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Explaining skin permeation of 2-butoxyethanol from neat and aqueous solutions

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ABSTRACT

Absorption of 2-butoxyethanol (BE) from neat and aqueous solutions of BE was measured through rat skin *in vitro* and *in vivo* and through silicone membranes. Like previous studies in human and guinea pig skin, BE flux increased proportional to BE concentration only when the weight fraction of BE (w_{BE}) < about 0.2. The flux of BE was relatively constant for $0.2 < w_{BE} < 0.8$, and it decreased dramatically for $w_{BE} > 0.8$. Experimental values of thermodynamic activity for BE and water in aqueous solutions of BE are presented. Except when the water content in the vehicle is small, skin is fully hydrated and the flux of a BE through it is proportional to the thermodynamic activity of BE. When $w_{BE} > 0.8$, there is a sharp drop in the activity-normalized BE flux through skin, which coincides with a decrease in water activity from 0.9 at $w_{BE} = 0.8$ to zero for neat BE. These observations are consistent with reduced BE flux arising from skin dehydration. From an analysis of previously published data, the activity-normalized flux of BE through hydrated human skin was determined to be 2–4 mg cm^{-2} h^{-1}, which is in reasonable agreement with predictions of its maximum flux.

1. Introduction

Although chemical permeation through skin often will increase with increasing concentration in a given vehicle, this is not always the case. Dermal absorption of a chemical will exhibit a non-linear dependence on concentration if the skin is changed, either reversibly or irreversibly, by the chemical or other components in the vehicle, or if concentration of chemical in the vehicle is not proportional to thermodynamic activity (Cussler, 1997). Thus, the thermodynamic activity of the permeating chemical must be known before it is possible to conclude that improved or reduced absorption has occurred because the skin has been changed. Non-linear relationships between thermodynamic activity and concentration reported as mass per volume or mass fraction are not restricted to non-ideal mixtures. Even for an ideal solution, concentrations will not be proportional to thermodynamic activity for all possible concentrations if the saturation concentration of chemical is larger than 10 wt% and the molecular weights of the chemical and vehicle are significantly different (see discussion in Section 2). With solutions of 2-butoxyethanol (BE) in water as an example, methods are described for calculating thermodynamic activity when solutions are or are not ideal, and for using these activity values to discern whether or not skin was changed by the solution.

Water and BE (Fig. 1) are miscible in all proportions at skin temperature, and the flux of BE across skin from human and other animals, both *in vitro* and *in vivo*, has been reported for various concentrations in water ranging from less than 1% (on a weight basis) to neat BE (Dugard et al., 1984; Jakasa et al., 2004; Johanson et al., 1988; Johanson and Fernstrom, 1986, 1988; Korinth et al., 2005; Traynor et al., 2007a,b; Wilkinson and Williams, 2002). The experimental conditions in these different studies were not the same. The volume of solution applied varied from a few microliters per square centimeter to essentially an infinite dose. Evaporation was prevented in some studies but allowed in others, and the exposure time varied from one to several hours. In many of the studies, the reported absorption rates were not steady-state values. Despite differences in the experimental procedures and variability in the data, skin permeation consistently exhibited the non-linear variation with BE concentration that is illustrated in Fig. 2, which shows the flux through human skin *in vitro* (Traynor et al., 2007a,b) and through guinea pig skin *in vivo* (Johanson and Fernstrom, 1988). The key experimental parameters for these studies are summarized in Table 1.

The effect of BE concentration on BE permeation displays three distinct behaviors, which are identified in Fig. 2 as Regions I, II and III. In dilute BE solutions, the flux increases with increasing BE concentration (Region I). At concentrations between about 20 and 80 wt%, the flux appears to be nearly unaffected by concentration change (Region II). Finally, flux from neat BE is substantially smaller

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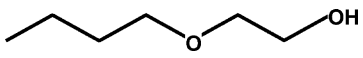

Fig. 1. Chemical structure of 2-butoxyethanol (BE).

(Region III) than from the 50% solutions and similar to that from the 10% BE solution. Similar observations have also been reported for other glycol ethers (Dugard et al., 1984; Traynor et al., 2007a; Venier et al., 2004; Wilkinson and Williams, 2002). It is important to note that the flux numbers presented in Fig. 2, which were taken without change from the original authors, may not have been at steady state. Because of this, measurements collected using the same procedures (i.e., within a study) can be meaningfully compared, but quantitative comparisons between different studies are not recommended.

Because results like those shown in Fig. 2 deviate from the expectation that dermal absorption of chemicals should increase proportionally with solute concentration in the vehicle, one paper concluded that the "applicability of Fick's law regarding the prediction of percutaneous absorption of liquid compounds ... [is] problematic" (Korinth et al., 2005). Other authors have attributed the non-linear effects of BE concentration to better partitioning of BE into hydrated stratum corneum, altered structure of lipid bilayers of the stratum corneum (Korinth et al., 2007), and skin dehydration by neat BE (Traynor et al., 2007a), although evidence supporting these hypotheses were not presented.

In this paper, thermodynamic activity data are presented, which show that aqueous solutions of BE are highly non-ideal. Except when concentrations of water are small, the expected effect of this non-ideal behavior is consistent with the concentration dependence of BE absorption observed in Fig. 2 and also in new measurements through rat skin. In contrast with skin, the concentration dependence of BE flux through silicone membranes is proportional to the thermodynamic activity of BE at all BE concentrations, including solutions containing little or no water. It follows from these observations and the thermodynamic activity of water that skin in contact with aqueous solutions of BE is fully hydrated except for solutions that are nearly pure BE, which most probably causes the skin, but not the silicone membranes, to dehydrate. This explains the dramatic reduction in BE flux through skin from neat BE compared to solutions of BE in water. Thus, the non-linear concentration variation of BE flux is not a problem with Fick's law, but with properly representing the effect of thermodynamic activity on solute permeation through skin from a non-ideal solution and recognizing that skin is changed when the concentration of water in the solution is very small.

2. Theory

According to Fick's law as it is usually written, the steady-state flux across skin of a chemical species j ($J_{ss,j}$) from a vehicle at

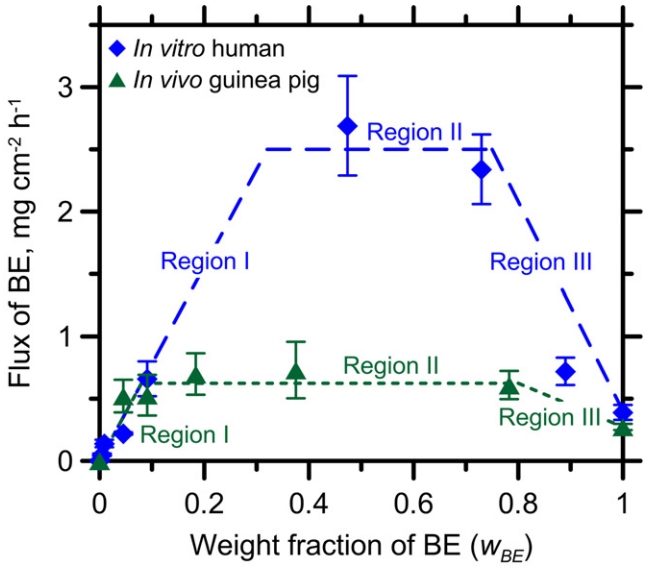

Fig. 2. Flux of BE (mean ± one standard error of mean) measured in human skin *in vitro* (from Traynor et al., 2007a,b) and guinea pig skin *in vivo* (from Johanson and Fernstrom, 1988) at various concentrations of BE in water with lines added to guide the eye.

Table 1
Summary of the key experimental parameters in the studies shown in Fig. 2.

Experimental parameter	Experiments	
Species	Human[a]	Guinea pig[b]
Type of experiment	*In vitro*	*In vivo*
Region	Breast	Back
Frozen	Yes	NA[c]
Skin thickness (μm)	320	NA
Volume applied (μL/cm^2)	200	320
Application time (h)	4	2
Occlusion	Partial; charcoal trap that allowed air passage	Complete
Sampled	Receptor solution	Blood
Sampling frequency	0.5 h for 3 h, then hourly	10–30 min
# of skin samples at each concentration	≥5	NA
# of subjects at each concentration	≥2	2; 4 for 10 vol% solution
How was flux calculated	From the approximately linear region of the cumulative penetrated-time curve; generally between 1 and 3.5 h	Uptake was estimated using first-order PK analysis of blood concentration; PK parameters (clearance and steady-state volume of distribution) were determined in separate experiments
Other notes		Solution at indicated BE concentration was applied for 2 h, then removed and after 2 h, neat BE was applied. In animals with neat BE applied in both exposures, uptake rate from second exposure was larger. Therefore, neat BE data are from the first exposure only

[a] Traynor et al. (2007a).
[b] Johanson and Fernstrom (1988).
[c] Not applicable.

concentration C_j to a receptor solution at sink conditions is represented as:

$$J_{ss,j} = P_j C_j \tag{1}$$

where P_j is the skin permeability coefficient of chemical species j, defined as

$$P_j = \frac{K_j D_j}{h} \tag{2}$$

In these equations, P_j has units of length per time consistent with units of mass per area per time for $J_{ss,j}$ and mass per volume for C_j.

The stratum corneum (SC) is the skin layer controlling skin permeation of chemicals like BE. Consequently, in Eq. (2) h is the effective thickness of the SC, K_j is the partition coefficient of the permeating solute j between the SC and the vehicle, and D_j is the effective diffusion coefficient of the permeating solute j in the SC.

