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Effects of Organic Solvents on the Barrier Properties of Human Nail

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Abstract

The effects of organic solvent systems on nail hydration and permeability have not been well studied. The objectives of the present study were to investigate the effects of binary aqueous organic solvent systems of ethanol (EtOH), propylene glycol (PPG), and polyethylene glycol 400 (PEG) on the barrier properties of nail plates. ^3H -water, ^{14}C -urea, and ^{14}C -tetraethylammonium ions were the probes in the nail uptake and transport experiments to study the effect(s) of organic solvents on nail hydration and permeability. Gravimetric studies were also performed as a secondary method to study nail hydration and the reversibility of the nail after organic solvent treatments. Both ungual uptake and transport were directly related to the concentration of the organic solvent in the binary systems. Partitioning of the probes into and transport across the nail decreased with an increase in the organic solvent concentration. These changes corresponded to the changes in solution viscosity and the barrier properties of the nail. In general, the effects for PPG and PEG were more pronounced than those for EtOH. Practically, these results suggest that organic solvents in formulations can increase nail barrier resistivity.

Keywords

hydration; human nail plate; transungual transport; ungual uptake; organic solvent effects

INTRODUCTION

The human nail plate contains a tightly woven network of lipids and keratin fibers cemented together by (hemi)desmosomes. Nail lipids contribute little to the overall permeability profile of the nail, but are important in imparting flexibility.¹ Proteins along with water are responsible for the permeability characteristics of the human nail. Specifically, the nail is composed of crystalline fibrous proteins embedded in an amorphous protein matrix. It has been hypothesized that these fibers overlap one another to create small, tortuous pore pathways which favor the permeability of small, hydrophilic molecules across the nail. As the nail becomes increasingly hydrated, the hydrogen bonds within the amorphous protein matrix are disturbed such that the pores expand for the permeation of molecules. In effect, the human nail behaves as a dense hydrogel.

The hydrogel characteristics of human nail are perhaps best shown by a study performed in the mid-1980s by Walters et al.² and Mertin and Lippold.³ In that study, up to fivefold higher permeability coefficients for dilute alcohols as compared with their neat counterparts were observed. Because water is believed to positively influence drug permeation through nail,⁴ special attention has been paid to the permeability of nail to water. In dry nails, Burch and Windsor⁵ observed diffusivity for water through nail at 2.5 mg/cm²/h. Similar findings were reported by Baden⁶ and Spruit.⁷ Walters et al.² found the permeability coefficient and flux for water to be 16×10^{-3} cm/h and 12 mg/cm²/h, respectively, whereas Smith et al.⁸ reported values of 11×10^{-3} cm/h and 10 mg/cm²/h, respectively, for hydrated nail. Using hydrated nail, Jemec et al.⁹ measured transonychia water loss (TOWL) and found that median TOWL was 1.9 mg/cm²/h. Differences in transungual water permeation are attributable to environmental factors such as temperature and humidity as well as intrinsic factors such as the condition of the nail.^{10,11}

As both nail hydration and iontophoresis facilitate transungual permeation, pharmaceutical scientists have recently begun studying the effects of water on the nail properties after iontophoresis. Dutet and Delgado-Charro¹² observed elevated TOWL values during the initial stages of direct current iontophoresis *in vivo*, which were attributed to increased hydration of the nail plate. These values returned to the baseline within 1–2 h after treatment.¹² In addition to the elevated TOWL values, the electrical resistance of the nail plate decreased rapidly during the first two hours of iontophoresis and then reached a constant value. This resistance profile was observed in both *in vitro* and *in vivo* nail experiments and could be explained by nail hydration.¹³

Transungual iontophoretic research is particularly important because nail diseases such as onychomycosis and nail psoriasis are becoming increasingly prevalent. Treatments for these diseases are typically by systemic oral medications and are riddled with adverse effects. The few topical therapies available have little efficacy given the relative impermeability of the nail. Additionally, these formulations contain organic solvents to increase the drug solubility, which likely introduce nail dehydration and decrease drug delivery across the nail. For example, Penlac[®] nail lacquer, the only US Food and Drug Administration (FDA) approved topical treatment for onychomycosis, contains 8% ciclopirox olamine dissolved in a solution of ethyl acetate and isopropyl alcohol,¹⁴ whereas Loceryl[®] or Curanail[®], distributed primarily in the United Kingdom, contains 5% amorolfine hydrochloride dissolved in a solution of glycerol triacetate, butyl acetate, and ethanol.¹⁵

Most nail transport experiments are performed under fully hydrated conditions, where the nail absorbs 30%–50% of its weight in water.^{8,10,16–20} Under physiological conditions, nail contains 7%–12% water and correspondingly is less permeable.^{21,22} Accordingly, a system should be devised that mimics *in vivo* conditions to study ungual transport better. To address this concern, Gunt and Kasting⁴ suspended excised nail over salt solutions, which created different relative humidities to examine the effect of hydration on ketoconazole diffusivity in human nail.

The objectives of the present study were twofold. First, this study was performed to further elucidate the effects of hydration on nail permeability. Second, this study was conducted to

help identify potential organic solvents that could be used in a topical drug formulation for the treatment of nail diseases to improve drug solubility, but with minimal nail dehydration. In addition, the present study provided the data that could assist the selection of an aqueous organic solution as a model system with comparable results to those of Gunt et al.¹⁰ to control nail hydration for future mechanistic studies of transungual transport. Gravimetric uptake studies and ³H–water uptake studies were first performed to examine the effects of organic solvents on the properties of the nail plate and to estimate nail hydration. Uptake and transport studies were then performed with ³H–water, ¹⁴C–tetraethylammonium (¹⁴C–TEA), and ¹⁴C–urea (¹⁴C–UR) to determine the impact of organic solvents on passive transport of neutral and positively charged permeants.

