This paper demonstrates that the effect of isopropanol on the permeant species in the formulation contributes to the flux enhancement. This demonstration is based on studies involving measurements of conductivity and pK_a as well as NMR spectroscopy in isopropanol–water mixtures. Increasing isopropanol concentration inhibits the proton dissociation of terbutaline and results in the ion associations between the protonated terbutaline and its counterion, sulfate anion. The species present in the formulation include protonated terbutaline, the negatively charged terbutaline–sulfate (1:1) ion pair, and the neutral terbutaline–sulfate (2:1) ion triplet. The results of the studies provide the basis for a quantitative evaluation of the species equilibria in solutions of terbutaline sulfate. The saturated terbutaline sulfate in 60% isopropanol produces the maximum concentration of the neutral ion triplet. This result is almost parallel to the terbutaline skin flux, which maximized at 60–80% isopropanol.

KEY WORDS: ionized terbutaline; counterion sulfate; aqueous isopropanol; ion associations and species equilibria; skin transport enhancement.

INTRODUCTION

Many pharmaceutical compounds are ionic salts or weak acids and bases which are ionized at physiological pH. Especially for those ionic drugs with an extensive liver first-pass effect and a short half-life, transdermal delivery is of great practical interest. Terbutaline, an established β_2-adrenoceptor agonist, has a very low bioavailability in oral and inhalation administrations (1). Like many other ionic compounds, when terbutaline is applied transdermally, its skin flux is inherently quite low (2,3). Recently, a formulation comprised of saturated terbutaline along with a counterion, sulfate, in aqueous isopropanol has been demonstrated to significantly enhance terbutaline flux through human skin *in vitro* (3,4). The maximum terbutaline flux, about 10–20 times greater than that in aqueous solution, was observed in 60–80% (v/v) isopropanol solutions. This enhancement was attributed to combined isopropanol effects on both the equilibria of permeant species in the formulation and the diffusion pathway barriers of stratum corneum (3,4). The solvent effects on the stratum corneum have been identified (2). However, the concept of cosolvents affecting the permeant species with an ionic equilibrium in the formulation has been generally ignored.

In solution chemistry, a considerable volume of literature has accumulated on the effects of solvent mixtures on ionic processes (5–8). The behavior of electrolyte solutions is determined by three factors in decreasing energetic contributions: ion–ion interaction, ion–solvent interaction, and solvent–solvent interaction. Unlike liquid water having a unique three-dimensional hydrogen-bonded structure, simple and monofunctional alcohols associate via short-lived polymer chains of hydrogen-bonded molecules (9). The water-miscible lower alcohols behave as "structure breakers" on water in water-rich regions and as hydrophobic "structure makers" in the alcohol-rich regions. Table I summarizes several important properties of isopropanol–water mixtures. Of these, the dielectric constant, as the most-used guide to solvent suitability and dissolving power, varies from 78.5 (in water) to 18 (in isopropanol). With ionic solutes, solvents of high dielectric constant facilitate dissolution by separating and solvating the ions (electrostatic effect). The hydrogen-bonding capability decreases about 70% on going from water to pure isopropanol (10). In short, the isopropanol–water mixtures affect the electrostatic aspects as the dielectric constant decreases from water to isopropanol. In addition to functioning as a dielectric medium, the proton-accepting tendency of the mixture is reduced as the water concentration/activity decreases.

As the first part of the study, this paper evaluates the base/acid ionization of terbutaline and ion association between the protonated terbutaline and the counterion sulfate anion in the isopropanol–water mixtures. The species equilibria and distribution are quantitatively determined. For comparison, another catecholamine, salbutamol (without counterion) is included. The chemical structures of terbutaline and salbutamol are shown in Fig. 1.

MATERIALS AND METHODS

Materials

Terbutaline hemisulfate salt and salbutamol free base (Sigma Chemical Company, St. Louis, MO) and HPLC-grade isopropanol and HPLC-grade phosphoric acid (85%, Fisher Scientific, Fair Lawn, NJ) were obtained commercially. HPLC-grade acetonitrile and potassium phosphate monobasic (J. T. Baker Inc., Phillipsburg, NJ), sodium gluconate, boric acid, sodium tetraborate decahydrate, glycerin, and *n*-butanol (Sigma Chemical Company) were used to prepare mobile phases for high-performance liquid chromatography (HPLC). Sodium chloride (Mallinckrodt Inc., Paris, KY) was used to make the calibration standard in the conductance measurements. Calibrating buffer solutions (pH 4, 7, and 10) were obtained from Baxter Healthcare Corporation (McGaw Park, IL). Deionized distilled water

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Table I. Some Properties of Isopropanol (IPA)–Water Mixtures as a Function of Isopropanol Concentration

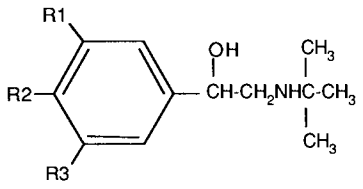
IPA (wt%)	IPA (vol%)[a]	X_{IPA}[b]	ρ (kg/L)[c]	η[c]	ε[d]	C_{H_2O} (mol/L)[e]
0	0	0	1	1	78.5	55.6
10	12.4	0.271	0.982	1.63	71.4	50.9
20	24.1	0.455	0.970	2.55	64.1	45.8
30	35.3	0.589	0.952	3.22	56.9	40.9
40	45.9	0.690	0.930	3.63	49.7	35.8
50	56.0	0.769	0.907	3.78	42.5	30.6
60	65.6	0.834	0.882	3.72	35.3	25.2
70	74.8	0.886	0.858	3.46	28.7	19.4
80	83.6	0.930	0.834	3.07	23.7	13.3
90	92.0	0.968	0.810	2.61	20.4	6.86
100	100	1	0.785	2.43	18.0	0