The driving force for permeation, however, is thermodynamic activity, not concentration (Higuchi, 1960). Thus, Eq. (2) represents a special case in which thermodynamic activity is directly proportional to concentration. In the more general representation of Fick's law specified by Eq. (3),

$$J_{ss,j} = T_j a_j \tag{3}$$

$J_{ss,j}$ is proportional to the thermodynamic activity (a_j), which is defined to be unity at the solubility concentration of species j in the same vehicle at the same temperature. When Fick's law is written in this way, T_j is defined as:

$$T_j = \frac{S_j K_j D_j}{h} \tag{4}$$

where S_j is the solubility concentration with the same units as C_j (i.e., mass of j per volume of vehicle), and S_j and K_j are for the same vehicle. Called the *transference* by Theeuwes et al. (1976), T_j represents the maximum steady-state flux that would be observed from any vehicle saturated with respect to species j as long as neither the solute j nor other components in the vehicle changes the skin significantly. Strictly speaking, Theeuwes et al. (1976) did not include h in their definition of the transference, because they were describing permeation through synthetic membranes that could be produced in a variety of thicknesses. However, because h for the SC is reasonably constant, it is convenient to include h in the definition of the transference used here. Note that this usage is consistent with the definitions of the *permeability* coefficient in the skin literature, which includes h, and the synthetic membrane literature, which does not (Cussler, 1997).

The approach represented by Eqs. (3) and (4) have been supported by several experimental studies in which membrane permeation of a solute was from equal fractions of saturation (i.e., equal values of a_j) in different vehicles. For example, the flux of progesterone through a synthetic membrane (ethylene–vinyl acetate copolymer) was the same at equal fractions of saturation in water, Dow 360 silicone oil, and the polyethylene glycol (PEG) 600 (Theeuwes et al., 1976). Similarly, the flux of methyl paraben through polydimethylsiloxane (PDMS) membranes from saturated solutions prepared in water, propylene glycol:water mixtures, polyethylene glycol (PEG 400):water mixtures and glycerin:water mixtures were the same (Twist and Zatz, 1986). In a comparable set of experiments on human skin, the transference of benzyl alcohol, calculated from the ratio of the flux and activity, was the same from neat benzyl alcohol as from 50 mol% solutions in isophorene, butyl acetate and butanol.

When the transference coefficient for solute j is not independent of the vehicle, then this indicates that the membrane is modified by the presence of that vehicle or the solute–vehicle combination (Theeuwes et al., 1976). For example, the flux of methyl paraben through PDMS from saturated solutions (i.e., $a_j = 1$) prepared in ethanol and water mixtures containing from 0 to 100 wt% ethanol increased with increasing ethanol (i.e., T_j was not constant), because ethanol swells the PDMS membrane (Twist and Zatz, 1986). In human skin, values of T_j for benzyl alcohol from 50 mol% solutions in toluene and propylene carbonate were inconsistent with the constant value of T_j observed for neat benzyl alcohol and 50 mol% solutions in isophorene, butyl acetate and butanol (Barry et al., 1985). These results signify skin interactions with toluene and propylene carbonate. Also, as long as the membrane is not changed by the applied formulation, Eq. (3) also describes absorption rates from supersaturated solutions, for which $a_j > 1$, even though these are not thermodynamically stable (Iervolino et al., 2001).

When the chemical components in the solute–vehicle solution do not interact, the solution is said to be ideal, and the thermodynamic activity a_j of component j is equal to the mole fraction of j (x_j) relative to the saturation mole fraction of j ($x_{j,sat}$) in the solution (Prausnitz et al., 1999):

$$a_j = \frac{x_j}{x_{j,sat}} \tag{5}$$

Ideal solutions are expected when the solute and vehicle components are chemically similar, such as decane dissolved in octane. If solute j and the vehicle are miscible in all proportions, as occurs for BE in water, then $x_{j,sat}$ is equal to one.

Ideal behavior is not expected for solutions with components that interact, for example, by hydrogen bonding as occurs for solutions of BE and water. For these solutions, a_j is defined as:

$$a_j = \frac{\gamma_j x_j}{x_{j,sat}} \tag{6}$$

in which solution non-idealities are described by the activity coefficient, γ_j, which depends on x_j. Since a_j is defined to be one at the solubility limit of species j, it follows that $\gamma_j = 1$ when $x_j/x_{j,sat} = 1$. Values of γ_j can be either larger or smaller than one and the magnitude relative to one is a measure of the extent of the non-ideal effect. If the solution is ideal at all solute concentrations, then $\gamma_j = 1$. In general, a_j for non-ideal solutions must be determined experimentally, although, as described below, it is sometimes possible to estimate a_j from data measured at different conditions or in the absence of any data.

The relationship of flux to concentration C_j depends on the relationship between C_j and x_j. For a mixture of solute j in a vehicle v, C_j (which has units of mass of j per volume of vehicle) is equal to the weight fraction of solute j in the solution (w_j, which has units of mass of j per mass of vehicle) divided by the density of the vehicle. Often the density is approximately constant for all possible concentrations of solute j. For aqueous solutions of BE at 20 °C, the density varies linearly with weight fraction of BE (within a few percent) from 1.0 g/mL for pure water to 0.9 g/mL for pure BE (Chiou et al., 2010).

From a mass balance, x_j is determined to be related to w_j as:

$$x_j = w_j \frac{MW_v}{MW_j} \left(\frac{1}{1 - w_j + w_j(MW_v/MW_j)} \right) \tag{7}$$

where MW_j is the molecular weight of species j and MW_v is the average molecular weight of the vehicle. Combined with Eq. (3), the steady-state flux of solute j is then:

$$J_{ss,j} = T_j \gamma_j \frac{w_j}{w_{j,sat}} \left(\frac{1 - w_{j,sat} + w_{j,sat}(Mw_v/Mw_j)}{1 - w_j + w_j(Mw_v/Mw_j)} \right) \tag{8}$$

For vehicles with multiple components, MW_v depends on the molecular weight of the components in the vehicle and either the

Table 2
Summary of the key experimental parameters in the rat skin and silicone membrane experiments presented in this paper.

Experimental parameter	Experiments		
Species	Rat	Rat	Silicone membrane
Type of experiment	*In vitro*	*In vivo*	*In vitro*
Region	Back	Back	NA[a]
Frozen	No	NA	NA
Skin (membrane) thickness (μm)	Full thickness (~650)	NA	500
Volume applied (μL/cm^2)	200	50	200
Application time (h)	4	4	4
Occlusion	Yes	Yes	Yes
Sampled	Receptor solution	Urine, feces, carcass and exhaled CO_2	Receptor solution
Sampling frequency	Hourly	Urine, feces and CO_2 continuously during exposure time; carcass at end of exposure time	0.25 h for 1 h, then every 0.5 h
# of samples at each concentration	1 or 2 samples per rat	NA	2
# of subjects at each concentration	3–4	4–6	NA
How was flux calculated	Maximum of the flux values calculated for each sampling interval. Generally for the last sample interval	Sum of radioactivity in excreta, exhaled CO_2 and carcass divided by exposure time and area	Maximum of the flux values calculated for each sampling interval

[a] Not applicable.

mole fraction or weight fraction of all components in the vehicle, excluding the solute *j*, as defined by Eq. (9):

$$Mw_v = \sum_{k=1}^{N_v} x_k Mw_k = \frac{1}{\sum_{k=1}^{N_v} w_k/Mw_k} \tag{9}$$

in which

$$\sum_{k=1}^{N_v} x_k = \sum_{k=1}^{N_v} w_k = 1 \tag{10}$$

and N_v is the number of components in the vehicle, excluding solute *j*.

As indicated by Eq. (7), x_j is proportional to w_j at all possible concentrations of *j* when the maximum concentration of *j* in the vehicle, which is its saturation concentration ($w_{j,sat}$) is small enough that *R*, defined in terms of the absolute value of the difference in molecular weights of the solute *j* and vehicle:

$$R = w_{j,sat}\frac{|MW_j - MW_v|}{MW_j} \tag{11}$$

is small. It follows that $x_j/x_{j,sat}$ will be within about 10% of $w_j/w_{j,sat}$, if $R < 0.1$. Thus, for ideal solutions, in which $\gamma_j = 1$ at all values of w_j, thermodynamic activity will be equal to $w_j/w_{j,sat}$ as long as $w_{j,sat} < 0.1\ MW_j/|MW_j - MW_v|$. For aqueous solutions, often MW_j for the dissolved solute is large compared with the molecular weight of water, which is 18, and $MW_j/|MW_j - MW_v|$ is approximately one. In this case, $R < 0.1$ when $w_{j,sat} < 0.1$. If the molecular weights of the vehicle and solute are similar, then $|MW_j - MW_v|$ approaches zero, which makes $MW_j/|MW_j - MW_v|$ large. In this case, $x_j/x_{j,sat} \approx w_j/w_{j,sat}$ for values of $w_{j,sat}$ that are larger than 0.1. For a completely miscible system (i.e., $w_{j,sat} = 1$), $x_j/x_{j,sat} \approx w_j/w_{j,sat}$ at all concentrations if $|MW_j - MW_v|/MW_j \leq 0.1$. Thus, if MW_v differs from MW_j by more than 10%, and *j* and *v* are miscible at all proportions (i.e., $w_{j,sat} = 1$), then $J_{ss,j}$ will never be proportional to w_j over all possible concentrations, even if the solution is ideal.

