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RESEARCH PAPER

Concentration Dependency in Nicotine Skin Penetration Flux from Aqueous Solutions Reflects Vehicle Induced Changes in Nicotine Stratum Corneum Retention

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ABSTRACT

Purpose This study sought to understand the mechanism by which the steady state flux of nicotine across the human skin from aqueous solutions is markedly decreased at higher nicotine concentrations.

Methods Nicotine's steady state flux through human epidermis and its amount in the stratum corneum for a range of aqueous nicotine solutions was determined using Franz diffusion cells, with the nicotine analysed by high performance liquid chromatography (HPLC). Nicotine's thermodynamic activity in the various solutions was estimated from its partial vapour pressure and stratum corneum hydration was determined using a corneometer. The amount of nicotine retained in the stratum corneum was estimated from the nicotine amount found in individual stratum corneum tape strips and a D-Squame determined weight for each strip.

Results The observed steady state flux of nicotine across human epidermis was found to show a parabolic dependence on nicotine concentration, with the flux proportional to its thermodynamic activity up to a concentration of 48% w/w. The nicotine retention in the stratum corneum showed a similar dependency on concentration whereas the diffusivity of nicotine in the stratum corneum appeared to be concentration independent. This retention, in turn, could be estimated from the extent of stratum corneum hydration and the nicotine concentration in the applied solution and volume of water in the skin.

Conclusions Nonlinear dependency of nicotine skin flux on its concentration results from a dehydration induced decrease in its stratum corneum retention at higher concentration and not dehydration induced changes nicotine diffusivity in the stratum corneum.

KEY WORDS nicotine thermodynamic activity · skin penetration flux · solute concentration · stratum corneum hydration · stratum corneum retention

INTRODUCTION

Topical or transdermal application of compounds through the skin is widely used to achieve both local and systemic effects. The major barrier for skin penetration is the outermost "dead" and desquamating layer, the stratum corneum. A number of transdermal therapeutic systems were developed and marketed in the 1980s, including nitroglycerin, scopolamine, clonidine, estradiol and nicotine (1). These were largely based on a series of compounds studied by Michaels *et al.* in 1975 (2). They showed a linear dependency between flux and solute concentration for the solid drugs: scopolamine, ephedrine, and chlorpheniramine and that their flux could be related to their degree of ionisation. Nicotine, an aid in the smoking cessation therapy (3–6), was shown to be absorbed through the skin as an adverse effect to being used as a topical insecticide (7–9). It was first used in a transdermal form as a smoking reduction and cessation aid in 1984 in a study in which 9 mg of nicotine base was applied in a 30% aqueous solution to intact skin on the underside of the forearm of a volunteer (10). The study was associated with significant levels of nicotine in the saliva between 30 and 90 min and an increase in both the pulse and systolic blood pressure. A

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subsequent study applied either 8 mg nicotine base in a 30% aqueous solution or an inactive placebo solution to intact skin of 10 cigarette smokers under a polyethylene patch and showed a reduced craving (11). It has been suggested that adverse reactions to topical nicotine is maximal with an aqueous 10% nicotine base in solution (12). However, these authors also reported that 50% nicotine base solutions caused a severe irritant reaction in subjects non responsive to the allergic patch test, possibly due to the high pH of the solutions. One of the first US patents applying this invention proposed a transdermal application pad with a reservoir for liquid nicotine base to be attached to the skin (13). In a subsequent patent, the polyurethane matrix layer component in a patch was specified as being between 5 and 50% nicotine and able to deliver nicotine through human skin over at least 24 h (14). A later US Patent has suggested that the concentration of nicotine in the patch reservoir should preferably at a thermodynamic activity of less than 0.50 (15). Nicotine patches are now widely used in a range of transdermal patches for smoking cessation (16–18).

In this study, we sought to understand the determinants of nicotine flux through human skin, given that has been suggested to have a maximal skin penetration at about 50% nicotine when a range of aqueous nicotine concentrations was applied to dermatomed abdomen and breast skin from human female donors in Franz skin diffusion cells (19) and many patents specify ~50% nicotine as an upper limit in nicotine concentration or in thermodynamic activity in transdermal nicotine systems (14,15). Nicotine (diacidic base, pKa 3.04 and 7.84), exists as a combination of unionised, monoprotonated and diprotonated forms depending on the pH of its environment. Its skin penetration flux is in the order: unionised nicotine > monoprotonated > diprotonated nicotine (20). It has been suggested that, when the different ionic forms of nicotine co-exist in solution, nicotine can partition into different parts of the stratum corneum (21). Similar fluxes of nicotine through human skin are found when nicotine was administered as a tartrate salt, salicylate salt and as the nicotine base at pH 8.5, where nicotine is predominantly (~82%) present in the unionized form. In contrast, the nicotine skin flux varies considerably for the different salts at pH 5, consistent with ion-pairing facilitating uptake of ionized nicotine (22). The dominant role of the unionised form of nicotine in determining its skin transport is evident in that the flux of a 20% nicotine acetic acid buffered solution at pH 4.6 (where nicotine is almost completely ionised into its mono or deprotonated forms), is 0.39% that of the flux for an unbuffered solution at pH 11.1 (where nicotine is fully unionised) (19), noting that its flux from the buffer solution could also involve ion-pair transport. A variation in nicotine skin flux has also been reported for various non-aqueous vehicles, with a reduced flux being reported when anionic polymers were also present in the vehicles (22). Given this data and that nicotine has been reported to show a parabolic relationship with its concentration in aqueous solutions (19) but that for most solutes a linear relationship exists between skin flux and thermodynamic activity up to the solute solubility limit (1, 2), we firstly sought to explore if the steady state nicotine skin flux data from a solution could be related to its thermodynamic activity in solution. We recognised that nonlinearity could occur when skin barrier properties has been adversely affected by the vehicle or the solute (23), often at higher concentrations as a consequence of damage (23), association in solution (24) or when there is super-saturation (25). Whilst, in principle, the ideal reference for maximal thermodynamic activity is a saturated drug solution where the drug is in solution in a molecular form (26) and where the thermodynamic activity is unity (27), such an approach was not possible here as nicotine was miscible with water at all concentrations at room temperature (25°C). One of the unique properties of nicotine is its closed "nicotine–water solubility loop" (28), whereby nicotine is miscible with water at ambient temperatures due to the formation of a nicotine covalent hydrate but at temperatures above a reported lower consulate temperature of 60.8°C becomes immiscible with water and then passes into one phase again above 208°C (29). Accordingly, we estimated the thermodynamic activity for aqueous nicotine solutions using the partial vapour pressure of nicotine for various aqueous solutions, with the maximal thermodynamic activity for pure nicotine being defined as unity. We then followed our earlier approach (30) and assumed that the major determinant of skin penetration was the diffusion of solute in the stratum corneum (D), its concentration in the stratum corneum C_m and its diffusion path length (h):