[a] Volume (%) of isopropanol, calculated as vol% = $100/\{(\rho_{IPA}/\rho_{H_2O})[(100/wt\%) - 1] + 1\}$.
[b] Mole fraction of isopropanol, calculated as $X_{IPA} = 1/\{(MW_{H_2O}/MW_{IPA})[(100/wt\%) - 1] + 1\}$, where MW represents molecular weight.
[c] ρ is the relative density (kg/L) and η is the relative viscosity (absolute viscosity ratio of a solution to water) at 20°C (19).
[d] Dielectric constant at 25°C (20).
[e] Water molar concentration, calculated as $C_{H_2O} = (100 - wt\%)/(0.1\ MW_{H_2O}\rho)$.

(Milli-Q Water System, Waters, Morrison, NJ) was used to prepare aqueous solutions and to rinse all glassware before use.

Potentiometric Determination of pK_a

Using very dilute solutions (the order of 10^{-4} *M*), the weak base/acid ionization constants (pK_a) were determined for terbutaline and salbutamol as a function of isopropanol concentration at 25 ± 1°C. Potentiometric titrations were carried out with an automatic titrator (DL40 Memotitrator, Mettler Instrument Corp., Hightstown, NJ). The detailed procedure was previously reported (11). For each titration the initial solution, with a total volume of about 50 ml, contained about 10 mg of either terbutaline sulfate or salbutamol, 5 ml of 1 *M* NaCl, and 0.1 ml of 0.1 *N* HCl. It was necessary to allow 1 min for equilibrium to be established prior to each new titrant addition in aqueous isopropanol solutions.



catecholamine	R1	R2	R3
Terbutaline	OH	H	OH
Salbutamol	CH_2OH	OH	H

Fig. 1. Chemical structures of the two catecholamines: terbutaline and salbutamol.

Conductivity Measurement

Solution conductivity ($\Omega^{-1} \cdot cm^{-1}$, Siemens/cm, or S/cm) was measured at room temperature and the temperature was corrected to the reference temperature of 25°C. A CDM83 conductivity meter (Radiometer America Inc., Cleveland, OH) was used with a T801 temperature sensor and either a CDC304 immersion cell with a cell constant of 1.00 cm^{-1} or a CDC314 flow and pipette cell with a cell constant of 3.16 cm^{-1}. Sodium chloride solution, 0.05% (w/w), was used as a reference, having a conductivity of 1015 μS/cm at 25°C. The apparent pH of each solution was also measured. The conductivity of the solvent mixture was in all cases subtracted from the observed conductivity in order to obtain the conductivity due to the dissolved solute. Equivalent conductivity, Λ, was calculated by the relationship

$$\Lambda = \frac{1000k}{c} \quad (1)$$

where k is specific conductance and c is the normal concentration of solute.

Nitrogen-15 Nuclear Magnetic Resonance (^{15}N-NMR) Spectroscopy

^{15}N-NMR spectra were obtained on a Varian XL-400 spectrometer (Varian Associates, Palo Alto, CA) operating at 40.5 MHz. Samples were contained in a 10-mm NMR tube fitted with a Teflon plug. All spectra were referenced to saturated ammonium chloride in D_2O (27.34 ppm) contained in a concentric capillary. A sweep width of 12315.3 Hz, a pulse width of 15 μsec (58°), and a 3-sec relaxation delay were used. Line broadening of 10 Hz was applied prior to Fourier transformation. Samples were run at 32°C with Waltz decoupling to avoid excessive heating of the sample. Aqueous samples of terbutaline sulfate and of salbutamol with isopropanol at 0, 20, 50, and 80% (v/v) concentrations were examined.

Solubility Measurement

The total solubility of each solute (terbutaline sulfate and salbutamol) was determined in isopropanol–water mixtures with varying isopropanol concentrations. An excess amount of the solute was introduced into 50-ml centrifuge tubes and sealed. The tubes were shaken at 25 ± 1°C in a thermostatically controlled water bath (Versa-Bath Model 236, Fisher Scientific) and then centrifuged with a centrifuge (Model 225, Fisher Scientific) at 7000 rpm for 5 min. The clear supernatant solution was appropriately diluted and analyzed by HPLC. The saturated concentration was determined after 24, 48, and 72 hr of equilibrium for the two solutes and all systems tested reached saturation within 24 hr. The apparent pH of each solution was also measured.