3. Methods

To assess whether skin is changed by contact with neat or aqueous solutions of BE, BE absorption through rat skin was determined and compared with absorption through synthetic silicone membranes and thermodynamic activity data from the literature. Rat skin was chosen for this study because it allowed testing for different responses of *in vivo* compared with *in vitro* skin. The key experimental parameters of the absorption experiments are summarized in Table 2. Additional details of the experimental methods and thermodynamic activity calculations are described here.

3.1. Butoxyethanol absorption experiments

3.1.1. Chemicals

Radiolabeled 2-butoxy-[^{14}C-1]ethanol ([^{14}C]BE) with a specific radioactivity of 2.00 GBq/mmol (54 mCi/mmol) was purchased from Amersham Pharmacia Biotech (Buckinghamshire, England). Purity of the unlabeled BE purchased from Across (France; specified as 99%) was verified by gel permeation chromatography (GPC) to be 99.6%. Aqueous solutions at various concentrations of BE were prepared gravimetrically one day before an experiment keeping the amount of radioactivity approximately constant for all BE concentrations. On the day of an experiment, radiochemical concentration was determined on two aliquots and radiochemical purity was assessed by high performance liquid chromatography (HPLC). All other chemicals had the highest purity available. Silicone membranes (500 μm thick) were a copolymer of dimethyl and methylvinyl siloxane purchased from Perouse Plastic (France).

3.1.2. Animals

Male Sprague-Dawley rats, were obtained from Iffa Credo (Saint-Germain-sur-l'Arbresle, France). For the *in vitro* experiments, rats were about 4 weeks in age and weighed approximately 100 g; for the *in vivo* studies, the rats were 8–10 weeks in age and weighed 250–300 g. The animals were acclimatized to laboratory conditions for at least 4 days prior to initiating the studies in rooms with a 12-h light/dark cycle designed to control relative humidity at 50 ± 5% and temperature at 22 ± 1 °C. Commercial food pellets (UAR Alimentation-Villemoison, Epinay-sur-Orge, France) and tap water were available *ad libitum*. The animal facilities, experimental laboratory and animal use protocols were approved by French governmental authorities under the number C84-547-10 in accordance to the ordinance N° 10-DDP-53.

3.1.3. In vivo experiments

One day before the dose was administered, hair on the middle of the back was removed with electric clippers and a circular ring (3.5 cm^2) was glued onto the site with DELO-CA® cyanoacrylate (DELO GmbH & Co., Landsberg, Germany). The applied dose (50 μL/cm^2) of neat or aqueous solutions of [^{14}C]BE (25–90%;

V/V) was delivered inside the ring, which was covered with a Tedlar® membrane cap to prevent evaporation. Individual doses were determined by weighing the syringe (Hamilton 250 μL) before and after each administration. For each rat, the radiocarbon dose was about 3.7 MBq/kg (100 μCi/kg).

Immediately after the dose was administered, rats were placed in individual glass metabolic cages for collection of urine and feces at 4 °C. Air in the cage was pumped through a water trap, which collected [^{14}C]BE that escaped from the application site, and a CO_2 trap containing about 500 mL of CARBOSORB® (Perkin Elmer Life Sciences, Courtaboeuf, France), which collected exhaled $^{14}CO_2$. Data were excluded for any rats in which the radioactivity in the water trap exceeded 5% of the applied dose, indicating excessive BE leakage from the occluded ring. For comparison, after intravenous administration of [^{14}C]BE, no radioactivity was observed in the water trap and about 2–3% of the administered dose was collected as $^{14}CO_2$.

At the end of the 4-h exposure, animals were euthanized with pentobarbital (Ceva Santé Animale, Libourne, France), and 1 mL of water was introduced through the Tedlar® membrane, which was then cut with a scalpel and the solution removed using a pipette followed by 5 dry cotton swabs. The skin around the application site was washed with a moistened cotton swab to detect leakage of the tested compound onto skin outside the ring. The materials from each step of the removal process were analyzed separately. The average absorption flux was calculated as the systemically absorbed fraction of radioactivity in the applied dose (urine, feces, carcass and $^{14}CO_2$ trap) multiplied by the mass of BE applied per area and divided by the total 4-h exposure time. Flux was measured in 4–6 rats at each BE concentration. The experiment using neat BE was repeated twice.

3.1.4. In vitro experiments

In vitro absorption through silicone membranes and rat skin was determined in static diffusion cells (Special Verre, Geipolseim, France) with a diffusion area of 1.76 cm^2 and receptor volume of 5.1 mL. The receptor fluid was a saline solution (0.9% NaCl) containing 1% penicillin–streptomycin. The diffusion cells were maintained at a temperature of 36 °C with a circulating water bath, yielding a temperature of 32 ± 1 °C at the surface of the skin or membranes.

Silicone membranes were hydrated in water solution for a night before experiments. Full-thickness rat skin was collected from rats sacrificed with pentobarbital. The entire dorsal region was shaved, cut from the animal, and excess subcutaneous tissue was carefully removed. The skin sample was then cut into one or two circular sections and the thickness of each was measured with a plate thickness gauge (Prost-Bourillon, Lunéville, France). The circular sections were then placed stratum corneum side up, in the diffusion cells. The integrity of the skin samples was assessed by determining the trans-epidermal water loss (TEWL, Tewameter®, TM210, Courage + Khazaka) after an equilibrium time of 2 h. Results from samples with TEWL greater than about 13 g m^{-2} h^{-1} and anomalously larger BE flux values were excluded.

Neat or aqueous solutions (1–90%; V/V) of [^{14}C]BE (200 μL/cm^2) were applied onto the skin (n = 4 to 6 from at least 3 animals at each concentration) or silicone membranes (n = 2 at each concentration) and occluded with paraffin film. To assess the experimental variability, measurements of 3–6 samples (from at least 3 animals) were repeated twice for 25 vol% BE and three times for neat and 50 vol% BE. These replicates are reported as independent measurements. An aliquot (400 μL) of receptor fluid was collected and replaced with fresh solution using an automatic fraction collector (Gilson FC 204, Middleton, WI, USA) each hour in the skin experiments and each 0.25 h up to 1 h and then every 0.5 h in the silicone membrane experiments. Four hours after the beginning of the exposure, the unabsorbed dose was removed with water (1 mL) and the exposed skin or silicone membrane surface was dried with cotton swabs and the skin was digested in KOH (25%; W/V). The radioactivity contained in the receptor fluid samples, the washing water, and the skin homogenates was measured by adding 10 mL of liquid scintillation solution (Pico Fluor 30, Perkin Elmer Life Sciences).

The reported fluxes are the maximum of the flux values calculated from the difference in the percent of the applied dose collected in the receptor solution over each sampling interval for each diffusion cell. Steady state was approached but probably not achieved in 4 h for all samples. This may have caused a larger variability in these *in vitro* experiments than was observed in the comparable *in vivo* experiments, which was related in part to variation in the skin thickness.

3.1.5. Analysis of radioactivity

Total radioactivity was determined by direct liquid scintillation counting (urine, feces, plasma, washing cage) or digestion in a 20% (W/V) solution of KOH (carcass, skin) with liquid scintillation solution (Pico Fluor 30, Perkin Elmer Life Sciences). Counting efficiency was determined by quenching the correction curves for the various addition and scintillation fluids with a liquid scintillation spectrophotometer (CA 1900, Perkin Elmer Life Sciences).

3.2. Thermodynamic activity

If a solute j has some volatility, then its thermodynamic activity in a vehicle at a specified temperature can be determined by measuring its partial pressure relative to the saturation pressure of the neat solute. Often, this is accomplished using headspace analysis, in which the equilibrium vapor phase above the sample in a closed container is analyzed by gas chromatography (Al-Khamis et al., 1982). This approach was used in the benzyl alcohol experiments in human skin described above (Barry et al., 1985), as well as the study of methanol, butanol and octanol uptake into hairless mouse skin from mixtures of saline and dimethyl sulfoxide (Kurihara-Bergstrom et al., 1986). In this approach, vapor phase interactions of the solute and vehicle are neglected.

For a completely miscible binary liquid mixture of species j and v like BE and water, the thermodynamic activity of j and v also can be determined experimentally by measuring at constant temperature the total vapor pressure above the liquid solution for compositions of species A varying from zero (i.e., pure B) to 100% (i.e., pure A) (Prausnitz et al., 1999). In this approach, it is not necessary to determine the equilibrium composition of the vapor phase, because its composition can be calculated by application of thermodynamic rules (i.e., the Gibbs–Duhem equation); see Section 6.7 in Prausnitz et al. (1999).

The total vapor pressure measured at 25 °C for solutions of BE and water has been reported by Koga (1991) along with calculated values of the partial pressures and excess partial molar free energies ($G^E_{M,BE}$ and $G^E_{M,w}$ for BE and water, respectively). From $G^E_{M,BE}$ and $G^E_{M,w}$ the activities of BE and water (a_{BE} and a_w) can be calculated as a function of the mole fraction of BE (x_{BE}) as follows (Prausnitz et al., 1999):

$$a_{BE} = x_{BE} \exp\left(\frac{G^E_{M,BE}}{RT}\right) \tag{12}$$

$$a_w = (1 - x_{BE}) \exp\left(\frac{G^E_{M,w}}{RT}\right) \tag{13}$$

where R is the real gas constant and T is absolute temperature in consistent units. Values of $G^E_{M,BE}$ and $G^E_{M,w}$ as a function of x_{BE} from Koga (1991) as well as calculated values for a_{BE} and a_w are provided in Supplementary materials.