$$J_{ss} = \frac{DC_m}{h} \tag{1}$$

We also assumed that C_m is dependent on both the thermodynamic activity of the solute in the vehicle and on the amount of vehicle retained in the stratum corneum. The steady state penetration flux J_{ss} of nicotine through human epidermal membranes was measured using Franz diffusion cells and HPLC, and collected stratum corneum strips were used to measure nicotine retention. A corneometer and a pH skin meter were used to measure stratum corneum water content and pH skin surface, respectively. The partial vapour pressure of nicotine was used to estimate its thermodynamic activity.

MATERIALS AND METHODS

Materials

(–) Nicotine, deionized water, phosphate buffer saline (PBS pH 7.4) sachets, HPLC grade methanol and acetonitrile were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia).

Human Skin Preparation

Abdominal skin was obtained from a female donor who underwent plastic surgery. Consent was collected from the patient based on ethical approval granted by The Queen Elizabeth Hospital and University of South Australia Human Research Ethics Committee. Human epidermal membrane was prepared using a heat separation technique (31) stored at −20°C and used within 8 months of collection.

In Vitro Human Skin Studies

The skin was thawed at room temperature, cut into pieces to allow between 3 and 5 replicates for any given study and mounted between the donor and receptor chambers of the Franz cells. The exposed skin surface area was 1.33 cm^2. Phosphate buffered saline (PBS) pH 7.4 was used as receptor phase. A magnetic stirrer bar was put at the bottom of each receptor phase chamber. The cells were put in the water bath thermostated at 35°C which provided a temperature of 33± 1°C in the receptor phase and 32±1°C on the skin surface. The cells were left for 30 min before dosing. A 400 μL aliquot of aqueous nicotine solutions at concentrations of 2, 10, 33.33, 48, 80.5 and 100% was applied to different skin specimens via the donor chamber individual Franz cells and the chamber was covered to minimise evaporation. During each experiment, 200 μL samples were removed from the sampling arm of the Franz receptor chamber and replaced by an equivalent volume of a nicotine-free receptor solution at predetermined times.

Sample Analysis

The nicotine concentration in the samples was analysed using a Shimadzu HPLC system using similar HPLC conditions for nicotine analysis as reported by Ho and Chien (32). The column used was a Microsorb C18 with phosphate buffer (10 mM pH 7.4): methanol: acetonitrile (30:35:35) mobile phase pumped at a flow rate of 1 mL/min and detected by an ultra violet detector at 260 nm. The retention time for nicotine was 4.0 min. The analysis method yielded a linear standard curve from 1.5 to 100 μg/ml (r^2=0.99), with recovery between 95 and 110% and intra-and inter-day variabilities of 0.53 to 1.93% and 1.79 to 3.66%, respectively.

Thermodynamic Activity Analysis

Partial vapour pressure data from a range of nicotine concentrations in water at 25°C were extracted from the data published by Norton *et al.* (33). The thermodynamic activity of nicotine in solution was estimated as the product of activity coefficient and its corresponding solute concentration expressed as a mole fraction (34).

Stratum Corneum Hydration and Skin Surface pH

On completion of the *in vitro* diffusion studies, the donor phase chambers in the Franz cells were emptied and any remaining solution on the skin surface was gently wiped off with a tissue. Skin hydration, which expresses the electrical capacitance of the stratum corneum arising from the high dielectric constant of water digitally in arbitrary units (AU), was measured by a corneometer probe (Derma Unit SSC 3, Caurage and Khazaka electronic GmbH, Cologne, Germany) (35) placed on the epidermal skin surface. The stratum corneum water content (μg) was then estimated from the changes in capacitance values and the reported linear relationship between capacitance and membrane sorption (36). The water fraction in the stratum corneum was calculated as the estimated stratum corneum water content for a given nicotine concentration divided by the maximum value for all studies. A flat glass electrode probe attached to the Derma Unit SSC 3 device was also placed on the stratum corneum skin surface to record its pH. The present studies were conducted at a room temperature of 25±2°C and at a relative humidity of 40–55%, as deduced by monitoring these parameters throughout the various studies.