HPLC Analytical Methods

Terbutaline and salbutamol were assayed by reverse-phase HPLC with a Model 712 WISP, Model 510 programmable solvent pump (Waters, Milford, MA) and a Spectroflow 783 programmable absorbance detector (KRATOS Analytical Instruments, Bristol, CT). A 4.6-mm-ID × 15-cm

Zorbax Phenyl column (Du Pont Instruments, Chadds Ford, PA) was used at a wavelength of 212 nm for terbutaline and 280 nm for salbutamol, respectively. The mobile phase was made of a water–acetonitrile mixture (85:15, by volume) with 0.01 *M* potassium monobasic phosphate, adjusted by phosphoric acid to pH 3.1, and then filtered and degassed. The flow rate of the mobile phase was 1.5 ml/min and the retention times were 2.2 min for terbutaline and 2.4 min for salbutamol. Sulfate was assayed by HPLC ion chromatography with a Model 712 WISP, a Model 510 programmable solvent pump, and a Model 431 conductivity detector (Waters). A 4.6-mm-ID × 5-cm IC-Pak A anion column (Waters) was used. Borate/gluconate eluent served as the mobile phase, which consisted of 0.08% (g/ml) sodium gluconate, 0.09% (g/ml) boric acid, 0.125% (g/ml) sodium tetraborate decahydrate, 1.25% (ml/ml) glycerin, 2% (ml/ml) *n*-butanol, 12% (ml/ml) acetonitrile, and about 80% (ml/ml) water. After being filtered and degassed, the mobile phase has a pH of 8.0 and a conductance of 1100 μS. The flow rate of the mobile phase was 1.0 ml/min and the retention time was 6.0 min.

RESULTS AND DISCUSSION

Apparent pK_a and pH in Isopropanol–Water Mixtures

Since the values of both pK_a (acidity/basicity) and pH measured in part- or nonaqueous solutions are not entirely predictable, we consider the difference between the apparent pK_a and the apparent pH, which is equal to that between the intrinsic (thermodynamic) pK_a and the intrinsic pH (12):

$$(pK_a - pH)_{intrinsic} = (pK_a - pH)_{apparent} \quad (2)$$

That is, although the apparent pK_a values obtained in the presence of alcohols do not represent the true thermodynamic values due to the potentiometric titration curves based on glass electrodes readings, the errors introduced are compensated by the fact that the apparent pH readings for the same solutions were also carried out in the presence of alcohols.

Terbutaline and salbutamol are weak bases and even weaker acids in water (Fig. 1). The effect of isopropanol concentration on the apparent pK_a values has been demonstrated (Fig. 2A). The pK_{a2} (for the first phenol) is significantly raised with increasing isopropanol concentration, while pK_{a1} (for the protonated β-hydroxyethylamine) is slightly lowered above 60% isopropanol concentrations. Similar phenomena have been found for other weak acids/bases in solutions of isopropanol (13). The results are consistent with the fact that (a) the pK_a values are dependent on the solvent, which functions both as a proton acceptor/donor and as a dielectric medium (14), and (b) the increased isopropanol concentration results in a decrease both in proton-accepting tendency (water activity) and in the medium dielectric constant (Table I). Lowering the dielectric constant of the medium, for example, would be expected to have a much larger effect on the electrostatic part of the free energy change with weak acid than with weak base. In the former case, dissociation involves the creation of electric charges in a medium of lowered dielectric constant, whereas the latter is an isoelectric process. The values of apparent solution pH for the two compounds are shown in Fig. 2B.

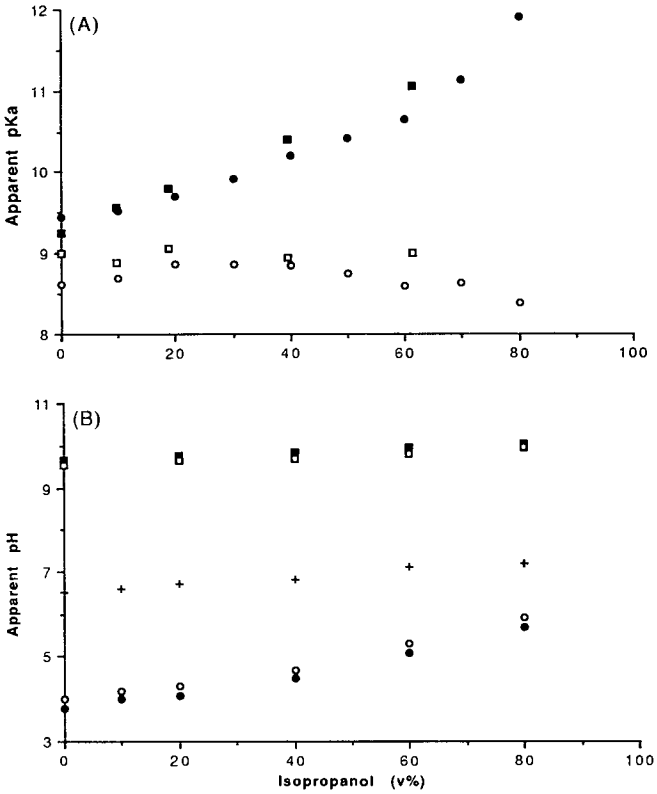


Fig. 2. (A) Apparent pK_a (acid/base ionization constant) of terbutaline (circles) and of salbutamol (squares) in isopropanol–water mixtures at 25 ± 1°C. pK_{a1} (open symbols) is for the protonated β-hydroxyethylamine and pK_{a2} (filled symbols) for the phenol. pK_{a3} for the second phenol of terbutaline, 11.0 in pure water, was not determined in isopropanol–water mixtures. The variations of data points are <5% (n = 3). (B) Apparent pH of isopropanol–water mixtures saturated (filled symbols) and 10% saturated (open symbols) with terbutaline sulfate (circles) and with salbutamol (squares) at 25 ± 1°C. The apparent pH of the solvent is also shown with symbols (+). The variations of all data points are within ±0.1 unit (n = 4).