Similar experiments were also conducted by Scatchard and Wilson (1964) for eight different concentrations of BE in water at 5, 25, 45, 65 and 85 °C. They reported equilibrium pressure and mole fractions of water in the vapor (y_w) and liquid (x_w), from which a_{BE} and a_w were calculated:

$$a_{BE} = \frac{(1-y_w)p}{p_{sat,BE}} \quad (14)$$

$$a_w = \frac{y_w p}{p_{sat,w}} \quad (15)$$

where $p_{sat.BE}$ and $p_{sat.w}$ are the equilibrium pressures reported at $x_w = 0$ and 1, respectively. They also reported their results in terms of the following two equations:

$$\frac{G^E_{M,BE}}{RT} = -d\left[\ln(1-c(1-x_{BE})) + x_{BE}\left(\frac{cx_{BE}}{1-c(1-x_{BE})} - \frac{b(1-x_{BE})}{1-bx_{BE}}\right)\right] \quad (16)$$

$$\frac{G^E_{M,w}}{RT} = -d\left[\ln(1-bx_{BE}) - x_{BE}\left(\frac{cx_{BE}}{1-c(1-x_{BE})} - \frac{b(1-x_{BE})}{1-bx_{BE}}\right)\right] \quad (17)$$

where the coefficients b, c and d are defined at temperature in Kelvin as:

$$b = -0.4087 + \frac{42.5}{T} \quad (18)$$

$$c = 0.4335 + \frac{364.7}{T} - \frac{64,400}{T^2} \quad (19)$$

$$d = 1.465 \quad (20)$$

At 25 °C $b = -0.2662$ and $c = 0.9323$. The vapor–liquid equilibrium data from Scatchard and Wilson (1964) and calculated values for a_{BE} and a_w are provided in Supplementary materials.

Experimental measurements of BE flux through skin are compared to a_{BE} at the same composition. Concentrations of BE reported in dermal absorption studies as volume fraction (v_{BE} = vol.%/100) have been converted to w_{BE} using the densities of BE and water (ρ_{BE} and ρ_w, respectively) as follows:

$$w_{BE} = \frac{v_{BE}\rho_{BE}}{v_{BE}\rho_{BE} + (1-v_w)\rho_w} \quad (21)$$

For comparing the Koga (1991) activity data with dermal absorption results, values of a_{BE} at each w_{BE} in the dermal absorption study were calculated by linear interpolation as follows:

$$a_{BE} = a_{BE,k} + (a_{BE,k+1} - a_{BE,k})\left(\frac{x_{BE} - x_{BE,k}}{x_{BE,k+1} - x_{BE,k}}\right) \quad (22)$$

where x_{BE} was calculated from w_{BE} using Eq. (7), and $a_{BE,k}$ and $a_{BE,k+1}$ are the activity data calculated from Koga (1991) at $x_{BE,k}$ and $x_{BE,k+1}$, respectively, which are the mole fractions of BE just below and above x_{BE}. Activity estimates from Scatchard and Wilson (1964) were calculated using Eqs. (16) and (17) combined with Eqs. (12) and (13).

4. Results and discussion

The concentration variation of BE absorption into rat skin and silicone membranes are presented and compared to the results shown in Fig. 2 for human and guinea pig skin (from Traynor et al., 2007a,b and Johanson and Fernstrom, 1988, respectively). These results are then compared to the thermodynamic activity of BE to assess whether the observed non-linear concentration dependence of BE absorption is due to changes in either the skin or the silicone membrane.

4.1. Butoxyethanol absorption

In Fig. 3 the average flux measured over 4 h in rats *in vivo* is compared with the maximum flux observed over 4 h in rats *in vitro*.

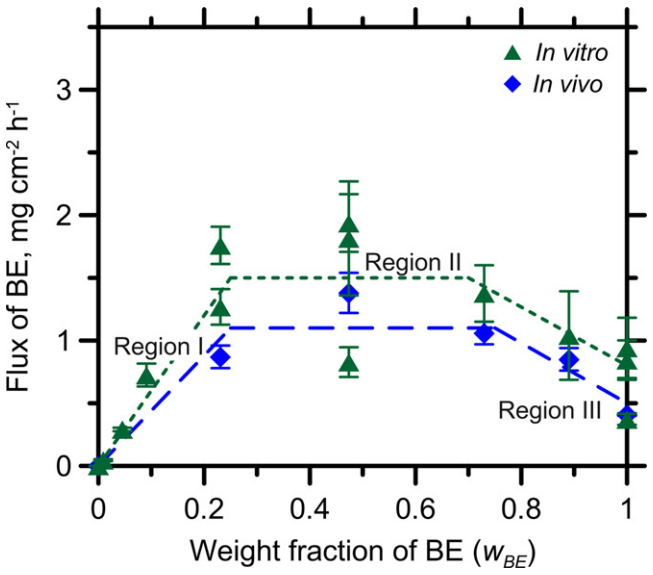

Fig. 3. Flux of BE (mean ± one standard error of mean) measured in rat skin *in vitro* and *in vivo* at various concentrations of BE in water with lines added to guide the eye.

These results show good correlation between the *in vivo* and *in vitro* measurements and the same dependence on BE concentration that was observed in the previously published human and guinea pig studies presented in Fig. 2. Variability in the *in vitro* rat experiments was larger than in the *in vivo* rat experiments. Almost certainly this was because some measurements deviated from steady state more than others. For example, the repeated measurements in Fig. 3 at $w_{BE} = 0.23$, 0.47 and 1.0, which are low relative to the other measurements at these concentrations, were all determined in skin samples that were thicker (i.e., 0.88 ± 0.09, 1.44 ± 0.19, and 1.46 ± 0.20 mm, respectively) than the average thickness of the skin samples (0.65 mm) in the other experiments for $w_{BE} > 0.2$. The three measurements determined at $w_{BE} < 0.2$ also were determined in thicker skin samples (i.e., 0.93 ± 0.07, 0.96 ± 0.10 and 0.91 ± 0.03 mm for $w_{BE} = 0.0091$, 0.045 and 0.091, respectively).

In silicone membranes, the concentration dependence in BE flux was the same as in skin at low and intermediate BE concentrations, but differed from skin at high BE concentrations (Fig. 4).

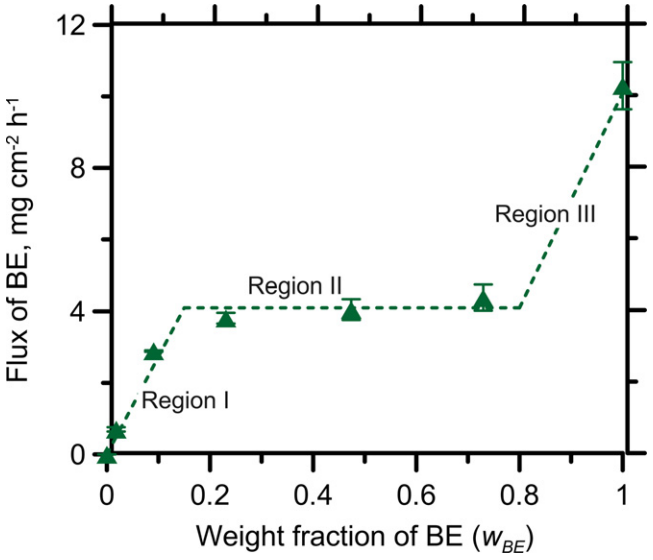

Fig. 4. Flux of BE (mean ± one standard error of mean) measured in silicone membranes at various concentrations of BE in water with lines added to guide the eye.

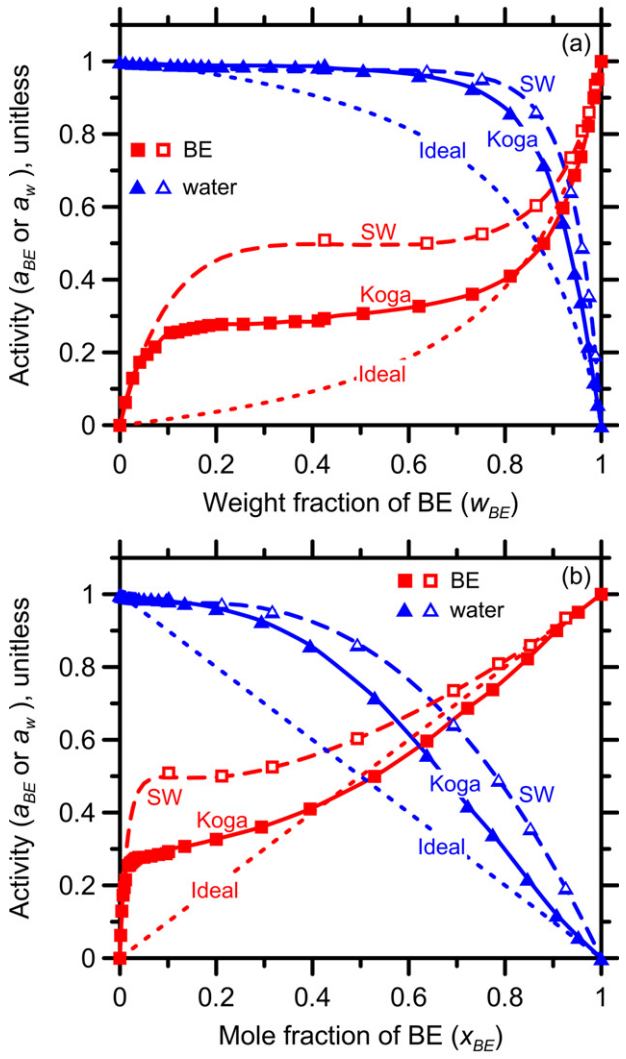

Fig. 5. Experimental values (symbols) of a_{BE} and a_w in aqueous solutions of BE at 25 °C from Scatchard and Wilson (SW) and Koga compared with those predicted if aqueous solutions of BE were ideal are plotted as a function of: (a) weight fraction of BE, and (b) mole fraction of BE. The solid curves are spline fits to the Koga data and the long dashed curves were calculated using Eqs. (16) and (17) from Scatchard and Wilson.

