# Self-Association of Nicotinamide in Aqueous Solution: Mass Transport, Freezing-Point Depression, and Partition Coefficient Studies[1]

**William N. Charman,[2,3] Christine S. C. Lai,[2] Barrie C. Finnin,[2] and Barry L. Reed[2]**

*Received July 30, 1990; accepted March 26, 1991*

The steady-state flux (SSF) of nicotinamide from an aqueous donor phase across a model Silastic membrane did not increase proportionally with increasing donor phase concentration. The suspected self-association of the drug in aqueous solution was evaluated by studying the concentration-dependent changes in (i) the molal osmotic coefficient of nicotinamide (freezing-point depression studies) and (ii) the partition coefficient between water and *n*-octanol. The freezing points of aqueous solutions of nicotinamide were measured and a plot of osmolality vs molality was nonlinear. The partition coefficient of nicotinamide, studied at 15, 25, and 32°C, also decreased with increasing concentration of drug. Mathematical models describing dimerization and higher orders of association were applied to the data. The results indicated the involvement of higher orders of association and it was found that an isodesmic (step-association) model was an adequate description of the freezing-point depression and partition coefficient data. The association constant, $K$, ranged between $1.59 \pm 0.02\ M^{-1}$ at the freezing point and $0.48 \pm 0.01\ M^{-1}$ as estimated from the partition coefficient data at 32°C. These models for the self-association of nicotinamide allowed estimation of the apparent concentration of "monomeric" nicotinamide in the donor phase solutions studied in the SSF experiments. When the SSF data were analyzed with regard to the concentration of monomeric nicotinamide in the donor phase, a relationship close to linearity was observed.

KEY WORDS: nicotinamide; steady-state flux; self-association; osmometry; partition coefficient; isodesmic.

## INTRODUCTION

Nicotinamide, the amide derivative of nicotinic acid, has potential as a therapeutic agent following topical administration to the skin. The therapeutic utility of nicotinamide includes the depigmentation of hyperpigmented skin and the treatment of acne vulgaris (1,2). The spectrum of activity observed with topically applied nicotinamide parallels that of nicotinic acid but, importantly, does not include the unwanted cutaneous flushing and vasodilation which are characteristic of nicotinic acid (3).

From a mechanistic standpoint, the *in vivo* penetration of a number of different esters of nicotinic acid has been studied by assessing the rate and extent of the induced vasodilation following their topical administration (4–6). These *in vivo* studies have enabled assessment of the relationship between the structure of the different nicotinate esters and the extent of percutaneous absorption. However, in terms of the clinical utility of topically applied nicotinic acid derivatives, it is the amide derivative which may prove most useful as it does not induce cutaneous flushing.

The percutaneous transport of nicotinamide has not been characterized to a sufficient extent to enable the rational design of topical formulations. Although the buccal transport of nicotinic acid and nicotinamide has been evaluated (7), the donor phase concentrations studied were one to two orders of magnitude below the concentrations employed in topical preparations.

The primary factor governing the transport of a drug across a membrane is the thermodynamic activity of the drug (in the formulation) at the surface of the barrier (8). Numerous formulation-related factors such as solubility, hydrogen ion concentration, cosolvent, and excipient selection can affect the rate and extent of transport from a topically applied formulation by changing the thermodynamic activity of the drug. A factor that can decrease the percutaneous transport of a drug is the formation of complexes or associated species of the applied drug (9,10). The formation of drug complexes or associated species will decrease the effective concentration of free drug in the formulation and hence lower the thermodynamic activity of the drug in the applied formulation.

Reports have suggested that nicotinamide (11,12) and structurally related compounds (13–15) can self-associate in aqueous solution. Therefore, attempts to optimize the transdermal transport of nicotinamide must include assessment of the suspected concentration-dependent self-association of the drug in aqueous solution.

The effect of self-association of nicotinamide on transport across a model membrane can be evaluated by determination of concentration-dependent changes in the steady state flux. Further, the self-association process can be investigated by determination of the concentration-dependent changes in the partition coefficient and freezing-point depression of nicotinamide.