MATERIALS AND METHODS

Materials

Ethanol (EtOH, ~95% ethyl alcohol and <1% each of methyl alcohol, methyl isobutyl ketone, and isopropyl alcohol), propylene glycol (PPG), and Carbowax[®] polyethylene glycol 400 (PEG) were obtained from Fisher Scientific (Fair Lawn, New Jersey). Phosphate buffered saline (PBS) tablets (0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride; pH 7.4) were purchased from Sigma–Aldrich (St. Louis, Missouri). Sodium chloride (NaCl, 99% purity) and tetraethylammonium chloride (TEACl, 99% purity, water content of ~10%, w/w) were obtained from Acros (Morris Plains, New Jersey). Silicone (MED-6033), used to construct nail adaptors, was from NuSil Silicone Technology (Carpinteria, California). ¹⁴C–TEA (1–¹⁴C, 1–5 mCi/mmol) and ³H–water (1.0 mCi/mL) were purchased from PerkinElmer Life and Analytical Sciences (Boston, Massachusetts). ¹⁴C–UR (50–60 mCi/mmol, 0.1 mCi/mL) was purchased from Moravek Biomaterials and Radiochemicals (Brea, California). The radioactive materials had a purity of at least 97%. All materials were used as received.

Solution Preparation and Characterization

Binary solvent systems were prepared by mixing EtOH, PPG, or PEG with distilled, deionized water (dI water) to the desired concentrations of 25%, 50%, 75%, and 90% organic solvent by volume. Prior to mixing, NaCl or TEACl was added to the aqueous phase in all binary solvent systems to obtain a final salt concentration of 0.075 M in the binary solutions. 0.075 M represented the maximum amount of NaCl that could be readily dissolved in the organic aqueous solutions prepared in the present study. NaCl solution served as the main control throughout the experiments and was prepared by mixing 0.075 M NaCl in dI water. PBS of pH 7.4 served as a control in the gravimetric studies and was prepared by dissolving PBS tablets in dI water as per manufacturer specifications. The final pH and conductivities of the solutions were determined using a pH/CON 510 benchtop meter (Oakton Instruments, Vernon Hills, Illinois). The densities of the solutions were determined gravimetrically by weighing 1 mL of the solution dispensed by a calibrated pipette on a mass balance. The viscosities of the solutions were measured using an Ostwald viscometer (Barnstead International/Thermo Scientific, Dubuque, Iowa) and the relationship:

(1)

where η is the viscosity, ρ is the density, t is the retention time, and the subscript r refers to a reference sample, which in this case is dI water.

The organic solvents EtOH, PPG, and PEG were selected because of their miscibility with water, low cost, low toxicity, and tolerable odor. All three organic solvents studied have high polarities and functional groups available for hydrogen bond formation to water. It is acknowledged that the behavior of PEG is more complex than that of EtOH and PPG because it is a polymer. Nevertheless, for simplicity, EtOH, PPG, and PEG will all be referred to as organic solvents. These organic solvents are commonly used at various concentrations in the transdermal drug and cosmetics industries, and are approved by the FDA. Other solvents such as ethyl acetate, acetone, and acetonitrile were considered in a preliminary study (data not shown), but excluded. Ethyl acetate has low to moderate water solubility and is a known skin irritant. Likewise, acetone and acetonitrile are skin irritants. Acetone also has high volatility, whereas acetonitrile has an unpleasant odor.

Nail Sample Preparation

Frozen, human cadaver fingernails (male, age 52–90 years) were acquired from Science Care Anatomical (Phoenix, Arizona). Nail plates were inspected visually for abnormalities such as cracking or discoloration and were stored in a -20°C freezer until use. Prior to use, the nails were thawed at room temperature in PBS. The nails were then cleaned with forceps to remove residual tissues, rinsed with PBS, and wiped dry with Kimwipes[®]. The thickness of the nail plates was measured using a micrometer (Mitutoyo, Kawasaki, Japan); the thickness range was 0.45–0.62 mm. For use in the hydration and uptake studies, the nail plates were cut into three to five nail samples. These cuts were always parallel to the line of growth, that is, from what would have been the attached end to the free end. The use of human tissues was approved by the Institutional Review Board at the University of Cincinnati (Cincinnati, Ohio).

Nail Gravimetric Studies

The gravimetric studies were divided into two stages (Fig. 1). During the first stage (Stage I), clean nail samples were weighed and immersed in 1-mL aliquots of test solution. Prior to equilibration, the nail samples were dried in a 60°C oven for 24 h to obtain an initial dry weight. The test solutions included all 12 binary solvent systems (25%, 50%, 75%, and 90% EtOH, PPG, and PEG with 0.075 M NaCl) and the three neat organic solvents. NaCl solution and PBS served as the controls. After equilibration for 3 days (Stage IA) and 14 days (Stage IB), the nail samples were removed from the solution, blotted dry with Kimwipes[®] (Kimberly-Clark Professional, Roswell, Georgia), and quickly weighed (i.e., wet weight, w_{wet}). Subsequent to Stage IB, the nail samples were dried in a 60°C oven until a constant weight was achieved (i.e., dry weight, w_{dry}). The specific uptake volume (V_{sp}) in the nail samples is defined as the difference in the wet and dry weights, normalized by the dry weight of the nail and corrected by the density of the test solution (ρ_{test}):

(2)

Immediately after Stage I, the nail samples were soaked in 1 mL NaCl solution for 48 h in Stage II. After removal from this solution, the nails were wiped dry, weighed, and oven dried overnight. Stage II was performed to assess the integrity and reversibility of the nails after the treatment with the aqueous organic test solutions. Water contents (expressed as V_{sp}) were determined using Eq. 2. Both uptake and hydration were performed at room temperature ($20 \pm 2^\circ\text{C}$) and at a relative humidity of $35 \pm 10\%$.