To assess the relative contributions of electrostatic and nonelectrostatic effects on the increase in pK_{a2} upon addition of isopropanol, we may plot (pK_{a2} − pH) values against the reciprocal of the dielectric constant (1/ε) (15). As shown in Fig. 3, the (pK_{a2} − pH) values increase linearly with the reciprocal of the dielectric constant (1/ε). The three solute/solvent compositions (terbutaline in aqueous isopropanol, terbutaline in aqueous ethanol, and salbutamol in aqueous isopropanol) have the same trend quantitatively. Similar results were reported with benzoic acid in isopropanol–water mixtures (13). It is clear that the change in dielectric constant is the primary effect accounting for the variation of pK_{a2} values.

Ion Associations in Isopropanol–Water Mixtures

In solutions which contain ionic species, the conductivity is dependent largely on the population of ions. The process of charge neutralization by ion associations can be observed via conductivity measurements. As shown in Fig. 4, terbutaline sulfate and salbutamol showed the same trend; either increasing isopropanol concentration or decreasing

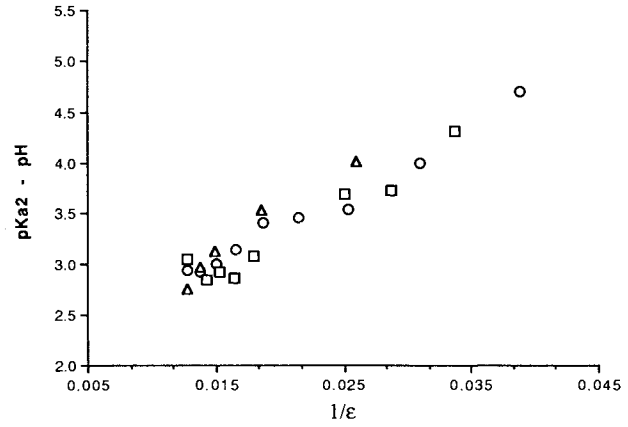


Fig. 3. Dependence of the apparent pK_{a2} (acidity) on the solvent dielectric constant for terbutaline in isopropanol–water mixtures (○), for salbutamol in isopropanol–water mixtures (△), and for terbutaline in ethanol–water mixtures (□).

solute concentration substantially reduced the solution conductivity.

The solvent affects conductance through its dielectric constant, its viscosity, and its specific interaction with ions (14). The ion–solvent interactions are reasonably assumed to be the same in all ranges of isopropanol–water mixtures because of the relatively large size of the catecholamine ions compared to that of water and isopropanol. Walden's rule, that the product of viscosity (η) and equivalent conductivity (Λ) is constant, is obeyed for large ions in a wide range of similar mixed solvents (7). Figure 5 shows the plots of the product of the total equivalent conductivity and viscosity (Table I) vs the isopropanol concentration for both salbutamol and terbutaline sulfate in their very diluted solutions. A continuous decrease in the Walden's product for salbutamol (Fig. 5B) is consistent with the (pK_a − pH) values (Fig. 2), suggesting an increased fraction of uncharged species or zwitterion at higher isopropanol concentrations. Walden's product for terbutaline sulfate decreases significantly for mixtures containing greater than 60% isopropanol (Fig. 5A). This result indicates the formation of neutral species caused by a lower medium dielectric constant in 60–80% isopropanol.

There is little doubt that ion association commonly occurs in electrolyte solutions, especially with a relatively low dielectric medium. The significant decrease in K_{a_2} (acidity of the phenol) of the two catecholamines with increasing isopropanol concentration demonstrates ion association between the catecholamine anion and the smallest ion, the proton (Figs. 2 and 3). These increased ion associations have also been found with several other drug salts in aqueous methanol, aqueous ethanol, and other mixed solvents (8). ^{15}N-NMR spectral data for terbutaline sulfate in isopropanol–water mixtures are shown in Fig. 6. The results indi-