The aim of the present work was to evaluate the mass transport of nicotinamide across a model membrane and the suspected self-association of nicotinamide in aqueous solution.

## MATERIALS AND METHODS

*Chemicals.* [*carbonyl*-$^{14}$C]Nicotinamide (sp act, 56 mCi/mmol) was obtained from Amersham International (U.K.), and unlabeled nicotinamide B.P. from Roche Products (NSW, Australia). Analytical reagent-grade sodium chloride, dextrose monohydrate, *n*-octanol, and isopropyl myristate were obtained from Ajax Chemicals (Melbourne, Australia). Silastic membrane (0.005 in. thick) was obtained from Dow Chemical Company (MI, USA), and prior to use, the powder coating was removed by washing with water. Scintillation fluid (Picofluor 30) was obtained from United Technologies Packard (Downers Grove, IL). Water was obtained from a Milli-Q (Waters Associates, Milford, MA) wa-

[1] Presented in part at the Fifth Annual Meeting of the American Association of Pharmaceutical Scientists, Las Vegas, Nevada, November 1990.

[2] School of Pharmaceutics, Victorian College of Pharmacy, Parkville, Victoria 3052, Australia.

[3] To whom correspondence should be addressed.

0724-8741/91/0900-1144$06.50/0 

ter purification system. All other chemicals were of standard analytical grade.

In Vitro *Diffusion Studies*. An automated flow-through cell diffusion apparatus was utilized to study the permeation of [$^{14}$C]nicotinamide across a standard Silastic membrane. The diffusion apparatus, originally described by Cooper and Berner (16), was based upon the recent modifications described by Akhter *et al.* (17). The membrane was sandwiched between the donor chamber and a flow-through receptor chamber. The volume of the donor chamber was 400 μl and the available area for diffusion was 78.5 mm$^2$. The diffusion cells were mounted on a hollow bar and the complete assembly was maintained at 32 ± 0.5°C by the circulation of water from a thermostated bath. Receptor fluid was pumped through each diffusion cell by means of a multichannel peristaltic pump (Watson/Marlow No. 202, UK) at a flow rate of 3 ml/hr to ensure sink conditions. To prevent the formation of bubbles at the interface of the membrane and the receptor chamber, the receptor fluid was initially degassed and then passed through a bubble trap prior to entering the receptor chamber. One-milliliter aliquots of receptor fluid were continuously collected (20-min periods) in scintillation vials for the duration of the diffusion experiment. The vials were automatically positioned beneath the diffusion cells. A 6-ml aliquot of scintillation fluid (Picofluor 30) was added to each sample vial prior to analysis using a Packard Tricarb 2000 (CA, USA) liquid scintillation counter. Each vial was counted for 5 min using the window setting appropriate for $^{14}$C. The results are expressed as disintegrations per minute (dpm) and were standardized using the external standard technique.

*Osmometric Studies*. Osmotic measurements were performed using a Fiske Osmometer (Model 130, Fiske Associates, MA, USA) which was calibrated with aqueous solutions (100 to 900 mosmol/kg) of sodium chloride and dextrose. All measurements were made with 2-ml samples and were replicated five times for each solution. The concentration range of nicotinamide studied was between 0.05 and 1.0 mol/kg.

*Partition Coefficient Studies*. The partition coefficient of nicotinamide between water and *n*-octanol was studied as a function of temperature and concentration of nicotinamide. The organic phase and water were presaturated with each other by overnight equilibration in a separating funnel. Different concentrations of nicotinamide (0.005 to 1.7 *M*) were prepared in the aqueous phase, which had been presaturated with *n*-octanol. The different concentrations of nicotinamide were then spiked with [$^{14}$C]nicotinamide and an appropriate aliquot was taken to determine the specific activity of the aqueous phase. A 2-ml aliquot of the aqueous nicotinamide solution and a 2-ml aliquot of *n*-octanol were transfered to a glass centrifuge tube, which was capped, and allowed to equilibrate at either 15, 25, or 32°C in a temperature-controlled water bath. The mixture was continuously inverted by hand for 5 min and then replaced in the water bath for an additional 2 hr before centrifugation at 2000*g* for 15 min in a temperature-controlled centrifuge to separate the two phases. Aliquots of known volume were taken from each phase, before and after shaking, and subjected to liquid scintillation counting as described.