Partition Studies

Clean nail samples with known weights were oven dried at 60°C until a constant dry weight was achieved (in less than 24 h). The nail samples were then equilibrated in 3 mL of test or control solution for 48 h at room temperature. ^{14}C -UR, ^{14}C -TEA, and ^3H -water were selected as the probes: ^{14}C -UR represents a polar neutral molecule, ^{14}C -TEA a positively charged molecule, and ^3H -water a small polar molecule that can provide insights into nail hydration. Prior to nail immersion, the test and control solutions were spiked with 0.5 μCi ^3H -water, ^{14}C -TEA, or ^{14}C -UR. The test solutions used in the uptake studies matched those used in the nail gravimetric studies. NaCl solution without an organic solvent served as the control. After equilibration, the nail samples were removed from the solution and blotted dry with Kimwipes[®]. The nails were then weighed and transferred to the extraction solution. Extractions were performed in 1 mL of NaCl solution for 24 h. This extraction procedure was repeated until the extracted amount was less than 10% of the amount in the first extraction; generally two extractions were sufficient. Following extraction, the nail samples were dissolved using 2 mL of Solvable[®] (PerkinElmer Life and Analytical Sciences, Shelton, Connecticut) in an oven at 50°C for 48 h. Ten milliliters of liquid scintillation cocktail (Ultima Gold[™], PerkinElmer Life and Analytical Sciences) were added to each extraction solution and the samples were assayed by a liquid scintillation counter (Beckman Coulter LS6500, Fullerton, California). Twenty microliters of equilibration solution were also withdrawn and mixed with 1 mL of NaCl solution and 10 mL of liquid scintillation cocktail for liquid scintillation counting. Fifteen milliliters of liquid scintillation cocktail were added to the dissolved nails to reduce counting efficiency loss by the Solvable[®]. Prior to counting, all samples were placed in a dark box overnight to reduce the chemiluminescence effects incited by Solvable[®]. The loss of counting efficiency due to the addition of Solvable[®] was checked with standards and found to be generally less than 5%. The whole extraction procedure was also checked in a recovery experiment. Total recovery of the extraction method for ^3H -water, ^{14}C -TEA, and ^{14}C -UR was found to be approximately 85–100%. The partition coefficient (K) was calculated and expressed as the ratio of the total amount of permeant per mass of the dried nail samples to the amount of permeant per gram of the bulk equilibrating solution:

(3)

where A_{Total} is the total amount of permeant extracted and C_{eq} is the concentration of permeant (w/w) in the equilibrating test solution. The dry weight of the nail is used in Eq. 3 to avoid the interference of the organic solvent effects on the nail wet weight in the analysis of the effects of the organic solvents upon permeant partitioning.

Transport Studies

The impact of the organic solvents on passive ^3H -water transport across nail was investigated *in vitro* utilizing the five-stage treatment protocols described in Figure 2a. The five stages were alternated between three control stages and two treatment stages. During the control stages (Stages I, III, and V), the nail was treated on both sides with NaCl solution. During the treatment stages (Stages II and IV), the nail was treated on both sides with the binary organic aqueous solutions of 0.075 M NaCl prepared using EtOH, PEG, or PPG and dI water. The concentrations of the organic solvents were 25% and 75% (v/v), respectively (six test solutions). All donor solutions were mixed with 1 μCi ^3H -water per milliliter. All stages had durations of 12 h. Between the stages, the diffusion cells were rinsed three times and the nails were allowed to equilibrate in the solution of the upcoming stage for 12 h.

The impact of the organic solvents on passive TEA transport across nail was investigated *in vitro* through the two-stage treatment protocols described in Figure 2b. The nail was treated on both sides with one of the six test solutions (25% or 75% EtOH, PPG, or PEG) during Stage I, and with NaCl solution during Stage II. A two-stage control protocol was performed in parallel, in which nails were treated with NaCl solution during both Stages I and II. All donor solutions were mixed with 0.5 μCi ^{14}C -TEA per milliliter. Both stages had durations of 84 h. Between Stages I and II, the donor and receptor chambers were rinsed and the nails were allowed to equilibrate in the control solution for 48 h. To ensure that both the organic solvents and permeant were removed, the solution in the chambers was replaced with fresh solution periodically during this equilibration period.

In all transport experiments, the nail plates were mounted between side-by-side diffusion cells using custom-made silicone nail adaptors such that the dorsal nail plate faced the donor chamber. The diffusion cells had a solution volume of 2 mL and an effective diffusion area of 0.64 cm^2 . Constant stirring and room temperature ($20 \pm 2^\circ\text{C}$) were maintained during both the equilibration periods and transport experiment stages. The donor and receptor chambers were sealed with Parafilm® (Pechiney Plastic Packaging, Menasha, Wisconsin) to prevent solvent evaporation. Prior to the transport experiments, the nails were equilibrated for 24 h in test or control solution. ^3H -water and TEA transport experiments were then performed according to the five-stage and two-stage protocols described above, respectively. During transport, samples were withdrawn at predetermined time intervals. Specifically, 10 μL of the donor solution and 1 mL of the receptor solution were withdrawn and 1 mL fresh solution was added to the receptor to maintain a constant volume in the receptor chamber.

Ten milliliters of liquid scintillation cocktail were added to each sample vial and the samples were assayed by the liquid scintillation counter. The cumulative amount of permeant delivered across the nail (Q) was plotted against time (t). The steady-state flux (J) was calculated using the slope of the linear portion of this curve and the diffusion area (A):

$$J = \frac{Q}{A \cdot t} \quad (4)$$

Statistical Analysis

Data were analyzed using SAS[®] 9.1 Software (SAS Institute Inc., Cary, North Carolina) after outliers were removed. Specifically, one way and two way analyses of variance were performed using the proc glm function, and significant differences were confirmed using the lsmeans/pdiff function, which executes all pairwise comparisons. Statistically significant differences were established when p value was less than 0.05. Outliers were identified using the quartile or fourth spread method in Microsoft[®] Excel 2007 (Microsoft Corporation, Redmond, Washington). Overall, less than 10% of the experimental values were removed and no more than one value was removed from a given set of data. The means $\pm$ standard deviations of the data are presented.