Stratum Corneum Nicotine Retention

Stratum corneum nicotine retention was estimated as we have described previously (30). In order to minimise nicotine evaporation, tape stripping was done immediately after the penetration studies completed. In brief, the epidermis was glued using cyanoacrylate glue onto a thin section of plastic card with the stratum corneum side up. A strip disc (D-Squame sampling disc, Cuderm Co., USA) was put onto the stratum corneum surface and pressed by a pressure device (D-500 D-Squame, Cuderm Co., USA) over a fixed period of application. The process was repeated until 10 strips were obtained. The protein content in each of stripped-stratum corneum was then estimated (D501-D-Squame Scan 850A instrument, Cuderm Co., USA) to enable variations in the amount of each tape strip to be adjusted for. The first strip was discarded and each subsequent tape was placed into 80% methanol, mixed in a rotating mixer and then centrifuged. The supernatant was collected and analysed by HPLC after appropriate dilution.

Data Analysis

The steady state flux, J_{ss} (μg/cm^2/h), was calculated from the linear portion of the cumulative amount of solute penetrating per unit area of skin (μg/cm^2) plotted against time (hours). The resultant flux was then related to both nicotine concentrations in the solution and thermodynamic activity in solution. It was assumed that a linear flux–thermodynamic activity

relationship would be found unless significant nicotine-solvent-skin interactions existed and induced changes in either the uptake into and/or diffusivity of nicotine in the stratum corneum (37). The stratum corneum nicotine retention was defined as the cumulative amount of nicotine recovered from tape strips 2–10 (30). The apparent diffusion coefficient (D*), diffusivity divided by path length (D/h) (cm/h), was derived from J_{ss} and stratum corneum nicotine retention (R_m) (μg) using Eq. (2) (30).

$$D^* = \frac{D}{h} = \frac{J_{ss}}{R_m} \quad (2)$$

The nicotine concentration in stratum corneum strips (weight fraction) was calculated as the ratio of the amount of nicotine retained in the stratum corneum and the estimated water amount in stratum corneum.

RESULTS

Skin Penetration Parameters

The estimated skin penetration steady state flux, J_{ss}, nicotine thermodynamic activity, stratum corneum hydration and nicotine retention in the stratum corneum values for each solution studied are shown in Table I. It is evident that the values for J_{ss}, nicotine thermodynamic activity, stratum corneum hydration and nicotine retention in the stratum corneum vary by 4.25, 12.5, 2.43 and 3.22 times, respectively.

Flux

Figure 1a shows that the nicotine epidermal flux is as a function of nicotine aqueous concentration, with the maximum flux being reached at an aqueous nicotine concentration of 48%. At higher aqueous nicotine concentrations, the nicotine epidermal fluxes decline with increases in nicotine concentration to reach a limiting flux for pure nicotine that is similar to that found for 2% aqueous nicotine. Whilst general shape of the flux profile obtained is similar to that reported by Zorin (19), the overall flux values in this study are slightly lower consistent with the lower temperature used here relative to that work. The fluxes for the single donor used here had coefficients of variation (SD/mean X100) for the various studies of 0.3 to 16% (Table I).

pH

The pH of the bulk nicotine solutions and the skin surface pHs for various nicotine concentrations are shown in Fig. 1b. It is evident that nicotine exists in mainly the unionised form at the surface and predominantly in the unionised form in the aqueous solutions where the pH could be measured.

Stratum Corneum Retention

Figure 1c shows that the relationship between nicotine stratum corneum retention and donor nicotine concentration is a similar parabolic shape as found for the epidermal flux—concentration profile (Fig. 1a). Figure 1c also shows that maximum retention occurs at the 48% aqueous nicotine solution used in this study.

Apparent Diffusion Coefficient

Figure 1d shows that the apparent nicotine diffusivity (D*) remained unchanged across the various aqueous nicotine concentrations. This suggests that diffusivity was not a major determinant in defining nicotine flux from various aqueous nicotine solutions.

Estimated Thermodynamic Activity and its Relationship with Flux and Nicotine Stratum Corneum Retention

Figure 2a presents the estimated nicotine thermodynamic activity from partial vapour pressure data provided by

Table I Steady State Nicotine Flux (J_{ss}), Nicotine Thermodynamic Activity, Stratum Corneum Hydration, Estimated Diffusivity (D*) and Nicotine Amount Retained in Stratum Corneum From Various Aqueous Nicotine Concentrations

Nicotine concentration (% w/w)	J_{ss} (μg/cm²/h)[a]	Nicotine thermodynamic activity (AU)	Stratum corneum hydration (AU) [a]	Nicotine estimated diffusivity (cm/h) [a]	Nicotine stratum corneum retention (μg) [a]
2	67.9 ± 2.84 (3)	0.08	36.6 ± 1.45 (3)	2.3 ± 0.49 (3)	29.8 ± 8.27 (3)
10	149.6 ± 5.75 (3)	0.19	30.7 ± 2.82 (3)	3.7 ± 0.50 (3)	40.1 ± 5.95 (3)
33.33	238.6 ± 10.89 (5)	0.22	29.5 ± 3.07 (5)	2.7 ± 0.29 (3)	86.9 ± 7.42 (3)
48	259.1 ± 28.66 (4)	0.26	26.1 ± 2.83 (4)	2.6 ± 0.15 (3)	96.3 ± 0.65 (3)
80.5	151.6 ± 0.46 (4)	0.46	17.4 ± 1.76 (4)	4.5 ± 1.66 (3)	36.0 ± 16.14 (3)
100	60.9 ± 9.63 (5)	1	15.0 ± 1.30 (5)	1.8 ± 0.35 (3)	33.7 ± 5.29 (3)