*Regression Analysis*. Linear regression, calculated using the least-squares estimation method, was performed on a CYBER computer system using a standard statistical package, LINREG. Nonlinear regression analysis, based on the Gauss–Newton algorithm, was performed using a nonlinear least-squares program, MULTI (18).

## THEORETICAL

The following section presents the derivation of the equations for the modeling of the osmometric and partition coefficient data in terms of either a dimer or an isodesmic (step-association) model.

*Osmometric Studies*. The development of the equations for the osmometric data are based upon the work of Schellman and Ts'o *et al.* (19,13). The relationship between osmotic molality ($\bar{m}$) and the stoichiometric molality ($m$) is defined as

$$\bar{m} = \nu \cdot \phi \cdot m \tag{1}$$

where $\nu$ is the number of active colligative species per molecule and $\phi$ is the molal osmotic coefficient. The relationship between the molal osmotic coefficient, $\phi$, and the molal activity coefficient, $\gamma$, can be derived from the Gibbs–Duhem equation (20) and is given by

$$\ln \gamma = (\phi - 1) + \int_0^m (\phi - 1)\, d\ln m \tag{2}$$

The isodesmic or step-association process (19) is assumed to occur via stepwise equilibria according to

$$A_{i-1} + A_1 \rightleftharpoons A_i \tag{3}$$

and therefore,

$$k_i = m_i/(m_{i-1} \cdot m_1) \tag{4}$$

where $i = 2$ to $n$, $A_1, A_2, \ldots, A_n$ denote the monomer, dimer, and $n$-mer, respectively, of the dissolved compound, and $m_1, m_2, \ldots, m_n$ represent the equilibrium molalities of the respective species. The partial association constants, $k_i$, are assumed to be equal to each other, i.e., $k_2 = k_3 = \ldots = k_n = K$. The osmotic molality, $\bar{m}$, of the solution is expressed as

$$\bar{m} = m_1 + k_2(m_1)^2 + k_2k_3(m_1)^3 + \ldots + k_2k_3 \ldots k_n(m_1)^n \tag{5}$$

and the stoichiometric molality, $m$, is, by definition;

$$m = m_1 + 2k_2(m_1)^2 + 3k_2k_3(m_1)^3 + \ldots + nk_2k_3 \ldots k_n(m_1)^n \tag{6}$$

Differentiation of (5) with respect to $m_1$ is equal to the division of (6) by $m_1$, i.e.,

$$d\bar{m}/dm_1 = m/m_1 \tag{7}$$

For nonelectrolytes,

$$\bar{m} = \phi \cdot m \tag{8}$$

Differentiation of (8) and combination with (7) yield

$$d \ln (m_1/m) = (\phi - 1)\, d \ln m + d\phi \tag{9}$$

Integration of (9) (and noting that $m_1/m$ and $\phi$ approach unity at infinite dilution) gives

$$\ln(m_1/m) = (\phi - 1) + \int_0^m (\phi - 1)d \ln m \quad (10)$$

Comparison of (2) and (10) yields the relationship

$$m_1/m = \gamma \quad (11)$$

If the assumption is made that the model is isodesmic (i.e., $k_2 = k_3 = \ldots = k_n = K$), then division of (5) by $m_1$ and subsequent application of the geometric series rule give

$$\bar{m}/m_1 = [1 - (Km_1)^n]/1 - Km_1 \quad (12)$$

If $n$ is large and there is no restriction on the size of the associated species (and provided that $Km_1 < 1$), Eq. (12) becomes

$$\bar{m}/m_1 = 1/(1 - Km_1) \quad (13)$$

Differentiation of (13) and combination with (8) and (11), which converges to $1/(1 - Km_1)^2$ if $Km_1 < 1$, produce the relationship