Theory and Equations

The diffusion of a molecule in solution can be affected by the temperature and viscosity of the solution as well as the size, charge, and concentration of the diffusing molecule. Under ideal condition, the free diffusivity (D_i) of a charged molecule in a solution is related to the effective electromobility of the molecule by the Einstein equation²³:

$$D_i = \frac{RT}{z_i F} u_i \quad (5)$$

where F is the Faraday constant, R_{gas} is the gas constant, T is the absolute temperature, z_i and u_i are the valence of the molecule and its effective electromobility, respectively, and subscript i refers to molecule species i . In a solution with a low Reynolds number and assuming that the molecule is spherical in shape, the diffusivity of the molecule can be determined using the Stokes-Einstein equation²⁴:

$$D_i = \frac{k_B T}{4 \pi \eta r_i} \quad (6)$$

where k_B is the Boltzmann constant, η is the solution viscosity, and r_i is the Stokes-Einstein radius of the molecule. The free diffusivity of a charged molecule in a solution is also related to the molar conductivity of the solution (Λ) by the Nernst-Einstein equation:

$$D_i = \frac{RT}{F} \frac{\Lambda}{z_i} \quad (7)$$

In transport across a porous membrane, the effective diffusivity of a charged molecule or its membrane diffusivity is related to the free diffusivity of the molecule. The effective diffusivity can be calculated from the steady-state flux of the molecule (J) across a membrane in transport experiments. According to Fick's laws, the steady-state flux is proportional to the effective diffusivity taking into account factors such as membrane thickness (h) and concentration of the permeant in the donor chamber (C_D). Hence, the permeability coefficient (P) is related to the effective diffusion coefficient (D_{eff}) and effective partition coefficient (K_{eff}) as¹⁹

$$P = K_{\text{eff}} D_{\text{eff}} \quad (8)$$

where K_{eff} is related to the amount of permeant in the nail (A_{Total}), nail wet weight, and permeant concentration in the donor chamber:

$$K_{\text{eff}} = \frac{A_{\text{Total}}}{W_{\text{nail}} C_D} \quad (9)$$

The permeability coefficient (P) can be further expressed as

$$P = K_e K_H \epsilon_p D_H r_i \quad (10)$$

where K_e is partition coefficient due to charge-charge interactions, K_H is the partition coefficient related to other permeant-to-nail interactions such as size exclusion in the transport pathway, ϵ_p is the combined porosity and tortuosity factor for the membrane (porosity divided by tortuosity), and D_H describes the hydrodynamic part of the hindered transport factor for diffusion.²⁵

The relationship between permeability and viscosity can be highlighted by substituting the expression for permeant diffusivity given in Eq. 6 into Eq. 10 and taking the logarithmic value of that expression such that

$$\log P = \log K_e K_H \epsilon_p D_H r_i + \log D_{\text{eff}} \quad (11)$$

A plot of $\log P$ versus $\log 1/\eta$ will be a straight line with a slope of unity for an inert membrane under the condition when the parameters ϵ_p , D_H , K_e , K_H , and r_i are constant.

RESULTS

Effects of Organic Solvents on Solution Properties

The properties of the binary organic aqueous solvent systems and the neat solvents used in these studies are given in Table 1. PBS data is provided as a control. All solutions were clear

and colorless except the TEA solutions of greater than or equal to 50% PEG, which were clear and yellow. For all solutions studied, the pH values measured were between 4 and 8. These values reflect the use of dI water and the organic solutions without the presence of a buffer. Similarly, the densities of all solutions studied were within 20% of 1 g/mL with the EtOH solutions being on the lower end and the PEG solutions being on the higher end of the density spectrum. The viscosity of the systems was related to the organic solvent concentration. For PPG and PEG, viscosity increased with an increase in the organic solvent concentration of the system. For EtOH, viscosity was parabolic with variations between 1 and 3 cP and maxima at around 50% EtOH. For the organic solvent systems studied and NaCl and TEACl control solutions, the conductivity was also a function of the concentration of the organic solvent. Conductivity was observed to decrease when the organic solvent concentration in the system increased.

Effects of Organic Solvents on the Specific Uptake Volume of the Nail

Figure 3 presents the ratio of the specific uptake volumes of the nail under a test condition of EtOH, PPG, or PEG compared with the nail treated with NaCl solution (the baseline). No significant difference was found between the average V_{sp} in NaCl solution and that in PBS. The figure shows an inverse relationship between the nail specific uptake volume ratio and the concentration of the organic solvent of the test solution used during Stages IA and IB in all systems. Specifically, the specific uptake volume of the nail decreased when the concentration of the organic solvent increased. For all organic solvents studied, the specific uptake volume of the nail returned to within $\pm 30\%$ of the control levels during Stage II, suggesting that the effects of the organic solvent are not irreversible and the exposure to organic solvents does not significantly affect the integrity of the nail. Likewise, all dry weights of the nails were within $\pm 10\%$ of their initial values after the organic solvent treatments. It should be noted that at high organic solvent concentrations, curling of the nail samples was observed. This effect disappeared when the nails were rehydrated during Stage II.

Effects of Organic Solvents on the Partitioning of Water, TEA, and UR into Nail

Figure 4 demonstrates the effect of neat and diluted EtOH, PPG, and PEG on the partitioning of water, TEA, and UR into human nail. The partition coefficients for water, TEA, and UR into nail from NaCl solution (the control) according to Eq. 3 were 0.7 ± 0.1 , 0.8 ± 0.1 , and 0.7 ± 0.4 , respectively. For water in all solvent systems, the partition coefficients of water into the nail at the 25% organic solvent concentration were essentially the same as the control and increased slightly with an increase in the concentration of the organic solvent between 50% and 75%. The effects of the organic solvents upon water partition coefficient were different at the concentration of 90% ($p < 0.05$). For TEA in EtOH, PPG, and PEG and UR in PPG and PEG, the results show a general trend of decrease in the partition coefficients of TEA and UR with an increase in EtOH, PPG, and PEG concentration. As the concentration of the solvents increased, the effects of the solvents became apparent. For UR in EtOH, the partition coefficients for the 75%–100% EtOH conditions were elevated over those at 25% and 50% EtOH. It should be pointed out that the control TEA uptake value (i.e., 0% organic solvent) is lower than the literature values¹⁹ due to the difference in the definitions of partition coefficients and the equations used in the

calculations. When TEA uptake was calculated using the method in the previous study, the value increased to 1.1 ± 0.1 and was consistent with the previous result.