[a] = mean ± standard deviation

() = replicates

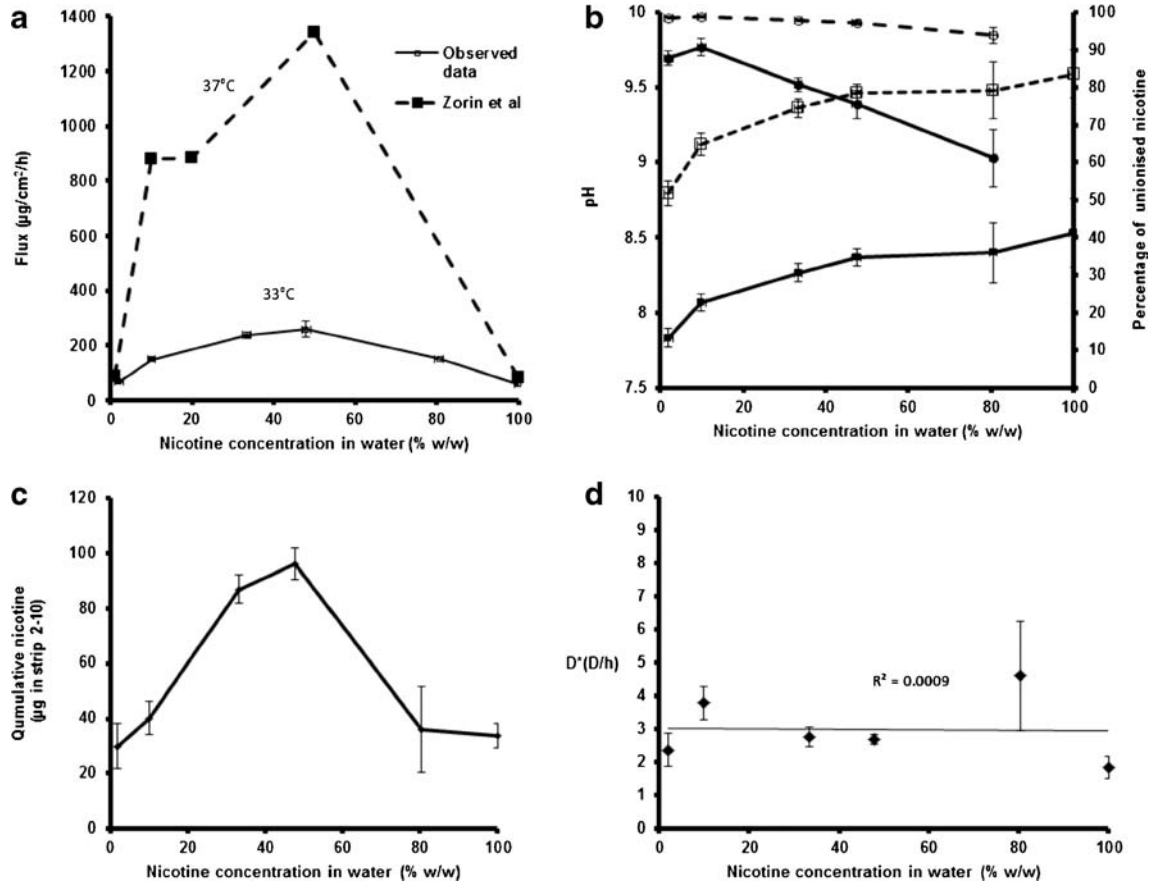

Fig. 1 Steady state nicotine flux ($\mu g/cm^2/h$) as a function of aqueous nicotine concentrations (% w/w) obtained from single donor in this study (*solid line*) and by Zorin *et al.* obtained from 17 human female donors (dashed line) (19) (**a**); pH and percentage of unionised nicotine of both nicotine solutions (● pH; ○ percentage unionised) and skin surface (■ pH; □ percentage unionised) as a function of aqueous nicotine concentrations (% w/w) (**b**); total amount of nicotine in stratum corneum (μg, strips 2–10) *versus* aqueous nicotine concentrations (% w/w) (**c**); apparent diffusivity coefficient (D*) as a function of aqueous nicotine concentrations (% w/w) (**d**).

Norton (33). The observed vapour pressure for nicotine in an aqueous solution positively deviated from ideal behaviour, the deviation being most evident at low nicotine concentrations. It is also evident in Fig. 2b that nicotine flux does not increase linearly with nicotine thermodynamic activity level. Linearity in flux was only evident up to 0.26 X the maximum nicotine thermodynamic activity of 1. Beyond this thermodynamic activity, the flux gradually decreases with a further increase in thermodynamic activity. A similar parabolic-like relationship was also obtained for a plot of nicotine stratum corneum retention *versus* nicotine thermodynamic activity (Fig. 2c).

Water Thermodynamic Activity and Stratum Corneum Hydration (Water Amount in Stratum Corneum)

The dependence of water thermodynamic activity in solution on nicotine concentration, based on the composition of the donor nicotine—water solutions and corresponding water vapour pressure data from Norton *et al.* data (33), as a function of nicotine concentration is shown in Fig. 3a. It is evident that the derived experimental water thermodynamic activity is higher than that predicted by Raoult's law, corresponding to a non-ideal behaviour of nicotine in water mixtures with a positive deviation from Raoult's law predictions. However, it is evident that water thermodynamic activity is relative high and constant (*i.e.* >0.9 of maximal thermodynamic activity), for nicotine in water concentration of 0 to 78%. Above this higher concentration, the thermodynamic activity of water rapidly reduces with increasing nicotine concentrations to become zero for pure nicotine solutions. The stratum corneum water content, based on the capacitance of the stratum corneum, as a function of nicotine concentration is shown in Fig. 3b. This figure shows that the stratum corneum hydration decreases as the aqueous nicotine concentration increases. It is also evident that, at high nicotine concentrations (above 78%), the stratum corneum is dehydrated *i.e.* a lower water content exists in stratum corneum than in the epidermis control (117.09 ± 12.88 mg). Figure 3c shows a plot of the nicotine retained in the stratum corneum, following normalisation for water content in the stratum corneum based on the observed maximum water content in the stratum corneum, *versus* nicotine thermodynamic activity in the