$$Km = (1 - \phi)/\phi^2 \quad (14)$$

For a dimer model where $K = k_2$ and $k_3 = k_4 = \ldots = k_n = 0$, and with substitution of $n = 2$ into (12) and similar combination with the analogous forms of (8) and (11) for a dimeric model, it can be shown that

$$Km = (1 - \phi)/(2\phi - 1)^2 \quad (15)$$

*Partition Coefficient Studies.* The general form of the equilibria describing the association of a solute present within a particular solution phase conforms to the following general scheme:

$$c_1 + c_1 + \ldots \rightleftarrows c_n \quad (16)$$

and

$$\beta_n = c_n/(c_1)^n \quad (17)$$

where, $c_1$, $c_2$, . . . , $c_n$ refer to monomeric, dimeric, and $n$-meric forms of the solute in the aqueous phase, and $\beta_2$, $\beta_3$, . . . , $\beta_n$ refer to the respective association constants.

The equation relating the observed partition coefficient for a solute between an organic phase (in which only monomer is present) and an aqueous phase in which association occurs, conforms to the following general scheme (21):

$$P_0/P = 1 + [2\beta_2(c_1)_{org}]/P_0 + [3\beta_3(c_1)^2_{org}]/P_0^2 + \ldots + [n\beta_n(c_1)^{n-1}_{org}]/P_0^{n-1} \quad (18)$$

where $P$ and $P_0$ are the observed and intrinsic partition coefficients [$P = (\text{conc.})_{org}/(\text{conc.})_{aq}$], respectively; $\beta_n$ denotes the particular association constant for the formation of the $n$-mer, and $(c_1)_{org}$ refers to the concentration of the solute (present in the monomeric form) in the organic phase.

The simplest form of association would be the formation of dimers in the aqueous phase, and therefore when $n = 2$, Eq. (18) yields

$$P_0/P = 1 + [2\beta_2(c_1)_{org}]/P_0 \quad (19)$$

For the step-association model described in (4), where all the partial association constants are equal (i.e., $\beta_1 = \beta_2 = \ldots = \beta_n = K$), the equation describing the equilibria in terms of partition coefficient data is of the general form

$$P = (c_1)_{org}/[c_1 + 2K(c_1)^2 + 3K^2(c_1)^3 + \ldots + nK^{n-1}(c_1)^n]_{aq} \quad (20)$$

Substitution of the definition of the intrinsic partition coefficient [i.e., $P_0 = (c_1)_{org}/(c_1)_{aq}$] into (20) yields:

$$P_0/P = [1 + 2K(c_1) + 3K^2(c_1)^2 + \ldots + nK^{n-1}(c_1)^{n-1}]_{aq} \quad (21)$$

which is equivalent to

$$P_0/P = d/dKc_1\,[1 + (Kc_1) + (Kc_1)^2 + \ldots + (Kc_1)^{n-1}]_{aq} \quad (22)$$

By application of the geometric series rule, and assuming no restriction on the size of the associated species (and provided that $Km_1 < 1$), Eq. (22) becomes

$$P_0/P = 1/(1 - Kc_1)^2_{aq} \quad (23)$$

However, as it is possible to measure only the concentration of monomer in the organic phase Eq. (23) becomes

$$(P/P_0)^{0.5} = 1 - K(c_1)_{org}/P_0 \quad (24)$$

## RESULTS

In Vitro *Diffusion Studies.* The concentration-dependent steady-state flux (SSF) of nicotinamide from an aqueous donor phase across a Silastic membrane was studied at 32°C. The steady-state flux at different donor phase concentrations of nicotinamide was determined by regression analysis of the slope of the linear portion of the receptor phase nicotinamide concentration–time profile. Figure 1 depicts the relationship between SSF ($\mu g \cdot cm^{-2} \cdot min^{-1}$) and concentration of nicotinamide in the donor phase. The steady-state flux profile exhibited pronounced curvature at the higher donor phase concentrations, indicating nonideal transport kinetics. The lag times for the different donor phase concentrations were calculated by extrapolation of the linear portion of the concentration–time profile to the abscissa. The lag times, although variable, were essentially independent of concentration and displayed a mean of 117 ± 16.6 min (mean ± SE, $n = 8$). Figure 2 describes the SSF profile of nicotinamide across a Silastic membrane from a donor phase of $n$-octanol. The concentration range studied