Specifically, compared with the average flux in Region II, BE flux from the neat solution increased more than two-fold in silicone membranes compared with a two-fold decrease in rat skin. Similar observations have also been reported for PDMS membranes (Traynor et al., 2007a). The mechanism of this dramatically different behavior in skin and silicone membranes at large BE concentrations was explored by comparing BE flux with the thermodynamic activity driving forces for BE and water.

4.2. Thermodynamic activity

The experimental values of BE and water activity from Koga (1991) and Scatchard and Wilson (1964) are plotted in Fig. 5 as a function of w_{BE} and also x_{BE}. The experimental measurements from Scatchard and Wilson (1964) are shown as symbols on the curves for water and BE, labeled as SW, which were calculated using Eqs. (16) and (17) in combination with Eqs. (12) and (13). Although the concentration dependence of the two data sets are qualitatively similar, a_{BE} values from Scatchard and Wilson are larger than those from Koga for $w_{BE} < 0.9$ for unknown reasons (Koga, 1991). It is important to notice, however, that the experimental measurements from Scatchard and Wilson (1964) do not extend below $w_{BE} = 0.4$. Therefore, the exact shape of the SW curve depicting a_{BE} for $w_{BE} < 0.4$ is not supported by experiments. In particular, the lower bound of the plateau in a_{BE} may deviate by an unknown amount from $w_{BE} \approx 0.2$, which is indicated by the SW curve in Fig. 5.

As also shown in Fig. 5, the values of a_{BE} and a_w that would occur if BE and water formed an ideal mixture (short dashes), deviate significantly from both sets of experimental values. If BE and water did form an ideal solution, both a_{BE} and a_w would vary linearly with the mole fraction of BE, but not with w_{BE}. This is because the molecular weights of water and BE are different (i.e., 18 differs from 118 by 85%), which causes x_{BE} to vary non-linearly with w_{BE} as indicated by Eq. (7).

Experimental values of a_{BE} increase almost proportional to w_{BE} for w_{BE} less than about 0.1. For $0.1 < w_{BE} < 0.9$, a_{BE} is almost constant, which coincides with the appearance of micellar-type supramolecular aggregates at $x_{BE} \approx 0.02$ (i.e., $w_{BE} \approx 0.12$) as observed in measurements at 25 °C by a variety of techniques including light scattering, neutron scattering and calorimetry (Darrigo et al., 1991a,b; Onori and Santucci, 1997). When w_{BE} increases above 0.12, the added BE molecules form new aggregates or are incorporated into existing aggregates, which might be as large as 1.8–2.0 nm in radius (Darrigo et al., 1997). As a result, the concentration of single, non-aggregated BE molecules stays relatively small and almost constant until w_{BE} is so large that the ratio of water to BE molecules is too small to energetically favor continued aggregation of BE. In addition, the concentration of the aggregates also stays small because each aggregate contains a large number of BE molecules (Darrigo et al., 1997). For w_{BE} less than about 0.8, a_w is almost the same as neat water because the concentrations of both single molecules of BE and BE aggregates are small. The observation that a_{BE} is also approximately constant for w_{BE} values between 0.1 and 0.8 is consistent with approximately constant values for both a_w and the concentration of single BE molecules.

4.3. Thermodynamic activity and flux of BE

In Figs. 6–10, the flux data presented in Fig. 4 for silicone membranes and in Figs. 2 and 3 for guinea pig, human and rat skin are shown normalized by w_{BE} and a_{BE}. According to Eq. (6), the transference, which is equal to the ratio of the steady-state flux and activity, should be constant as long as neither the solute *j* nor other components in the vehicle change the skin significantly. This should also be true for flux values that are not at steady state as long as the experimental procedures were the same for all measurements.

In the skin experiments, the ratio of the flux to w_{BE} decreased with increasing BE concentration over the full range of w_{BE} (Figs. 7, 9 and 10), except for the two measurements from *in vitro* rat skin for $w_{BE} < 0.1$. These measurements, as well as the replicates with low normalized flux values at $w_{BE} = 0.23$, 0.47 and 1, were determined in thicker pieces of skin, which may have deviated from steady state more than the other measurements. In the silicone membrane experiments, the ratio of the flux to w_{BE} also decreased with increasing BE concentration, except for the measurement from neat BE, which was larger than the measurements at w_{BE} equal to 0.47 and 0.73 (Fig. 6).

The activity-normalized flux of BE, however, shows a different behavior. For the silicone membranes (Fig. 6), the activity-normalized flux of BE was essentially constant for all BE concentrations, including neat BE. Although the variability was larger, the activity-normalized flux of BE through skin from all three species also was essentially constant except when w_{BE} was greater than about 0.8. At $w_{BE} > 0.8$, a_{BE} increases significantly with w_{BE}, while the flux of BE through skin decreases dramatically. This indicates that, in the presence of BE with little water, the stratum corneum of rats, guinea pigs and humans behaved differently than when it was in contact with BE solutions containing at least

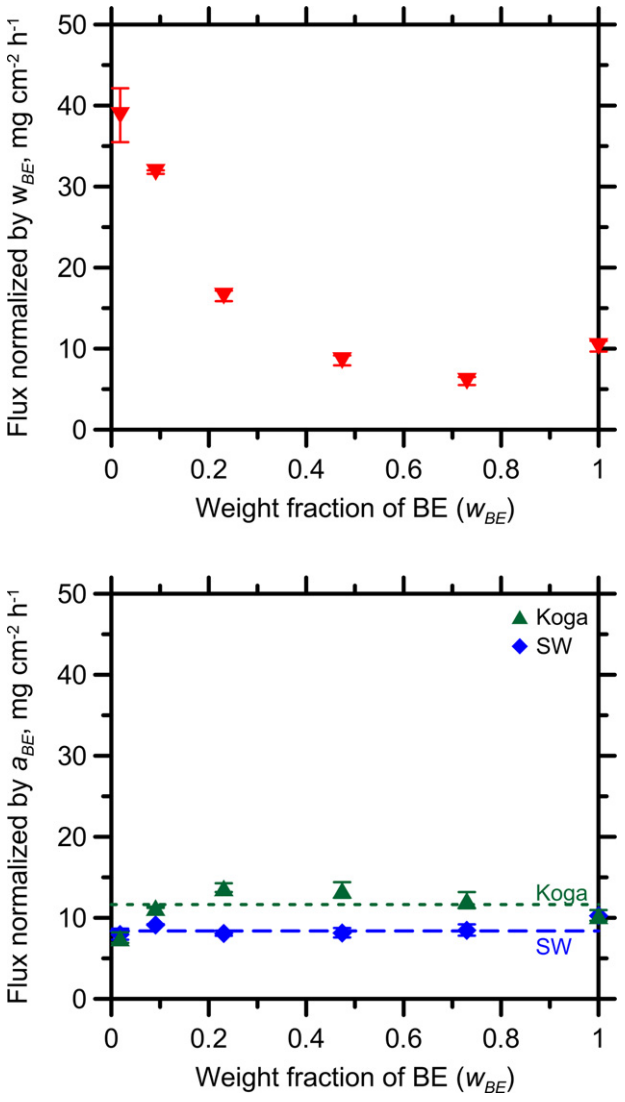

Fig. 6. Flux of BE (mean ± one standard error of mean) measured in silicone membranes at various concentrations of BE in water normalized by either w_{BE} or a_{BE}. The dashed lines correspond to the average activity-normalized flux of BE from all solutions with $w_{BE} < 0.8$ calculated using the activity data from Koga and the BE activity equation from Scatchard and Wilson (SW).

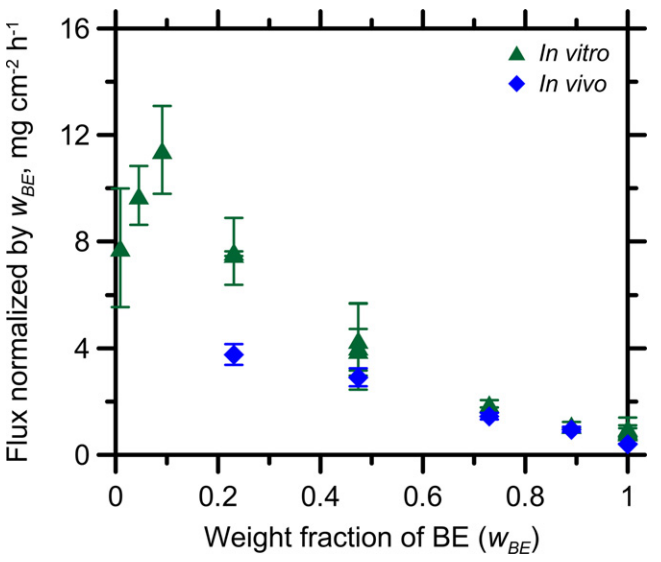

Fig. 7. Flux of BE (mean ± one standard error of mean) measured in rat skin *in vitro* and *in vivo* at various concentrations of BE in water normalized by w_{BE}.