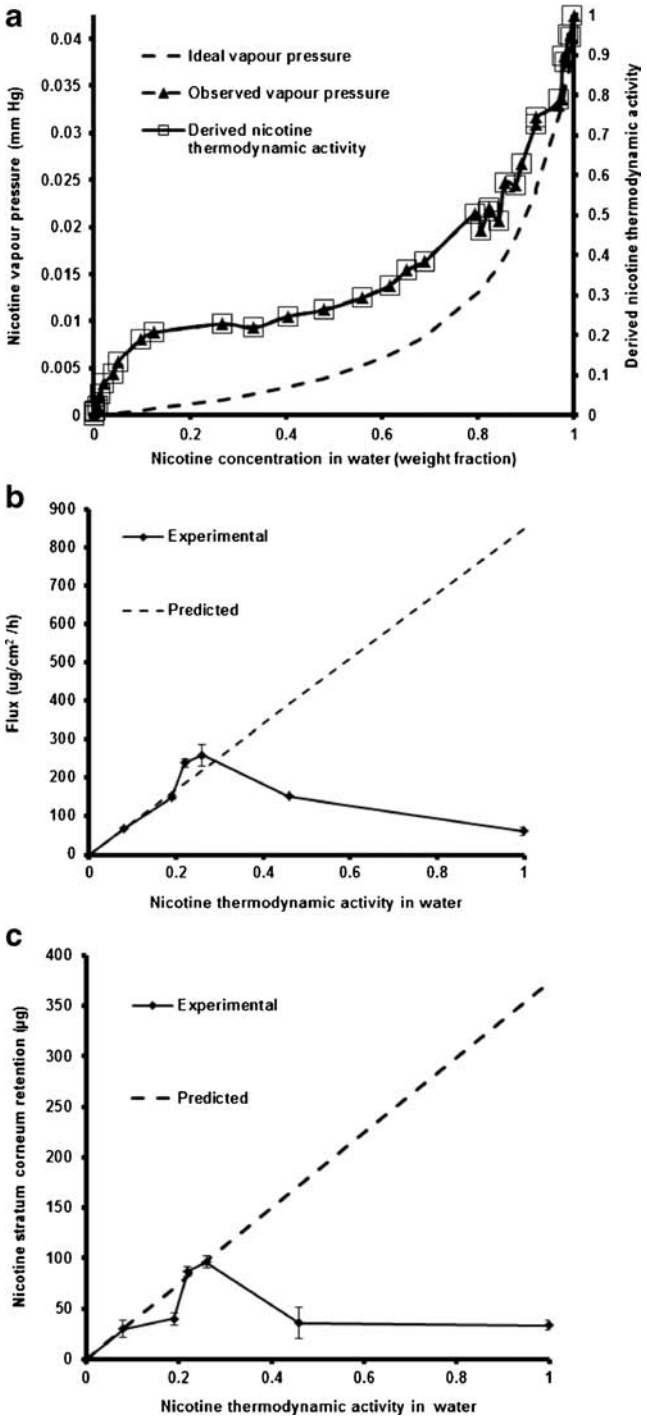

Fig. 2 Derived thermodynamic activity of nicotine as a function of aqueous nicotine concentrations expressed in weight fraction calculated from partial vapour pressure from Norton (33) data (**a**); steady state nicotine flux ($\mu g/cm^2/h$) as a function of nicotine thermodynamic activity in water (**b**); stratum corneum nicotine retention (μg) as a function of nicotine thermodynamic activity in water (**c**).