Effects of Organic Solvents on the Transport of ^3H -Water across Nail

Figure 5 shows the permeability coefficients of the nail for water in the binary EtOH-, PPG-, and PEG-water solvent systems. At the solvent concentrations studied, EtOH had a small effect on the permeability of nail to water. Specifically, the permeability coefficients of water across nail decreased by ~20 to 50% at 25% and 75% EtOH. PEG at 25% also had a small effect (~30%) on the permeability of the nail to water. None of these values differed with statistical significance from the three control (NaCl solution) stages. At 25% PPG, 75% PPG, and 75% PEG, however, the organic solvent had a statistically significant impact on the permeability of nail to water. The permeability coefficients of water decreased by approximately 2 to as much as 80 times in these organic solvent systems. All reduction in permeability resulting from the organic solvent treatments was reversible as evidenced by the comparable permeability coefficients of the nail for water in all control stages ($p > 0.05$).

Effects of Organic Solvents on the Transport of TEA across Nail

Figure 6 presents the permeability of nail to TEA in the presence of (a) binary solvent systems at 25% and 75% EtOH, PPG, and PEG and (b) NaCl solution following the treatment with these binary organic aqueous solvent systems. The permeability coefficients from the two-stage control experiment (Controls 1 and 2; $1.0 \pm 0.6 \times 10^{-7}$ and $1.1 \pm 0.7 \times 10^{-7}$ cm/s, respectively) are also shown in Fig. 6b to check for nail integrity during the lengthy procedures in these transport experiments. All three organic solvents, regardless of concentration, had a statistically significant impact on the permeability coefficients of TEA across nail (Fig. 6a). Particularly, the nails treated with EtOH had permeability coefficients for TEA approximately an order of magnitude lower than the control, whereas the permeability coefficients in PPG and PEG systems were 10–40 times lower than the control. Despite the rather large reduction in TEA permeability, the effects of the organic solvents were at least partially reversible (Fig. 6b). All permeability coefficients for TEA determined in Stage II in which the nail was treated with the control solution after the organic solvent solution experiment were within 50% of the controls in Stages I and II.

DISCUSSION

Effects of Organic Solvents on Permeant Mobility

Solution conductivity is a measure of how well a solution transmits electricity and is therefore directly related to the ion concentration and the mobility of the ions in the solution. Because the mobility of the ions is related to their diffusion coefficients (Eq. 5), solution conductivity can be used to measure the diffusion coefficients of the ions in the solution (Eq. 7). As was demonstrated in the present and other studies, organic solvents affect the conductivity of the solution. Particularly, the conductivity decreased as the concentration of the organic solvents increased in the present study. In Table 1, because the ion concentrations of NaCl and TEACl are the same in both NaCl and TEACl solution systems, the difference between the NaCl and TEACl conductivity reflects a difference in the mobilities of Na and TEA due to the different Stokes–Einstein radii of Na and TEA—

roughly 0.25 and 0.36 nm,²⁶ respectively. The decrease in the conductivity of NaCl and TEACl solution with increasing organic solvent concentrations in the systems can be attributed to the increase in the viscosity (η) of the systems; the diffusivity of a solute is inversely proportional to the viscosity of the solution (Eq. 6). Figure 7 shows the ratios of the conductivity of the test solutions to that of the control solution versus the concentration of EtOH, PPG, and PEG in the solutions. The ratios of the inverse of the solution viscosity ($1/\eta$) of the test solutions to that of the control versus EtOH, PPG, and PEG concentrations are also plotted in the same figure for comparison. From the figure, the viscosity of the organic solvent systems of NaCl is essentially the same as those of TEACl at the same organic solvent concentrations as the relative low ion concentrations in the solution are not expected to significantly affect the solution viscosity. The solution conductivity ratio profiles of NaCl and TEACl systems generally also follow the same trend. The similarity between the NaCl and TEACl conductivity profiles suggests similar effects of the organic solvents upon Na mobility in the solution to that of TEA at the same organic solvent concentration.

In Fig. 7, according to the Stokes–Einstein relationship, the superimposition of the plot of $1/\eta$ ratios and that of the conductivity ratios is anticipated. The deviation between the conductivity and viscosity plots suggests that mechanisms such as a change in the Stokes–Einstein radii and/or association of the ions in the solution could be involved. From Eqs. 6 and 7, the higher conductivity than that expected from the change in $1/\eta$ when the organic solvent concentration increases in Fig. 7 signifies a decrease in the hydrodynamic radii of the ions. Although the decrease in the dielectric constant of the solution due to the incorporation of the organic solvent could lead to the formation of ion pairs, the data suggest that ion pair formation that significantly affects TEA mobility was unlikely in the present PPG and PEG organic solvent systems. For example, solution conductivity will decrease when ion pairs are formed because ion pairs have larger Stokes–Einstein radii and are uncharged. This would have an opposite effect to the deviations generally observed in the $1/\eta$ and conductivity plots in Figs. 7b and 7c. In summary, the mobilities of charged molecules in a solution decrease in the presence of organic solvents such as EtOH, PPG, and PEG, and this could affect the permeation of the molecules across a porous membrane such as the nail plate.

Effects of Organic Solvents on Nail Hydration and Uptake Properties

Previous hydration studies have suggested that the water content in the nail plate can be used as a marker to screen for the effectiveness of transungual delivery systems under the assumption of a correlation between nail hydration and its permeability.^{27,28} The present study employed a similar method of gravimetric measurements to study the effects of organic solvents upon the specific uptake volume of the nail, assuming that the specific uptake volume represents the uptake of both the organic solvent and water into the nail (i.e., liquid content or porosity of the nail). The specific uptake volume of the nail decreased as the concentration of the organic solvent increased in the solution, suggesting a decrease in the volume of the solution filled pores available for the permeation of ions and drugs into the nail structure. The specific uptake volume versus the organic solvent concentration relationship can generally be explained by the reduction of water activity in the solvent

system. As the concentration of the organic solvent in the solution system increases, the activity of water and the driving force decrease for water uptake into the nail. In addition, the specific uptake volume of the nail was shown to be solvent dependent (on the type of the organic solvent) in the present study. The profiles of the changes in specific uptake volume of the nail treated with PPG and PEG solutions show similar effects, but differ from the nail treated with EtOH solutions (Fig. 3).