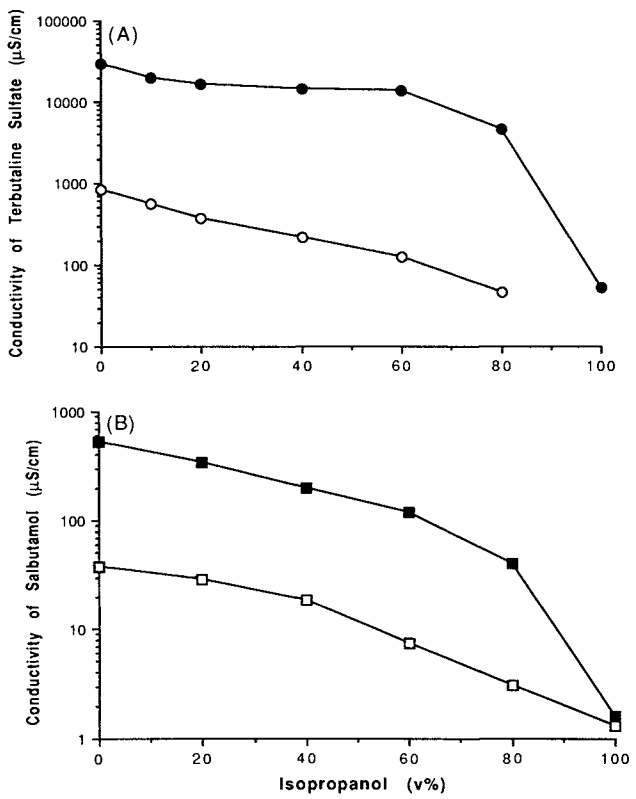


Fig. 4. Conductivity of terbutaline sulfate (A) and of salbutamol (B) in isopropanol–water mixtures at 25 ± 1°C. The saturated solution (filled symbols) and the diluted solution (open symbols; 3 mg/ml for terbutaline sulfate and 1 mg/ml for salbutamol). The variations of all data points are <5% (n = 3).

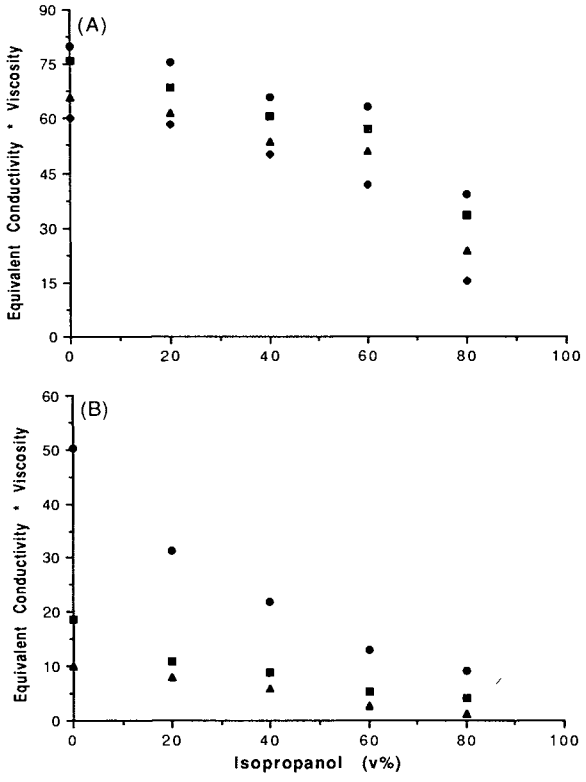


Fig. 5. The product of total equivalent conductivity and viscosity in the isopropanol–water mixtures at 25°C. Very dilute solutions are presented in this figure. The variations of all data points are <7% (n = 3). (A) Terbutaline sulfate: 0.04 mg/ml (●), 0.2 mg/ml (■), 1.0 mg/ml (▲), and 5.0 mg/ml (◆). (B) Salbutamol: 0.04 mg/ml (●), 0.2 mg/ml (■), and 1.0 mg/ml (▲).

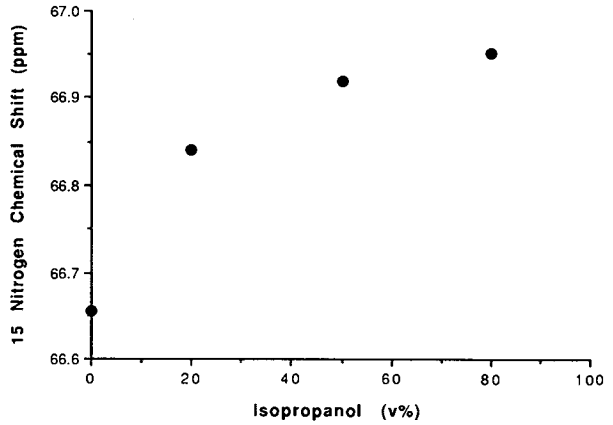


Fig. 6. ^{15}N chemical shift of terbutaline sulfate in isopropanol–water mixtures. Near-saturated solution was used for each sample.

cate a downfield shift of 0.3 ppm for the nitrogen resonance of terbutaline sulfate on increasing the isopropanol concentration. This deshielding influence on the nitrogen chemical shift suggests a stronger association between the acid proton and the amino nitrogen with increasing isopropanol concentration. These effects support greater ion pairing in isopropanol since stronger association between the acid proton and the amino nitrogen would be stabilized by tighter association with the sulfate anion in the lower dielectric medium. For salbutamol (in the absence of the counterion), however, an observed downfield shift is attributed to the decreased hydrogen bonding of the amine proton with the solvent lone pairs with increasing isopropanol concentration. Enhanced ion associations of saturated aliphatic amines and their hydrochlorides in methanol–water solutions have been reported in an earlier ^{15}N-NMR study (16). The NMR data may be explained by an ion association model proposed by Fuoss (17). Accounting for the ion–solvent interactions, the model introduced the concept of an intermediate transition state, the solvent-separated pairs ($A^+ \ldots B^-$), between unpaired ions, A^+ and B^-, and contact pairs, $[A^+B^-]^0$.

$$A^+ + B^- \rightleftharpoons (A^+ \ldots B^-) \rightleftharpoons [A^+B^-]^0 \qquad (3)$$

The first step describes the formation of contact pairs by a series of interchanges of sites between solvent molecules and ions and the second step depends on short-range ion–solvent and cation–anion interactions. However, the relaxation kinetics of ion associations indicates that the second step is the rate-limiting step (18).