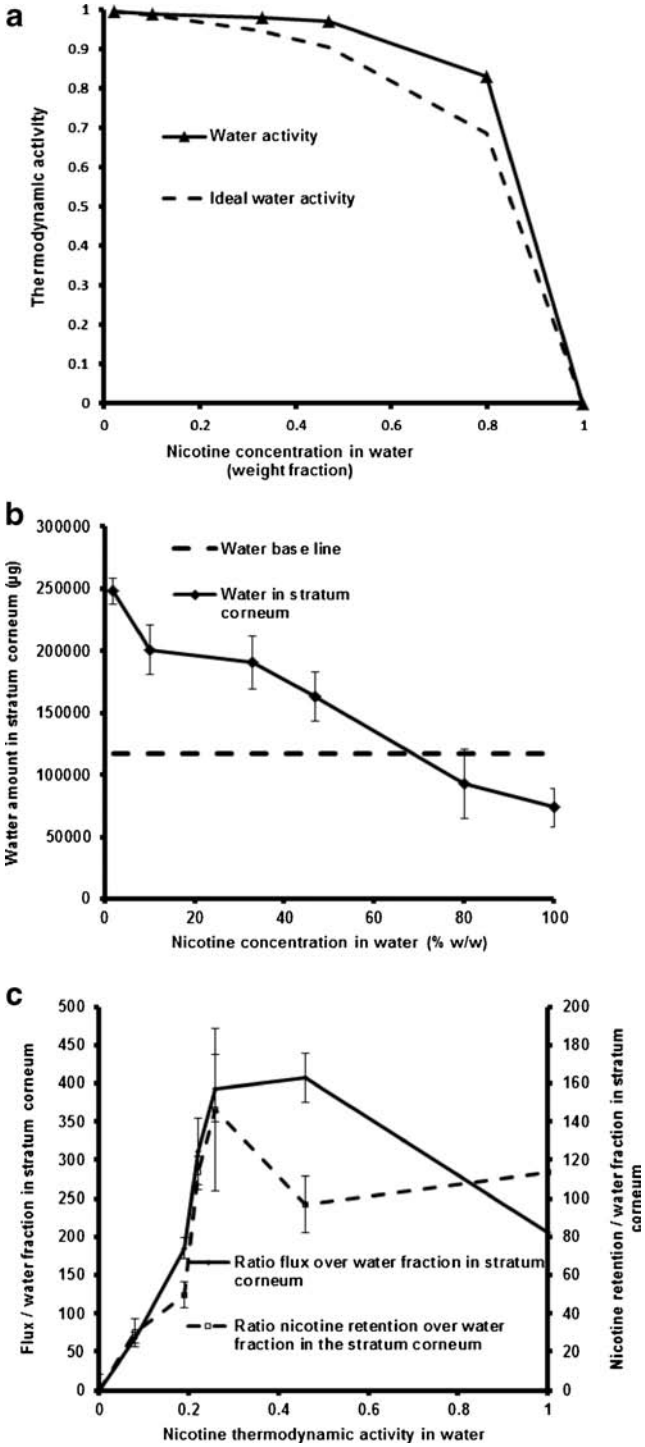

Fig. 3 Derived water thermodynamic activity (▲) and ideal water activity estimated from ideal nicotine behaviour (*dashed line*) based on Norton *et al.* (33) data as a function of nicotine concentrations in water (**a**); water amount in stratum corneum (*solid line*) and hydration base line (*dashed line*) as a function of nicotine concentrations (**b**); nicotine flux divided by water fraction in stratum corneum (*solid line*) and nicotine stratum corneum retention divided by water fraction in stratum corneum (*dashed line*) as a function of nicotine thermodynamic activity in water (**c**).

nicotine-water solutions. It is evident that the normalised nicotine stratum corneum retention and flux by accounting water fraction in stratum corneum showed linearity up to 0.26 nicotine thermodynamic activity which then seemed to be flat at higher thermodynamic activity. It implies that reduced stratum corneum nicotine retention and flux at higher

thermodynamic activity are likely caused by the lower amount of water in the stratum corneum.

DISCUSSION

Understanding solute-vehicle-skin interactions is essential for effective drug delivery design (37,38). Fick's first law of diffusion is generally used to interpret solute penetration across a membrane and assumes that flux is linear with solute concentration up to the solubility limit (2). An ill-defined phenomenon, however, has shown a parabolic dependence of steady state flux on solute concentration when solute-vehicle interactions occurs or solute-vehicle-skin interactions exists affecting stratum corneum properties (39–41). Parabolic behaviour for solute flux *versus* solute concentration in a given vehicle has now been shown by a number of authors (39,42–44). Our result study here confirms the parabolic relationship between nicotine skin flux and aqueous nicotine concentrations (Fig. 1a), reported previously by Zorin *et al.* (19). The difference in the magnitude of fluxes between the two studies is most likely due to the different human skin and temperatures used, *e.g.* 33°C used in this study *versus* 37°C used by Zorin *et al.* (19). Most of the available studies on nicotine skin penetration are only peripherally relevant to this work in that they have either been done mainly at low pHs or at low concentrations (20–22,45). They do, however, provide some insights into the variability in inter and intra-subject variability in nicotine skin penetration. As an example, Qvist *et al.* (45) reported a mean ± SD flux ($\mu g/cm^2/h$) data for 3 replicates from 3 separate donors of 277±15, 208±37 and 135±42. This data shows that inter-subject variability in nicotine skin penetration of 35% is greater than intra subject variabilities of 5, 18 and 31%. In Zorin's study (19), the average cumulative amount of nicotine flux from two different donors was found to have a two-fold difference. Accordingly, more precise results and a minimising of a potential type 2 statistical error (*i.e.* reaching a conclusion of no significant difference, when one actually exists) should occur if replicate studies for different treatment conditions were obtained avoiding inter-subject skin variability. Whilst such a precision may be warranted in seeking to understand why nicotine skin penetration flux has a parabolic dependency on nicotine concentration, it is also based on the nicotine flux concentration profile obtained using abdominal and breast skin from 17 women donors (19).

Analysis of the pH's skin surface showed that the skin's pH increased after the application of nicotine solution (Fig. 1b) compared to that of the pre-treated epidermis (pH of 5.6± 0.06). It is apparent, however, that there is a buffering occurring at the skin surface as the pH on the surface was lower than in the bulk solution (Fig. 1b). As shown in Fig 1b, nicotine is mainly unionised at the surface and even more so in solution, consistent with a buffering action of the stratum corneum itself. Further, analysis of the amount of nicotine in the strips (Fig. 1c) and the apparent diffusivity (Fig. 1d) suggests that the parabolic dependency of nicotine epidermal flux on nicotine solution concentration arises from a concentration—dependent partitioning into the stratum corneum and not due to effects of the various concentrations on the diffusivity of nicotine into the skin.