The effects of the organic solvents on the uptake of a neutral and positively charged permeants into the nail were also investigated. Similar trends were observed for the partitioning of TEA and UR into the nail even though the molecular size of UR is considerably smaller than that of TEA. This suggests permeant-to-pore charge interactions for TEA.¹⁹ The partition coefficients of ³H-water into the nail determined using Eq. 3 show a different trend from those of TEA and UR (Fig. 4). This can be attributed to the size exclusion effects upon permeant partitioning into the nail. The essentially same partition coefficients of ³H-water in the 0%–75% organic solvent systems in Fig. 4 suggest that the water content in the nail (or nail hydration) is generally proportional to the activity of water in the solvent system; that is, when the activity of water decreases with the dilution of water in the equilibrating solution, the amount of water in the nail decreases. This decrease in nail hydration could therefore lead to the decreasing partition coefficient versus organic solvent concentration profiles observed in the TEA and UR uptake experiments. In summary, when the concentration of the organic solvent increases in the equilibrating solution, the specific uptake volume, nail hydration, and permeant partition coefficient into the nail decrease. This supports the hypothesis of a correlation between nail hydration and permeant partitioning into the nail.

It should be pointed out that the results of the nail exposed to NaCl solution in the control experiments (0.7 ± 0.1 , 0.6 ± 0.1 , and 0.6 ± 0.1 g water/g dry nail for Stages IA, IB, and II) in the present study are higher than our previously reported value of 0.4 ± 0.1 g water/ g dry nail.^{8,18,19} These previous results were comparable to the sorption-desorption results reported by Gunt and Kasting.¹⁷ A number of reasons related to the experimental procedure (e.g., the drying and weighing of the nail plate) were considered in an effort to explain the discrepancy and later disproven by the validation of the procedure. It was therefore concluded that the increase in nail hydration compared to previous results is likely due to nail-to-nail variability. To ensure the best possible analysis of the data, the nail-to-nail variability was assumed to be constant throughout each set of experiments such that the control samples were always run in parallel with the test conditions. Additionally, the data were analyzed as ratios of the test values to the control values from NaCl solution.

In the current study, the observed visual curling of the nail samples exposed to organic solvents, particularly at high organic solvent concentrations, suggests protein denaturation. It is possible that the organic solvents can denature or disrupt the hydrogen bonds within the secondary and tertiary structures of the ungual proteins. However, during Stage II in the nail gravimetric studies and the control stages in the water transport experiments, the specific uptake volume and the permeability coefficients of the nail returned to the control values, respectively, suggesting that the effect of organic solvents is reversible; the overall primary

structure of the nail is maintained such that the secondary and tertiary protein structures return when the organic solvent is removed from the system.

Relationships between Permeant Diffusion, Nail Barrier, and Nail Permeability

The results in the present study demonstrate the effects of solution viscosity upon permeant electromobility and hence, its diffusion in solution. This is likely to affect the permeability of the nail plate (Eq. 10). Although the trend of decreasing solution $1/\eta$ (or conductivity) in Fig. 7 is consistent with that of the decreasing nail permeability when the organic solvent concentration increases in the solution, the increase in solution viscosity generally does not fully account for the decrease in the permeability of the nail in the present study. Figure 8 shows the log–log plot of TEA permeability versus solution $1/\eta$ (Eq. 11). As seen in this figure, the extent of the decrease in nail permeability is greater than that of solution $1/\eta$ when the organic solvent concentration increases in the solution systems. This can be explained by the alteration of the nail barrier such as a decrease in nail hydration, a decrease in the effective porosity of the nail, and/or an increase in transport hindrance of the permeation pathway in the nail. These hypotheses are further supported by a decrease in the available uptake volume in the nail and the corresponding decrease in water, TEA, and UR partitioning into the nail in the presence of the organic solvents in the present study.

To further study the effects of organic solvents upon the barrier properties of the nail, the mean effective diffusivity of water across human nail was estimated using Eq. 8 and assuming that the nail plate is a homogenous membrane with a thickness of 0.05 cm. The mean effective diffusivity of water across human nail was found to be between 4×10^{-9} cm²/s under 75% PEG conditions and 1×10^{-7} cm²/s under full hydration (NaCl solution control). These values are within the range of water diffusivity across nail under hydration at a water activity of 0.3–1.0 calculated using the free volume theory.¹⁰ The effective diffusivity of TEA across the nail ranged from 3×10^{-10} to 1×10^{-8} cm²/s (from under 75% organic solvent conditions to under NaCl solution control), which was approximately an order of magnitude lower than those of water for all conditions studied. The difference between nail permeability to water and TEA can be attributed to size exclusion and hindered transport of the permeants across the nail plate. As solution conductivity reflects the mobility of an ion in a solution and hence its diffusivity in the solution, the effective diffusivity of TEA across the nail was plotted against TEACl solution conductivity (Fig. 9) to examine the effect of hindered transport upon transungual TEA permeation. In theory, when the mobility of TEA in the organic solvent system decreases, TEA diffusivity in the solvent-filled pores in the nail would decrease proportionally if the nail barrier structure remains the same (e.g., same hindered transport). The line in Fig. 9 represents this proportional relationship relative to the permeability of fully hydrated nail for TEA in NaCl solution (control). The deviation of the effective diffusion coefficient data from the line in the figure suggests stronger transport hindrance on transungual TEA permeation in the organic solvent systems compared with that in the control. However, when the organic solvent concentration increased, smaller data deviation from the line was observed. This could be due to a lack of penetration of the organic solvent into the nail (a result of organic solvent exclusion from the nail) or a decrease in transport hindrance (due to nail barrier

alteration) at higher organic solvent concentrations. Future work is needed to investigate this behavior.