For terbutaline sulfate solution (Fig. 2B) where the concentration of the hydrosulfate ion (HSO_4^-) is negligible, the ion associations can be regarded as a stepwise process involving equilibria which may be written as

$$T + H^+ \overset{K_0}{\rightleftharpoons} TH^+ \qquad (4)$$

$$TH^+ + S^= \overset{K_1}{\rightleftharpoons} [TH^+S^=]^- \qquad (5)$$

$$[TH^+S^=]^- + TH^+ \overset{K_2}{\rightleftharpoons} [(TH^+)_2S^=]^0 \qquad (6)$$

where T, H^+, TH^+, $S^=$, $[TH^+S^=]^-$, and $[(TH^+)_2S^=]^0$ represent the uncharged and zwitterionic terbutaline, the proton, the protonated terbutaline, the sulfate anion, the terbutaline–sulfate (1:1) ion pair anion, and the neutral terbutaline–sulfate (2:1) ion triplet, respectively. The ion association constants for each step are K_0 (reverse of K_{a_2}), K_1, and K_2. An attempt is presented to estimate the ion association constants (K_1 and K_2) by a combined approach incorporating chemical equilibria–limiting equivalent conductivity–electrostatic effects (Appendix, A). The values of K_1 and K_2 are shown in Fig. 7. As expected, the factors which increase the ion association constant are (a) low dielectric constant, (b) small ionic radii, and (c) large charge valence.

Species Equilibria and Distribution in Isopropanol–Water Mixtures

Figure 8 presents the total solubility (saturated concentration) of salbutamol and of terbutaline sulfate in the isopropanol–water mixtures. The results indicate that the greater the dielectric constant of the isopropanol–water mixture, the greater is its ability to dissolve terbutaline sulfate electrolytes. An exact 2:1 (terbutaline/sulfate) molar concentration ratio is determined through all the range of isopropanol–water mixtures saturated with terbutaline sulfate. A large decrease in terbutaline solubility is observed when the isopropanol concentration is 60% and higher. In contrast, the solubility of salbutamol (without sulfate) gradually increases with increasing isopropanol concentration. We now consider the differences between salbutamol and terbutaline sulfate with respect to the distribution of species in solution.

For salbutamol in alkaline solution (Fig. 2B), the mole fraction for each species ($f_{HA\text{-}BH^+}$, $f_{HA\text{-}B}$, and $f_{^-A\text{-}B}$) can be written in terms of the (pK_a − pH) values:

$$f_{HA-BH^+} = \frac{1}{1 + 10^{-(pK_{a1}-pH)} + 10^{-(pK_{a1}-pH)-(pK_{a2}-pH)}} \qquad (7)$$

$$f_{HA-B} = \frac{10^{-(pK_{a1}-pH)}}{1 + 10^{-(pK_{a1}-pH)} + 10^{-(pK_{a1}-pH)-(pK_{a2}-pH)}} \qquad (8)$$

$$f_{^-A-B} = \frac{10^{-(pK_{a1}-pH)-(pK_{a2}-pH)}}{1 + 10^{-(pK_{a1}-pH)} + 10^{-(pK_{a1}-pH)-(pK_{a2}-pH)}} \qquad (9)$$

where HA-BH^+, HA-B, and $^-$A-B are the cation, the uncharged and zwitterionic salbutamol, and the anion of salb-

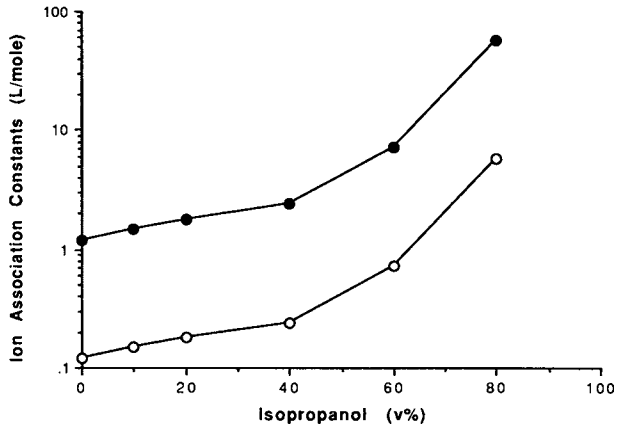


Fig. 7. Ion association constants, K_1 (filled symbols) and K_2 (open symbols), calculated as a function of isopropanol concentration.