As a major determinant of partitioning is solute–vehicle interactions (46), we examined whether the thermodynamic activity *i.e.* effective solute concentration may better describe the nicotine flux and partitioning dependency. It was recognised that such an approach had not only previously been used to estimate the thermodynamic activity of other a volatile solute skin penetrants (34,46) but that work showed that the benzyl alcohol vapour skin flux was directly proportional to its thermodynamic activity (34). These findings are consistent with the principle that skin flux for a solute is directly linear with the solute's thermodynamic activity in the applied vehicle unless solute-vehicle-skin interactions occur affecting the skin (25,34,46). Figure 2a shows that aqueous nicotine solutions exhibit non-linear vapour pressure behaviour in terms of Raoult's law predicted based on nicotine weight fraction in solutions. It is evident from Fig. 2a that nicotine has an increased escaping tendency from a nicotine–water mixture than would be predicted by Raoult's law, consistent with nicotine being a semi-volatile hydrogen bonded molecule with very different molecular properties to water, as a consequence, the strength of the adhesive binding of nicotine molecules to water is less than the cohesive binding between nicotine molecules or between water molecules. Noting that nicotine is mostly present in unionised form in all solutions tested and only unionised nicotine which is volatile measured in vapour pressure phase (47), the estimated nicotine thermodynamic activity values extracted from 25°C was used to relate nicotine flux across human skin measured at 32°C. The nicotine thermodynamic activity value at 32°C was assumed to be proportional to that found 25°C in this analysis. It is evident that both nicotine flux and stratum corneum retention is approximately proportional to nicotine thermodynamic activity in solutions up to a thermodynamic activity of 0.26, corresponding to an aqueous nicotine concentration is 48% (Fig. 2b and c).

The most likely reason for the significantly reduced nicotine flux at concentrations above 48% (or thermodynamic activity above 0.26) is the decrease in the solution water activity (Fig. 3a) and associated reduced stratum corneum water content (*i.e.* a lower stratum corneum hydration) compared to that of epidermal control (Fig. 3b). In analysing the skin flux of solutes showing a parabolic dependence on solute concentrations in a vehicle, Roberts (23) and Bunge (48) showed that this parabolic dependence did not exist for the same solute–vehicle formulation using a polymeric membrane. Indeed, both found an approximate linear dependency

of flux on concentration for a polymeric membrane and concluded that, at high concentrations, the solute may be dehydrating the skin. Our findings here provided direct evidence that dehydration of the stratum corneum is the cause for the parabolic dependency of nicotine flux on thermodynamic activity from various aqueous nicotine solutions. Consistent with the present findings that nicotine fluxes decrease when the water activity in solution falls below 0.9, Bunge *et al.* suggested that non-linearity of butoxyethanol flux on its thermodynamic activity over 80% buthoxyethanol solution might arise from dehydration of the skin as indicated by the drop of water activity from 0.9 at 80% butoxyethanol solution to zero at pure butoxyethanol (48,49). The dominant role of skin hydration on the skin flux found here is also consistent with other published results studies (48,50,51). Indeed, recent study by Bjorklund (51) showed that water gradient can be used to reversibly regulate drug transport across the skin independent of drug lipophilicity. They reported that skin permeability increased abruptly at high degrees of hydration corresponding to water thermodynamic activities above 0.96.

It is interesting to note that similar parabolic profile of flux *versus* thermodynamic activity was also found for nicotine amount in stratum corneum plotted against thermodynamic activity (Fig. 2c). It is therefore evident that the nicotine retained in the stratum corneum is also controlled by the reduced stratum corneum hydration arising from a decreased water activity in solutions at high nicotine concentrations (Fig. 3b). Indeed, when stratum corneum volume water changes were expressed in terms of the fraction of maximal stratum corneum hydration, the normalised flux and nicotine retention were actually linear up to 0.26 nicotine thermodynamic activity then shown to level off (Fig. 3c) instead of becoming significantly reduced (Fig. 2b and c). This indicates that the declining flux or stratum corneum retention at higher nicotine thermodynamic activities has arisen from the corresponding decrease stratum corneum hydration. It is possible that water in the stratum corneum promotes nicotine solubility in the stratum corneum by the formation of a nicotine covalent hydrate, which enables miscibility between nicotine and water at ambient temperatures (29). Accordingly, less water in the stratum corneum would result in a reduction in amount of the nicotine covalent hydrate formed and, in turn, a lower retention of nicotine in the stratum corneum. The finding also reinforces theories suggesting the uptake of vehicle components into the stratum corneum can alter percutaneous absorption as has also been shown, for example, in our recent work in Zhang *et al.* (52,53), where enhanced permeation of similar sized of phenolic compounds resulted from their solvent uptake into the skin.

One mechanism used to explain enhanced percutaneous absorption associated with solvent uptake into the skin is a solvent drag mechanism, *i.e.* the amount of solute passing through and/or retained in the skin is dependent on the amount of solvent penetrating into the stratum corneum carrying the soluble solute (37,52–54). This premise is unlikely to apply here as the amount of nicotine in the stratum corneum for a 10% nicotine solution (40.14 μg) is somewhat