10–20 wt% water. Moreover, this different behavior in the presence of solutions with large BE concentrations was not observed in silicone membranes.

For silicone membranes, the activity-normalized flux at all BE concentrations was approximately 8.4 mg cm^{-2} h^{-1} for a_{BE} from the SW equation and 11.6 mg cm^{-2} h^{-1} for a_{BE} from Koga. For rat skin, the average activity-normalized flux for $w_{BE} < 0.8$ was about 2.6 mg cm^{-2} h^{-1} and 2.2 mg cm^{-2} h^{-1}, respectively in the *in vitro* and *in vivo* measurements using a_{BE} from SW, and about 3.9 mg cm^{-2} h^{-1} and 3.6 mg cm^{-2} h^{-1} using a_{BE} from Koga. These numbers are about 4 times and 6 times (for SW and Koga, respectively) larger than the flux from neat BE. In both the silicone membrane and rat skin experiments, the variability in the activity-normalized flux numbers compared to the average (shown as the horizontal dashed lines in Figs. 6 and 8) was smaller for the activity data from SW compared with Koga.

The results in rat skin were consistent with the observations for guinea pig and human skin. For guinea pig skin (Fig. 9), the activity normalized flux was approximately 1.7 mg cm^{-2} h^{-1} for a_{BE} from SW and 2.3 for a_{BE} from the Koga; respectively, these ratios correspond to 6.3 and 8.5 times larger than the flux from neat BE (0.27 mg cm^{-2} h^{-1}). For human skin (Fig. 10), the activity-normalized flux was approximately 2 mg cm^{-2} h^{-1} for $w_{BE} < 0.1$, which is about 5-times larger than 0.39 mg cm^{-2} h^{-1} for neat BE. Compared to the measurements at $w_{BE} < 0.1$, the activity-normalized flux values at w_{BE} equal to 0.47 and 0.73 (50 and 75 vol%) were larger than expected for both the Koga and SW activity data. However, in keeping with the other studies, flux normalized by the SW activity data (5.4 and 4.5 mg cm^{-2} h^{-1} for w_{BE} equal to 0.47 and 0.73, respectively) differed from measurements at $w_{BE} < 0.1$ by a smaller amount than flux normalized by the Koga activity (8.9 and 6.5 mg cm^{-2} h^{-1}, respectively). In general, the BE flux measurements in the four skin studies shown in Figs. 8–10 are more consistent with the activity data from Scatchard and Wilson (1964).

Other authors have reported larger amounts of activity-normalized skin absorption for BE in water, usually measured at $w_{BE} = 0.47$, compared with neat BE (Jakasa et al., 2004; Korinth et al., 2005, 2007; Wilkinson et al., 2006). Furthermore, this behavior is not limited to BE. It has also been reported for five other glycol ethers (Venier et al., 2004): dipropylene glycol methyl ether, n-propoxyethanol, 2-isopropoxyethanol, 2-methoxyethyl acetate, and 2-(2-butoxyethoxy)ethyl acetate. These observations that the activity-normalized flux (i.e., the transference) from neat or nearly neat solutions was reduced compared with diluted solutions means that skin was changed by vehicles containing large concentrations of BE or these other glycol ethers. That this occurred in vehicles with low water activity and a large capacity to hold water is consistent with reduced skin hydration as the cause.

4.4. Thermodynamic activity of water and BE flux

The activity of water is the same as the relative humidity of a solution. Thus, a solution with $a_w = 1$ has a relative humidity of 100%. Because a_w remains nearly one for w_{BE} as large as 0.8, skin exposed to aqueous solutions of BE should be nearly fully hydrated as long as $w_{BE} < 0.8$. In addition, the permeation rate of water through the skin should be constant for $w_{BE} < 0.8$, which was observed by Traynor et al. (2007a). In their experiments on human skin *in vitro*, water flux from pure water was 4.72 ± 0.52 mg cm^{-2} h^{-1} (mean ± SEM for $n \geq 5$) compared with 5.17 ± 0.70 mg cm^{-2} h^{-1} when w_{BE} was 0.47. Interestingly, this was

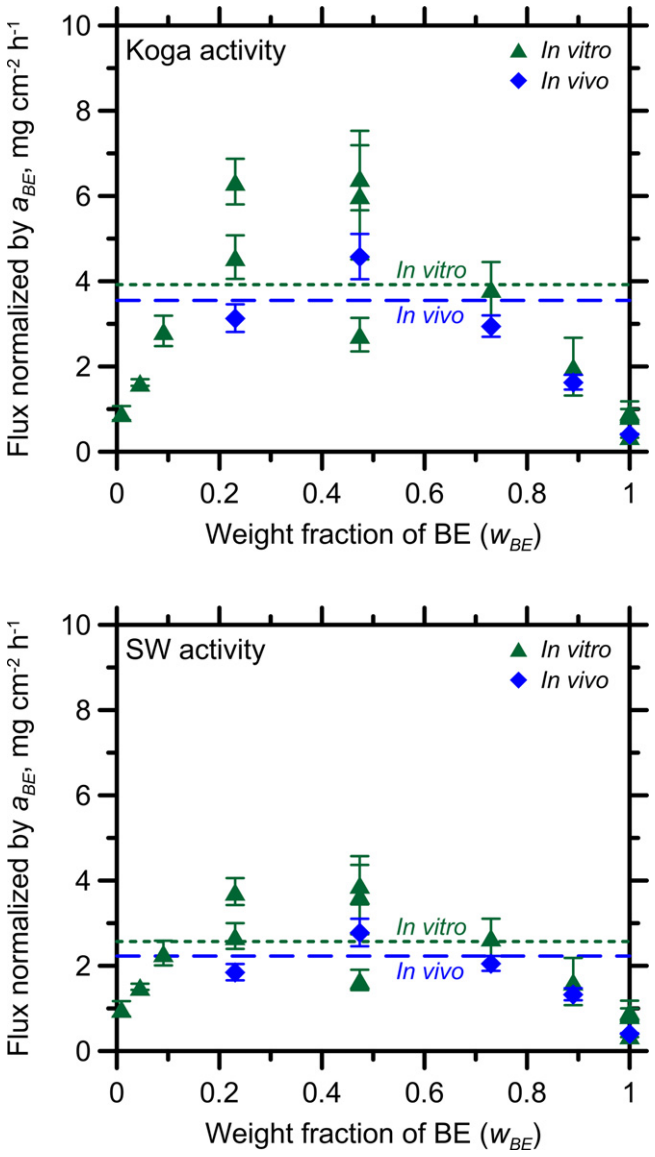

Fig. 8. Flux of BE (mean ± one standard error of mean) measured in rat skin *in vitro* and *in vivo* at various concentrations of BE in water normalized by a_{BE}. The dashed lines correspond to the average activity-normalized flux of BE from all solutions except those with $w_{BE} > 0.8$ calculated using the activity data from Koga and the BE activity equation from Scatchard and Wilson (SW).

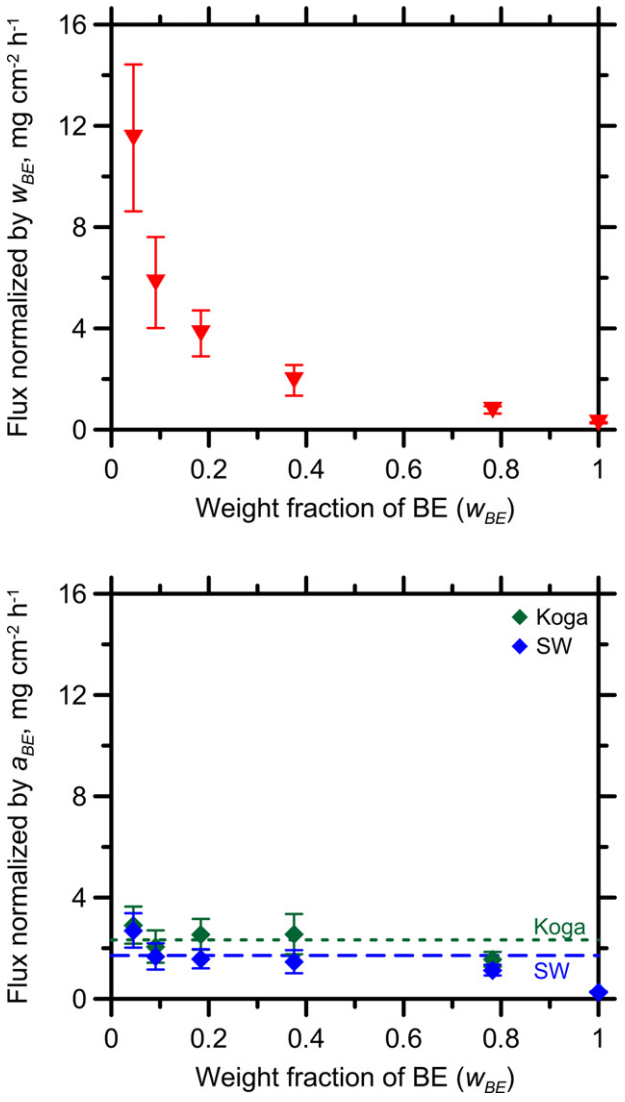

Fig. 9. Flux of BE (mean ± one standard error of mean) measured in guinea pig skin *in vivo* (from Johanson and Fernstrom, 1988) at various concentrations of BE in water normalized by either w_{BE} or a_{BE}. The dashed lines correspond to the average activity-normalized flux of BE from all solutions with $w_{BE} < 0.8$ calculated using the activity data from Koga and the BE activity equation from Scatchard and Wilson (SW).

also the case for 2-ethoxyethanol, in which the water flux was $5.28 \pm 0.40\,\mathrm{mg\,cm^{-2}\,h^{-1}}$ from a solution with $w_{BE} = 0.47$ (Traynor et al., 2007a).