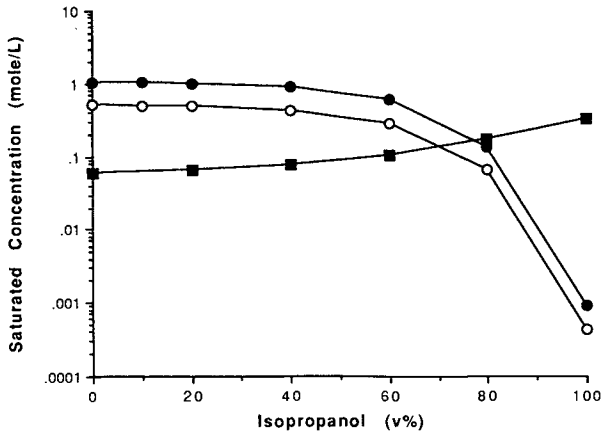


Fig. 8. Concentrations of terbutaline (●) and sulfate (○) of saturated terbutaline sulfate and of saturated salbutamol (■) in isopropanol–water mixtures at 25 ± 1°C. The variations of all data points are <10% (n = 3 for terbutaline sulfate and n = 2 for salbutamol).

utamol, respectively. The species distribution profiles in isopropanol–water mixtures were calculated by Eqs. (7)–(9) with correction of the ionic strength (similar to Appendix, B). As shown in Fig. 9 for both saturated and 10% saturated salbutamol, the species fractions are independent of the salbutamol concentration but vary with the (pK_a − pH) values. It is obvious that increasing isopropanol concentration favors the neutral and zwitterionic species in terms of the mole fraction (f_{HA-B}) as well as the mole concentration, the product of f_{HA-B} and the total salbutamol presented (Fig. 8).

For terbutaline sulfate in the acidic solution (Fig. 2B), where the three-step equilibria are described in Eqs. (4)–(6), the calculation procedure is described in the Appendix (B). Species concentration profiles for both saturated and 10% saturated terbutaline sulfate are shown in Fig. 10. The acidic pH and the amine pK_a result in a negligible (<0.08%) amount of the unchanged and zwitterionic species. The ion associations between the protonated terbutaline and the sulfate anion (either the 1:1 ion pair or the 2:1 ion triplet) are strongly dependent on both the terbutaline sulfate concentration and the isopropanol concentration. In the 10% saturated case, there are insignificant 2:1 ion triplets and about 5% (in water) to 15% (in 80% isopropanol) of the 1:1 ion pair. In the case of saturated terbutaline sulfate, however, the 1:1 ion pair is doubled and the 2:1 ion triplet is significantly formed at 60% and higher isopropanol concentrations. For each species, the effective concentration can be calculated combining the mole fraction and the total terbutaline solubility. For example, the maximum concentration of the 2:1 ion triplet occurs at about 60% isopropanol.

We conclude that, as a part of the isopropanol enhancement on skin flux of terbutaline (sulfate), isopropanol has two important effects with regard to the ionic interactions of the permeant species in the formulation. First, increasing isopropanol concentration inhibits the proton dissociation of

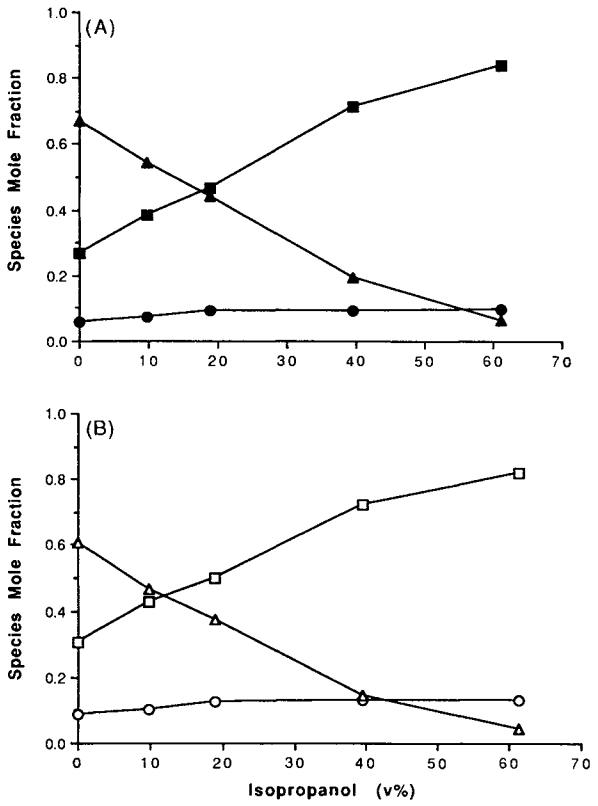


Fig. 9. Calculated mole fractions of salbutamol species in isopropanol–water mixtures for the saturated (A) and 10% saturated (B) cases: the cation [HA-BH$^+$] (triangles), the neutral and zwitterion [HA-B] (squares), and the anion [$^-$A-B] (circles).