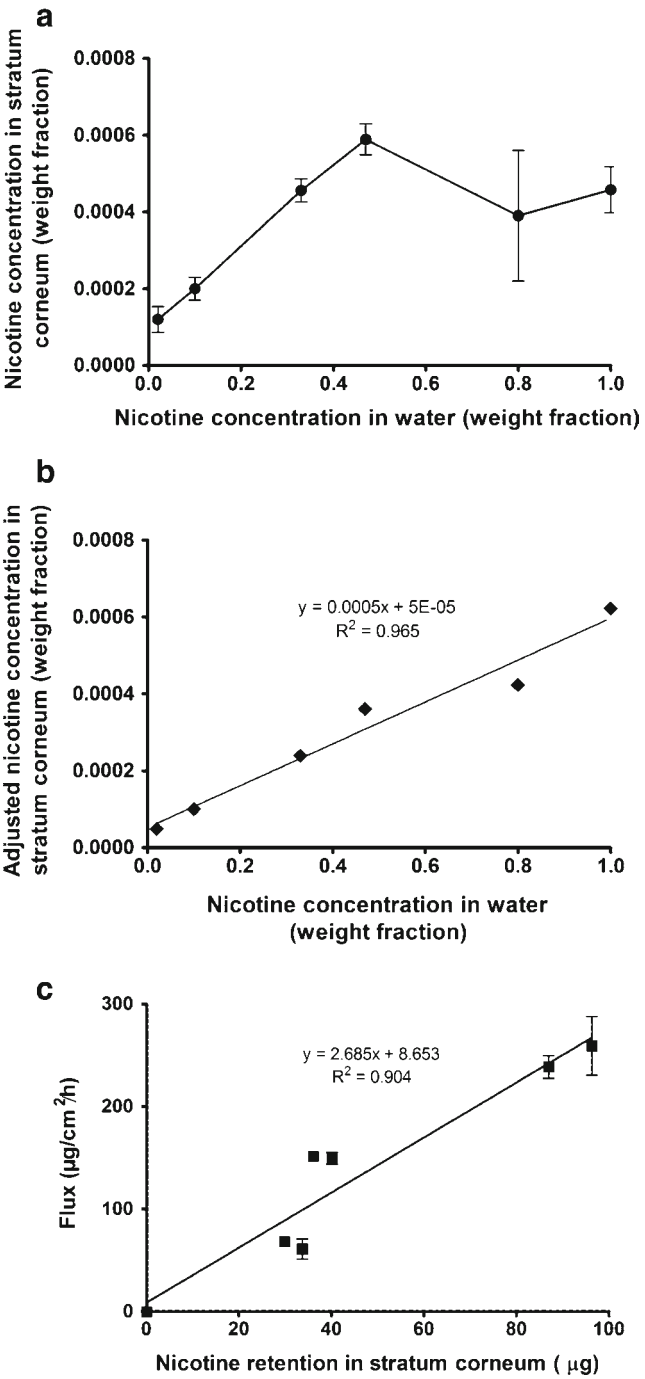

Fig. 4 Nicotine concentration in stratum corneum as a function of nicotine concentrations in water (**a**); Adjusted nicotine concentration in stratum corneum when water amount in stratum corneum is adjusted at the same amount as a function of nicotine concentrations in water (**b**); steady state of nicotine flux ($\mu g/cm^2/h$) as a function of nicotine stratum corneum retention (μg) (**c**).

less than the 22.3 mg of nicotine predicted for an uptake of 200.7 mg of water into the stratum corneum. Similar results were obtained for other nicotine concentrations in which the observed nicotine retained in the stratum corneum were less than 1% of the solvent drag predicted values. A smaller amount of nicotine retained in the stratum corneum than was estimated may reflect lower nicotine activity in the skin compared to that in the water vehicle. The linear relationship between nicotine retention in the stratum corneum and the water vehicle up to 48% (Fig. 4a) is consistent with a partitioning process. Deviations above that concentration are most likely caused by skin dehydration because if the nicotine amount in the stratum corneum is expressed in terms of the relative skin hydration, a linear relationship is found between nicotine concentration in stratum corneum and nicotine concentrations in water (Fig. 4b). In other words, the solute amount retained in stratum corneum is dependent on both solute concentrations in the vehicle and the amount of water in stratum corneum. This finding accords with our earlier work in which we showed that the hydrocortisone maximum flux was linearly related with the predicted vehicle volume sorbed by silastic membranes (37). The result is also consistent with observations by Twist and Zatz that partitioning rather than diffusivity is the major determinant for the maximum flux of parabens (55).

Consistent with a non-linear dependency in skin flux, reflecting a nonlinear dependency in the partitioning of nicotine into the stratum corneum from aqueous solutions, the epidermal flux is directly related to the nicotine amount retained in stratum corneum (Fig. 4c). This finding supports our earlier work that suggested solute retention in stratum corneum directly determines skin flux (30). These findings, however, contrast with polymer membrane studies in which a high concentration of a vehicle component has been shown to affect the solute diffusivity in the membrane (24).

These findings also assist in the understanding of earlier reported data on the skin flux and reactivity for nicotine. It is evident that high concentrations of nicotine can facilitate skin dehydration and a reduction of skin flux (Fig. 1a), justifying the patent recommendations that the nicotine concentration present be between 5 and 50% nicotine (14) or at a thermodynamic activity of less than 0.50 (15). Further, the greater adverse effects for nicotine at a concentration of 10% (12) reflect a higher skin penetration than for lower concentrations, whereas the high irritancy at 50% aqueous solution may not just be pH effects (12) but also a high concentration of nicotine in the stratum corneum (Fig. 4). Finally, the variations in nicotine skin flux from various non-aqueous vehicles from a 1% solution (22), is consistent with an apparent dependency of nicotine flux on its partitioning into the stratum corneum (Fig. 4) and, in turn, on the thermodynamic activity of nicotine in the vehicle.

CONCLUSIONS

In summary, the parabolic behaviour between nicotine epidermal flux on the concentration of nicotine in aqueous solutions was qualitatively similar to the findings reported by Zorin (19). The parabolic behaviour arises from a linear relationship between nicotine skin flux and nicotine thermodynamic activity in solution up to a nicotine concentration of 48%. Beyond that concentration the epidermal flux falls due to a decreased retention of nicotine in the stratum corneum as a result of stratum corneum dehydration. Consistent with a dominant partitioning process, the flux was directly related to the amount of nicotine retained in the stratum corneum and its hydration, with a relatively unchanged estimated diffusivity.

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