However, when $w_{BE} > 0.9$, a_w decreased sharply from about 0.7 or 0.8 (as reported in the Koga and SW studies, respectively) to zero for neat BE (i.e., $w_{BE} = 1$). Since the water activity in hydrated skin is larger than 0.8, there will be a driving force for water to diffuse from the skin into the vehicle when $w_{BE} > 0.9$; this driving force does not exist (or is much smaller) when $w_{BE} < 0.8$. If skin is exposed to neat or nearly neat BE, the driving force for water transfer from the skin will continue until enough water transfers so that w_{BE} is less than 0.9. This will cause skin hydration to decrease, which in turn causes a reduction in the BE flux. In contrast with skin, silicone membranes, which absorb little water, should be unaffected by changes in water activity. This is consistent with the experimental observation that the activity-normalized flux of BE through silicone membranes was constant for all BE concentrations, including neat BE.

Although the idea that dermal absorption increases with increased hydration is not new (Idson, 1973; Scheuplein and Blank, 1971; Wurster and Kramer, 1961), unambiguously linking changes in skin hydration and permeability has been difficult because, until recently, there have been few definitive experiments in which all experimental factors are held constant except water activity of the formulation applied to the skin surface. Commonly, the effects of increased hydration have been inferred from measurements with and without occlusive coverings, which have produced variable results (Bucks et al., 1991).

Recently, fluxes through pig skin of two compounds, metronidazole and methyl salicylate, were measured from solutions prepared in phosphate buffered saline (PBS) that did or did not contain one of three water-soluble polymers: PEG 1500, PEG 4000 or dextran 1500 (Bjorklund et al., 2010). The water activity of these vehicles was manipulated from 0.82 to 0.996 by changing the concentration of polymer without changing the activity of metronidazole or methyl salicylate. To accomplish this, concentrations of metronidazole or methyl salicylate were adjusted so that the fraction of saturation was the same in all polymer solutions. Constant activity

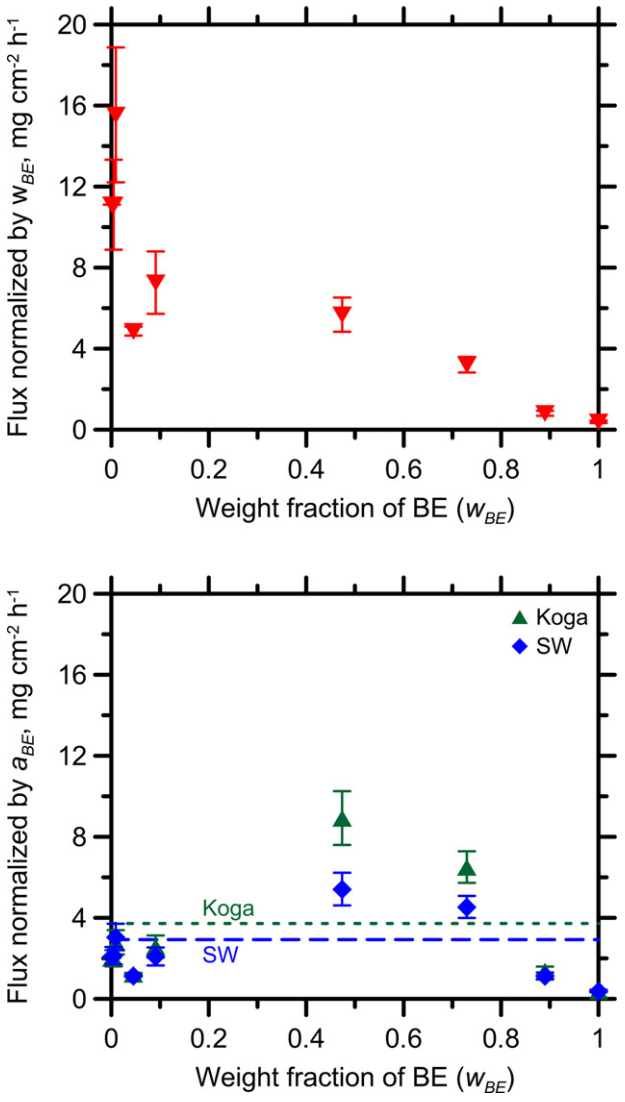

Fig. 10. Flux of BE (mean ± one standard error of mean) measured in human skin *in vitro* (from Traynor et al., 2007a,b) at various concentrations of BE in water normalized by either w_{BE} or a_{BE}. The dashed lines correspond to the average activity-normalized flux of BE from all solutions with $w_{BE} < 0.8$ calculated using the activity data from Koga and the BE activity equation from Scatchard and Wilson (SW).

of the chemical was confirmed by measurements of chemical flux through PDMS, which were the same from all formulations. For both chemicals, the flux through skin was larger from PBS than from solutions with $a_w < 0.9$. For metronidazole, which is relatively hydrophilic (the logarithm of the octanol–water partition coefficient, $\log K_{ow}$, is −0.02), flux increased almost 13 fold with increased water activity. This was larger than the 2.6 fold increase for methyl salicylate, which is more lipophilic ($\log K_{ow} = 2.55$). These results are consistent with factors of approximately 4–8 in the activity-normalized flux of BE ($\log K_{ow} = 0.83$, Hansch et al., 1995) observed in solutions with $a_w > 0.9$ compared with neat BE (Figs. 8–10).

If BE solutions containing little or no water exhibit reduced flux because the solution has low water activity and the skin is dehydrated, then one would expect that this should also occur when other solvents that are miscible with water in all proportions come into skin contact. This is apparently the case. With a striking similarity to the behavior of BE, the skin fluxes of both ethanol and nitroglycerin from aqueous solutions of ethanol were maximized when the solution contained only 50 vol% ethanol (Berner et al., 1989). A more thorough examination of the literature would undoubtedly reveal other examples.

The amount of reduced flux caused by skin dehydration in the presence of low water activity is likely to vary significantly between studies. Using BE as an example, if the vehicle volume is small, or if BE evaporates from the skin during the exposure, then less water will transfer before w_{BE} becomes <0.9. One would expect, therefore, that skin exposed to solutions with w_{BE} larger than about 0.9 will become desiccated to an extent that will depend on the starting value of w_{BE}, the volume of the vehicle on a given area of skin, and whether or not the applied solution is prevented from evaporating.

4.5. Experimental and predicted transference values

If flux through skin was measured at steady state, then the approximately constant activity-normalized flux for solutions with $w_{BE} < 0.8$ should be equal to the flux through fully hydrated skin from a saturated solution, which is the transference defined in Eqs. (3) and (4). Given this, it is interesting to compare the experimental results from Figs. 8–10 with estimates of the maximum BE flux calculated using a structure–activity equation to predict the permeability coefficient of BE through human skin from water (P_{BE}). In this scheme, P_{BE} is multiplied by the density of neat BE, which, because BE is completely miscible with water, is the maximum possible concentration of BE that can be achieved. For BE transfer across hydrated human skin, P_j is estimated to be 0.0015 cm/h using an often used structure–activity equation from Potts and Guy (Guy, 2010):

$$\log P_j = -2.7 + 0.71 \log K_{ow,j} - 0.0061\, MW_j \quad (23)$$

where j = BE, $\log K_{ow,BE} = 0.83$ and $MW_{BE} = 118.18$ Da. When multiplied by the density of neat BE (900 mg/mL), the steady-state flux from a saturated solution, which should represent the transference of BE through hydrated human skin, is predicted to be 1.4 mg cm^{-2} h^{-1}. This is a reasonable estimate of all the experimental results shown in Figs. 8–10 for the SW activity data, even though Eq. (23), which was developed using *in vitro* human skin data, is not generally applicable to other animals, especially rat skin, which is usually more permeable. Compared with the previously published human skin data (Fig. 10), this prediction of BE transference is close to the experimental value of 2.1 mg cm^{-2} h^{-1} calculated for $w_{BE} < 0.2$, and it is not too different from 2.9 mg cm^{-2} h^{-1}, which is the average for $w_{BE} < 0.8$. Overall, these results are within the expected predictive range of the method.

4.6. Thermodynamic activity calculations

Thermodynamic data for calculating the activity of penetrating solutes are not available for all compounds of interest or, even when experimental data exist, they may be difficult to find, or the data may not be at the conditions that are relevant to dermal absorption (e.g., 32 °C and one atmosphere). It is possible to estimate a_j for components in a mixture, with varying degrees of success, using thermodynamic model equations, which are described in the chemical engineering literature (see Prausnitz et al., 1999). Activity coefficient methods are recommended for liquid mixtures at atmospheric pressure, especially those that exhibit highly non-ideal interactions. These methods use a selected activity coefficient model (i.e., a set of equations) to estimate a_j by describing the interactions between all pairs of chemicals in the mixture. Thus, a mixture of three components A, B and C can be described using experimental data from mixtures of A and B, A and C, and B and C. In many of the models the interaction of A with B is not the same as the interaction of B with A (known as asymmetric binary interactions) leading to at least two and often several parameters required to describe the interactions for each component pair.