Formulation Factors and Clinical Implications

Antifungal agents and other medications used to treat nail diseases are predominantly large and lipophilic molecules, which are practically insoluble in water. Accordingly, topical nail formulations usually use a combination of one or more organic solvents to increase drug solubility. In the present study, EtOH, PPG, and PEG reduced nail hydration and permeability. Owing in part to data variability, it is difficult to define the interactions and exact relationships between the organic solvent systems and nail. Additionally, developing such relationships into a predictive model would require a mechanistic examination of the physicochemical properties of the solvent systems using other experimental techniques and perhaps several other solvent systems. Nevertheless, two practical implications arise from these results. First, any increase in drug solubility engendered by adding an organic solvent to a formulation to improve transungual drug delivery may be counteracted by the reduction in nail hydration and permeability caused by the solvent itself. Increasing the aqueous solubility of antifungal agents and other medications used in the treatment of nail diseases may therefore be best achieved through other approaches such as modulating the pH of the formulation. Second, binary aqueous organic solvent systems can be used in the methodology of *in vitro* assessments of *in vivo* nail permeability. By varying the concentrations and nature of the organic solvents, one would be able to select the water activity to which the nail is exposed. For example, according to the water permeability data in the present study and the results of Gunt et al.,¹⁰ it can be determined from the effective diffusivity of water across nail versus water activity relationship that the nail samples in the present experiment mimicked the water activity conditions of 0.3–0.4 for 75% PPG and PEG, 0.6–0.8 for 25% PPG and PEG, and 1 (liquid phase) for control and EtOH conditions.

CONCLUSIONS

The effects of the organic solvents EtOH, PPG, and PEG on the barrier properties of nail were determined. Permeant partitioning into and transport across the nail were shown to decrease as the concentration of the organic solvent in the binary solvent system increased. Decreases in permeant uptake and transport were due to both solvent–solution and solvent–nail interactions. More specifically, the changes in the solution properties such as viscosity and changes in the nail barrier properties such as nail porosity and hindered transport due to a decrease in nail hydration contribute to the observed decreases in nail partitioning and permeability compared with the NaCl solution control. In general, EtOH had a lesser impact on the barrier properties of the nail than PEG and PPG, indicating the interplay between molecular size of the solvent, solution viscosity, and their effects on nail porosity. Two important findings arise from these results. First, the direct contact of organic solvents to the nail plate in transungual delivery formulation may result in increased barrier resistivity of the nail. Second, organic solvent systems may be useful in manipulating nail hydration.

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Figure 1.

Schematic for the multistage protocol used in the gravimetric uptake study. Human nail clippings were equilibrated in test solution during Stages IA and IB and equilibrated in control solution during Stage II. Test solutions were prepared from ethanol (EtOH), propylene glycol (PPG), or polyethylene glycol 400 (PEG) and water to 25%, 50%, 75%, and 90% v/v; 100% EtOH, PPG, or PEG was also used as test solution in Stages IA and IB. The control solution was water with 0.075 M NaCl.

Figure 2.

Diagrams for the multistage protocols for transport of (a) ^3H -water and (b) ^{14}C -tetraethylammonium ion (TEA) across nail plates *in vitro*. Protocols and time points (in hours) are provided in chronological order from left to right. Fully hydrated nails were mounted between side-by-side diffusion cells prior to the start of the transport experiments. The nail plates were subjected to equilibration in test and control solutions. Water served as the control solution, whereas EtOH, PPG, or PEG at concentrations of 25% or 75% (v/v) served as the test solutions; 0.075 M NaCl was added to all solutions.

Figure 3.

Transungual solvent uptake as a function of the concentration of (a) EtOH, (b) PPG, and (c) PEG. Uptake values are presented as the ratio of the specific uptake volume (V_{sp}) of the nail under the test conditions versus the control hydration condition of 0.075 M NaCl in distilled, deionized water. Symbols: Stage IA (closed diamond), Stage IB (open diamond), and Stage II (closed square). Control V_{sp} value was 0.6 ± 0.1 mL solution/g dry nail from averaging the three V_{sp} values of 0.7 ± 0.1 , 0.6 ± 0.1 , and 0.6 ± 0.1 mL solution/g dry nail in Stages IA, IB, and II, respectively. The V_{sp} values in PBS were 0.5 ± 0.1 , 0.6 ± 0.1 , and 0.7 ± 0.1

mL solution/g dry nail in Stages IA, IB, and II, respectively. Data represent the mean and standard deviation of three to four nail samples.

Figure 4.

Partition coefficients of (a) water, (b) tetraethylammonium ion, and (c) urea into nail treated with neat and diluted ethanol (EtOH, open diamond), propylene glycol (PPG, open square), and polyethylene glycol 400 (PEG, open triangle) calculated using Eq. 3. Control data—presented as 0% organic—represent partitioning of each permeant into nail from NaCl solution. All solutions except the neat organic solvents had 0.075 M NaCl. Data represent the mean and standard deviation of three to nine nail samples.

Figure 5.

Permeability coefficients of water across nail plates treated on both sides with water (Controls 1–3) as well as (a) EtOH, (b) PPG, and (c) PEG diluted to 25% and 75% (v/v) with water. All solutions had 0.075 M NaCl. Data represent the mean and standard deviation of three to four samples.

Figure 6. Permeability coefficients of TEA across nail plates treated with (a) binary organic aqueous solvent systems prepared from EtOH, PPG, or PEG and (b) control (NaCl solution) following those treatments to test reversibility. All solutions had 0.075 M NaCl. Data represent the mean and standard deviation of three to four samples.

Figure 7.

Plots of the ratio of solution properties versus the concentration of (a) EtOH, (b) PPG, and (c) PEG mixed with water and 0.075 M NaCl or TEACl salt. Conductivity for NaCl solutions (closed diamond) and TEACl solutions (closed square) are presented as the ratio of the conductivity for the test condition to the control solution; 1/viscosity of NaCl solutions (open diamond) and TEACl solutions (open square) are presented as the ratio of the viscosity of the control solution to the test conditions. Data represent the mean of three to nine samples.

Figure 8.