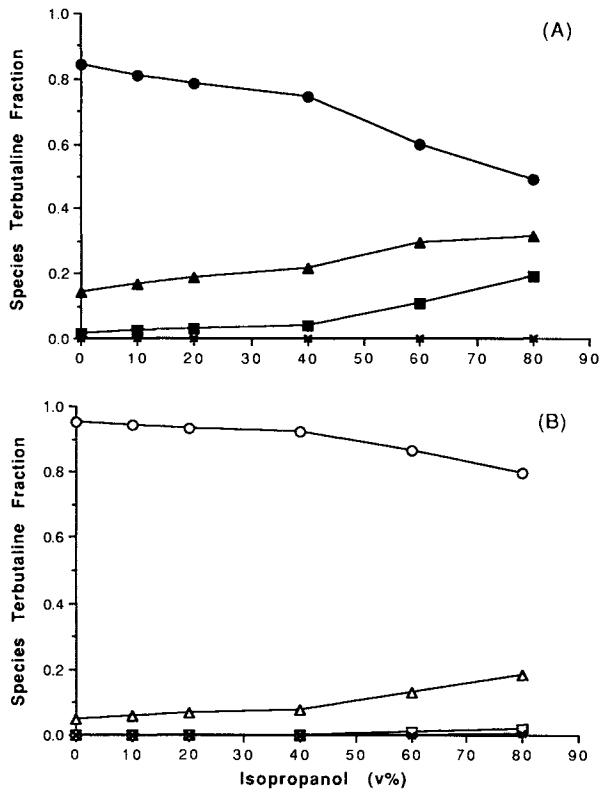


Fig. 10. Calculated mole fractions of terbutaline species in isopropanol–water mixtures for the saturated (A) and 10% saturated (B) cases: the 2:1 ion triplet [$(TH^+)_2S^=]^0$ (squares), the 1:1 ion pair [$TH^+S^=]^-$ (triangles), the protonated [TH^+] (circles), and the free base [T] (crosses).

terbutaline and salbutamol. The second role played by isopropanol is to increase ion pair formations between the protonated terbutaline and its counterion, sulfate anion. The ionic interactions and the species distribution of terbutaline sulfate in isopropanol–water mixtures have been quantitatively analyzed by considering the concepts of chemical equilibrium, limiting equivalent conductivity, and electrostatic effects. The analysis strongly supports the hypothesis that the species presented in the solution include protonated terbutaline, the negatively charged terbutaline–sulfate (1:1) ion pair, and the neutral terbutaline–sulfate (2:1) ion triplet. The saturated terbutaline sulfate in 60% isopropanol produces the maximum concentration of the neutral ion triplet. Formation of the neutral ion triplet may significantly increase the total terbutaline flux through skin. In fact, this result is almost parallel to the terbutaline skin flux, which maximized at 60–80% isopropanol. A quantitative separation of the isopropanol effect on the permeability coefficient for each species in the overall skin flux will be the subject of a future report.

APPENDIX: CALCULATIONS ON THE ION ASSOCIATION CONSTANTS AND SPECIES DISTRIBUTION OF TERBUTALINE SULFATE

The ion association constants describing species equilibria of terbutaline sulfate in Eqs. (4)–(6) are given by

$$K_0 = \frac{[TH^+]}{[T]\,[H^+]}\,\frac{\gamma_{TH^+}}{\gamma_{H^+}} \tag{A1}$$

$$K_1 = \frac{[TH^+S^=]^-}{[TH^+]\,[S^=]}\,\frac{\gamma_{[TH^+S^=]^-}}{\gamma_{TH^+}\gamma_{S^=}} \tag{A2}$$

$$K_2 = \frac{[(TH^+)_2S^=]^0}{[TH^+]\,[TH^+S^=]^-}\,\frac{1}{\gamma_{TH^+}\gamma_{[TH^+S^=]^-}} \tag{A3}$$

where brackets enclose concentrations and γ's are the corresponding activity coefficients. The neutral species, T and $[(TH^+)_2S^=]^0$ are assumed to have unity activity coefficient.

The total mole concentrations of sulfate (m_S) and of terbutaline ($m_T = 2m_S$) may be expressed as

$$m_T = [T] + [TH^+] + [TH^+S^=]^- + 2[(TH^+)_2S^=]^0 \tag{A4}$$

$$m_S = [S^=] + [TH^+S]^- + [(TH^+)_2S^=]^0 \tag{A5}$$

Equations (A1)–(A5) are used in both estimating the association constants from the conductivity data and determining the concentration for each species with the known association constants.

A. The Two-Step Ion Association Constants

A combined approach incorporating chemical equilibria–limiting equivalent conductivity–electrostatic effects is used to estimate the two-step ion association constants (K_1 and K_2). In the very diluted solution, where the values of the activity coefficient γ in Eqs. (A1)–(A3) are all unity, we may apply the mixture rule to the observed equivalent conductivity (Λ_{obs}) of terbutaline sulfate solution as

$$\Lambda_{obs} = \frac{[TH^+]}{3\,m_S}\Lambda_{TH^+} + \frac{4\,[S^=]}{3\,m_S}\Lambda_{S^=} + \frac{[TH^+S^=]^-}{3\,m_S}\Lambda_{[TH^+S^=]^-} \tag{A6}$$

where the individual limiting equivalent conductivity $\Lambda_{S^=} = 80$, $\Lambda_{TH^+} = \Lambda_{[TH^+S^=]^-} = 20$ according to Walden's rule ($\eta\Lambda$ = constant). Finally, based on the size of ion association, we assume that $K_1 = 10K_2$.

B. The Species Distributions of Terbutaline Sulfate

The species concentrations are calculated by Eqs. (A1)–(A5) with the known K_0, K_1, K_2, m_S, and apparent pH. The ionic activity coefficients (γ_z) are determined by the Davies equation (8),

$$-\log \gamma_z = Az^2\left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.2\,I\right) \tag{A7}$$

where z is the ion valency, I is the ionic strength

$$I = \frac{1}{2}\{[TH^+] + 4\,[S^=] + [TH^+S^=]^-\} \tag{A8}$$

and A is a constant dependent upon the dielectric constant (ϵ) and absolute temperature (T):

$$A = \frac{1.826 \times 10^6}{(\epsilon T)^{1.5}} \tag{A9}$$

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