The binary parameters in the activity coefficient model are estimated by regression of the activity coefficient model equation to experimental data of the component pairs. Binary parameters for the most common activity coefficient models (e.g., NRTL, UNIQUAC and Wilson) are available for many component pairs, and when not available, they can be estimated by regression to phase equilibrium experimental data. As with all models, binary parameters are only valid over the temperature and pressure ranges of the data. Even when the different activity coefficient models are regressed to the same binary component data, the calculated activity values from the models will differ because the functional form of the equations in each is different. Generally, it is not possible to predict these differences or to know which model will most closely match the experimental data. The Wilson, NRTL and UNIQUAC activity coefficient models are all commonly used for combinations of polar and non-polar compounds, including those with very strong non-ideality (Prausnitz et al., 1999). The NRTL or UNIQUAC models should be used for liquids with limited miscibility, which the Wilson model cannot describe (Prausnitz et al., 1999).

When binary parameters are not available and data for estimating them does not exist, group contribution approaches, such as the universal quasi-chemical functional activity coefficient (UNIFAC) method, can be used to make parameter predictions for the UNIQUAC equations based on the functional groups of each molecule. The approach is limited to molecules containing functional groups for which UNIFAC parameters are available (Prausnitz et al., 1999). This can be a problem for molecules containing chlorine, nitrogen or functional sub-groups that are sometimes found in less common cyclic groups. Insufficient binary interaction experimental data is available for compounds with these molecular groups to adequately develop the functional group interactions, resulting in what may be thought of as proximity affects (e.g., the —C—Cl bond at the end of an alkane chain compared to the same bond when a —CH_2Cl group is attached to an aromatic ring structure).

Commercial software packages are available for estimating thermodynamic properties of solutions, either as stand-alone programs or as modules in process simulation packages such as Aspen Plus (AspenTech, Burlington, MA). These packages usually contain libraries of binary parameters for common solvents and chemicals. Sometimes, binary parameters for chemicals that are not in the library can be estimated from other data that are available to the package. This was the case for BE and water, for which binary parameters used by Aspen Plus in three different activity coefficient models (Wilson, NRTL and UNIQUAC models) were estimated using the VLE-IG databank, developed by AspenTech for vapor–liquid equilibrium (VLE) applications using the Dortmund Data Bank.

Values of a_{BE} and a_w calculated by the Wilson, NRTL, UNIQUAC and UNIFAC models in Aspen Plus are compared with the Koga and SW data in Fig. 11. Calculated values for a_w by all activity coefficient models but UNIFAC are similar to each other and close to the experimental data from both Koga and SW. For a_{BE}, the NRTL and UNIQAC models closely represent the SW data, as does the Wilson model for $w_{BE} > 0.1$. However, a_{BE} values calculated by all activity coefficient models are larger than the Koga data for $0.2 < w_{BE} < 0.8$. The binary parameters used in the models were found to have been derived solely from the SW data and not the Koga data (Gmehling et al., 1981). This explains the disparate results in the Wilson model for $w_{BE} < 0.1$. Compared with binary parameter estimates for the other models, the Wilson model is affected much more by the lack of concentration data. Further research regarding vapor–liquid equilibra for the BE–water system would be to reconcile the differences in the SW and Koga data sets and then determine if the UNIQUAC model is the most suited for the general activity analysis. UNIQUAC is clearly suitable in describing the SW data.

Unlike the other activity coefficient models, the UNIFAC model can predict activity with no experimental data. Although group-contribution approaches are attractive because no experimental data are required, they provide only a rough and potentially unreliable approximation. Given this, it is not surprising that UNIFAC predictions deviate from the experimental data more than the other activity coefficient models. The prediction of BE activity by the UNIFAC model appears to be strongly influenced by the infinite dilution end-points, causing erroneous mid-region concentration predictions of the BE activity.

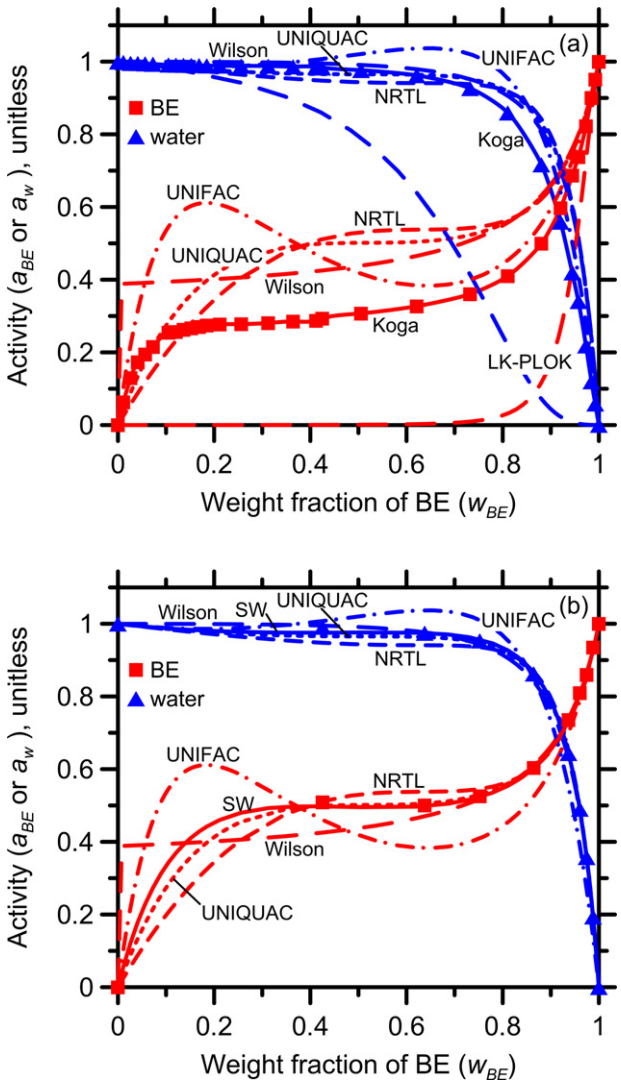

Fig. 11. Values of a_{BE} and a_w in aqueous solutions of BE calculated by four different models in Aspen Plus plotted as a function of the weight fraction of BE compared with: (a) experimental values from Koga (symbols) and spline fit to data; and (b) experimental values (symbols) and the activity equations from Scatchard and Wilson (SW).

The curves identified in Fig. 11a as LK-PLOK deviate significantly from the experimental data for both BE and water. These curves were calculated using an equation-of-state method, specifically the Lee–Kesler–Plöcker equation, which is an alternative to activity coefficient methods. Equation-of-state methods are useful for describing phase equilibria over wide ranges of temperature and pressure, but have limited capabilities for representing highly non-ideal chemical mixtures like alcohols and water. Calculations from the LK-PLOK method were included in Fig. 11 to illustrate the importance of using an appropriate method for the chemical mixture.

Binary parameters in packages like Aspen Plus are sometimes derived from data sources that may not be readily available (e.g.,

the data are proprietary or they were published in documents with limited access). Also, experimental data may have been measured at temperatures that are different from, but bracket the desired temperature. In these situations, it useful to compare values of a_j calculated by more than one activity coefficient model. As illustrated by the BE–water example in Fig. 11, calculations from more than one model may prove helpful for identifying deviant results. For example, confidence in a_{BE} from the Wilson model would be reduced because values calculated for $w_{BE} < 0.1$ deviated significantly from the NRTL and UNIQUAC values (which were in reasonably close agreement with each other for all w_{BE}) and for no reason that is apparent in the structure of the Wilson model and applicable to this binary system. For purposes of explaining the dermal absorption experiments, either the NRTL or UNIQUAC model calculations are sufficient.

5. Conclusions

Except when concentrations of water are small, the observed concentration dependence of BE absorption into skin is consistent with the non-ideal behavior of BE in water as reflected by its thermodynamic activity. Because the activity of water is almost one for $w_{BE} < 0.8$, skin in contact with these solutions should be fully hydrated. For these solutions, the transference for BE through skin, which is the ratio of its flux and thermodynamic activity, is nearly constant. However, the transference decreases significantly when w_{BE} is greater than about 0.8. This change in the transference coincides with a decrease in water activity from about 0.9 at $w_{BE} = 0.8$ to zero for neat BE, suggesting that BE flux decreased because the skin became dehydrated. This same behavior has also been observed in neat and aqueous solutions of other glycol ethers and ethanol. Moreover, these observations are consistent with a recently published study of the effect of water activity on the dermal absorption of metronidazole and methyl salicylate. The flux of BE through skin is consistent with Fick's law written in terms of the activity instead of concentration for $w_{BE} < 0.8$. Overall, the transference of BE through hydrated human skin was about 2–4 mg cm^{-2} h^{-1}, which is similar to the predicted maximum flux of BE, 1.4 mg cm^{-2} h^{-1}, calculated using the Potts and Guy equation and the density of neat BE.

When experimental activity data exist but are not available, calculations from appropriately chosen activity coefficient models may provide reasonable activity estimates. However, among the models that are appropriate for a given system, it is not possible to predict which will provide reliable results. One strategy for identifying unreliable results is to compare calculations from multiple activity coefficient models. When experimental data do not exist, rough predictions that will have unknown reliability are possible using group contribution type approaches such as UNIFAC.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2012.01.058.

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