Plot of TEA permeability coefficients versus $1/\text{viscosity}$ for 25% and 75% EtOH (open diamond), PPG (open square), and PEG (open triangle) solutions containing 0.075 M NaCl. The dashed line represents a proportional relationship between permeability and the inverse of the solution viscosity from the control condition in which nail was treated with NaCl solution (closed diamond). This line is presented to show the relationship derived from Eq. 11 (extended from the NaCl control data point) with an assumption that the nail barrier property is not affected by the organic solvents during the experiments and the deviation of the experimental data from this theoretical line. Data represent the mean of three to nine samples.

Figure 9.

Plot of effective diffusion coefficients of TEA in nail permeation versus the conductivity of 25% and 75% EtOH (open diamond), PPG (open square), and PEG (open triangle) solutions with 0.075 M TEACl. The dashed line represents a proportional relationship between effective diffusivity and solution conductivity from the control condition in which nail was treated with NaCl solution (closed diamond). This line is presented to show the relationship derived from Eq. 11 (extended from the NaCl control data point) with an assumption that the nail barrier property is not affected by the organic solvents during the experiments and the deviation of the experimental data from this theoretical line. Data represent the mean of three to nine samples.

Table 1

Properties of Binary Organic Aqueous Solvent Systems Prepared from Ethanol (EtOH), Propylene Glycol (PPG), or Polyethylene Glycol 400 (PEG) and Distilled, Deionized Water (dI Water)

	pH ^a	ρ^b (g/mL)	η^c (cP)	Conductivity ^d (mS/cm)
PBS ^e	7.2 ± 0.2	0.98 ± 0.01	— ^h	13.3 ± 1.6
Neat solutions ^f				
dI water	4.5 ± 0.1	1.00 ± 0.01	1	0.002 ± 0.002
EtOH	5.4 ± 0.3	0.80 ± 0.05	1.0 ± 0.3	0.05 ± 0.01
PPG	5.9 ± 0.2	1.03 ± 0.05	33 ± 4	— ^h
PEG	6.5 ± 0.1	1.14 ± 0.11	75 ± 8	— ^h
NaCl solutions ^g				
dI water + 0.075 M NaCl	5.4 ± 0.1	1.01 ± 0.01	1.1 ± 0.1	8.1 ± 0.1
25% EtOH	5.3 ± 0.1	0.98 ± 0.01	1.9 ± 0.2	6.2 ± 1.4
50% EtOH	5.1 ± 0.3	0.87 ± 0.01	2.4 ± 0.1	4.2 ± 1.0
75% EtOH	5.4 ± 0.3	0.85 ± 0.03	2.4 ± 0.1	3.1 ± 0.7
90% EtOH	4.6 ± 0.4	0.82 ± 0.01	1.8 ± 0.2	3.0 ± 0.7
25% PPG	5.2 ± 0.1	1.04 ± 0.01	2.7 ± 0.4	6.4 ± 1.7
50% PPG	4.4 ± 0.1	1.05 ± 0.04	5.4 ± 1.5	3.5 ± 0.7
75% PPG	4.7 ± 0.3	1.07 ± 0.01	9.8 ± 1.4	1.3 ± 0.1
90% PPG	6.5 ± 0.1	1.02 ± 0.04	14 ± 5	0.6 ± 0.1
25% PEG	4.8 ± 0.1	1.05 ± 0.01	3.5 ± 0.4	6.0 ± 1.2
50% PEG	4.5 ± 0.1	1.16 ± 0.15	9.5 ± 1.5	2.4 ± 0.1
75% PEG	4.7 ± 0.1	1.10 ± 0.08	31.8 ± 0.1	0.5 ± 0.1
90% PEG	5.0 ± 0.2	1.04 ± 0.06	57 ± 2	0.16 ± 0.01
TEACl solutions ^g				
dI water + 0.075 M TEACl	8.0 ± 0.4	1.01 ± 0.01	0.99 ± 0.01	5.78 ± 0.01
25% EtOH	6.3 ± 0.7	0.97 ± 0.01	2.2 ± 0.1	3.6 ± 0.1
50% EtOH	6.0 ± 0.3	0.98 ± 0.04	3.0 ± 0.1	2.3 ± 0.1
75% EtOH	5.1 ± 0.4	0.85 ± 0.01	2.37 ± 0.01	1.7 ± 0.1
90% EtOH	4.9 ± 0.5	0.82 ± 0.01	1.8 ± 0.1	1.6 ± 0.1
25% PPG	7.3 ± 0.1	1.05 ± 0.01	2.8 ± 0.1	2.9 ± 0.1
50% PPG	6.0 ± 0.3	1.03 ± 0.05	6.6 ± 0.1	1.2 ± 0.1
75% PPG	5.5 ± 0.3	1.01 ± 0.01	13.5 ± 0.1	0.5 ± 0.1
90% PPG	5.8 ± 0.2	1.04 ± 0.01	25.6 ± 0.3	0.5 ± 0.1
25% PEG	3.9 ± 0.1	1.02 ± 0.01	5.4 ± 0.3	3.6 ± 0.4
50% PEG	3.7 ± 0.1	1.08 ± 0.04	14 ± 3	1.2 ± 0.2
75% PEG	4.0 ± 0.1	1.11 ± 0.01	45.4 ± 0.1	0.4 ± 0.1
90% PEG	4.3 ± 0.4	1.15 ± 0.03	87 ± 1	0.15 ± 0.01

All measurements were made at 20 ± 2°C.

^aValue is apparent pH, mean $\pm$ standard deviation (SD), $n = 3-6$.

^bDensity, mean $\pm$ SD, $n = 3-6$.

^cViscosity, mean $\pm$ SD, $n = 3-9$.

^dSolution conductivity, mean $\pm$ SD, $n = 3-6$.

^ePhosphate buffered saline (PBS) is shown as a comparison with physiological conditions.

^fNaCl or TEACl was not added to the neat solvents.

^gNaCl or TEACl was added to the aqueous phase of the binary solvent systems prior to mixing for a final concentration of 0.075 M.

^hValue was not measured or not measurable.