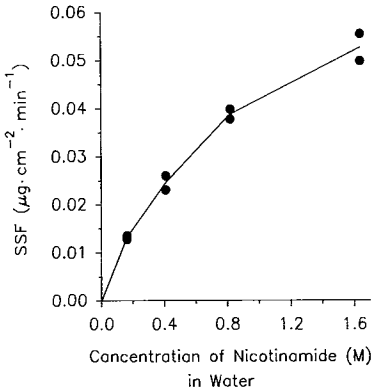


Fig. 1. Relationship between observed SSF and concentration of nicotinamide in an aqueous donor phase. Each point represents a separate permeation experiment and the line is drawn through the average of the data from the replicate experiments.

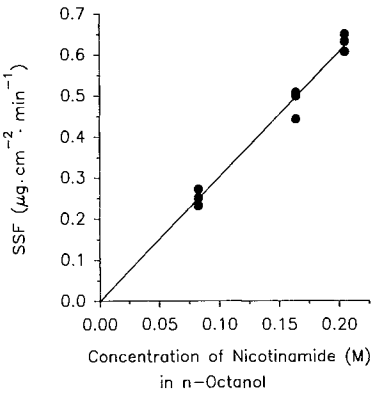


Fig. 2. Relationship between observed SSF and concentration of nicotinamide in a donor phase of *n*-octanol. Each point represents a separate permeation experiment and the line is the linear regression of the mean data from the replicate experiments.

Table I. Experimentally Determined Osmotic Molality ($\bar{m}$), Stoichiometric Molality ($m$), and Calculated Molal Osmotic Coefficients ($\phi$) for Aqueous Solutions of Nicotinamide at Their Freezing Point

| Molality (mmol/kg) | Osmotic molality (mosmol/kg) | Molal osmotic coefficient ($\phi$) |
|---|---|---|
| 50.0 | 47.4 | 0.948 |
| 100.0 | 92.4 | 0.924 |
| 150.0 | 131.4 | 0.876 |
| 200.0 | 165.0 | 0.825 |
| 300.0 | 228.8 | 0.763 |
| 400.0 | 284.5 | 0.711 |
| 500.0 | 337.4 | 0.675 |
| 600.0 | 386.4 | 0.644 |
| 700.0 | 434.0 | 0.620 |
| 800.0 | 475.8 | 0.595 |
| 900.0 | 514.4 | 0.572 |
| 1000.0 | 552.2 | 0.552 |

corresponded to the concentrations of nicotinamide in the *n*-octanol phase observed during the partition coefficient studies. In contrast to the data in Fig. 1, the permeation of nicotinamide from the *n*-octanol phase was linear as a function of donor phase concentration. The lag times for permeation of nicotinamide from the *n*-octanol donor phase displayed a mean of 16 ± 1.1 min ($n$ = 9).

*Freezing-Point Depression.* Figure 3 depicts the relationship between the experimentally determined osmotic molalities ($\bar{m}$) and the stoichiometric molalities ($m$) for aqueous solutions of sodium chloride, dextrose, and nicotinamide. The osmotic data including the calculated osmotic coefficients for nicotinamide are presented in Table I. Regression analysis was performed on the slope of the observed osmolality vs molality data for sodium chloride and dextrose. The slopes for the sodium chloride and dextrose solutions were 1.821 ± 0.005 and 1.029 ± 0.006, respectively ($n$ = 5). The profile for the aqueous solutions of nicotinamide was nonlinear and the slope was much less than unity at concentrations greater than 200 mmol/Kg.

*Partition Coefficient Studies.* The partition coefficient ($P$) of nicotinamide between water and *n*-octanol was studied as a function of concentration ($M$) and temperature (15, 25, and 32°C). As depicted in Fig. 4, the observed partition coefficient of nicotinamide decreased with increasing concentrations of nicotinamide in the organic phase. The data presented in Fig. 4 were fitted to a second-order polyexponential equation using nonlinear regression and the intrinsic partition coefficient ($P_o$) was determined from the calculated intercept at zero nicotinamide concentration. The intrinsic partition coefficients ($P_o$) of nicotinamide were 0.438 ± 0.004 ($n$ = 10), 0.435 ± 0.003 ($n$ = 9), and 0.420 ± 0.003 ($n$ = 19) at 15, 25, and 32°C, respectively.

## DISCUSSION

The *in vitro* steady-state flux of nicotinamide from an aqueous solution across a model Silastic membrane did not increase proportionality with an increase in the donor phase concentration of drug (Fig. 1). Although it is possible to invoke a number of explanations for the nonideal behavior, self-association of nicotinamide in the donor phase was initially considered because of the propensity of structurally similar compounds to exhibit self-association in aqueous solution (11,13,14). The low SSF value for nicotinamide across the Silastic membrane is not only a reflection of the potential self-association, but also a function of the high water solubility and low partition coefficient of the compound. The SSF data alone do not indicate whether the putative self-

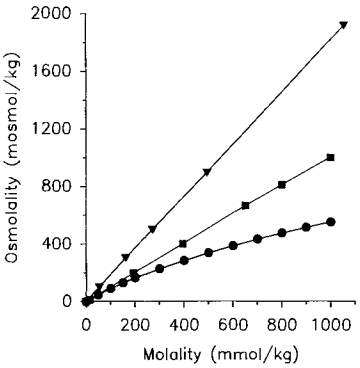


Fig. 3. Plot of experimentally determined osmolality as a function of the molality for aqueous solutions of sodium chloride (▼), dextrose (■), and nicotinamide (●). The standard deviations of the measurements are within the size of the symbols.

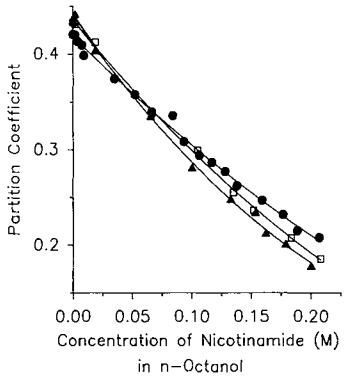


Fig. 4. Plot of partition coefficient ($P$) of nicotinamide between water and *n*-octanol as a function of concentration and temperature. (●) 32°C; (□) 25°C; (▲) 15°C.

association of nicotinamide is occurring in the donor phase and/or the membrane. The extent and nature of the putative self-association of nicotinamide were investigated by osmotic and partition coefficient studies.

The osmotic molalities of nicotinamide in aqueous solution were measured at the freezing point of the solutions. The osmometer was calibrated with sodium chloride and dextrose and the relationship between the osmotic and the stoichiometric molalities was consistent with the known osmotic and colligative properties of these solutes (22). The concentration-dependent decrease in the molal osmotic coefficients of nicotinamide (Table I) indicated a potential solute–solute interaction.

The change in partition coefficient of a drug as a function of concentration is often a useful indicator of potential solute–solute interactions in one or both of the chosen phases (21). Generally, a solvent in which the drug of interest exists predominantly in the monomeric form is chosen as one of the phases.

The *in vitro* diffusion profile of nicotinamide from *n*-octanol, depicted in Fig. 2, demonstrated a linear dependence of the SSF on the donor phase concentration of nicotinamide, indicating that it is unlikely that nicotinamide is present in an associated form in *n*-octanol. Further, concentration-dependent chemical shift data from NMR spectroscopy indicated that nicotinamide was not present in an associated form in *n*-octanol (23). Therefore, the partition coefficient of nicotinamide was determined between water and *n*-octanol. The profile of the partition coefficient data presented in Fig. 4 was consistent with self-association of nicotinamide occurring in the aqueous phase. The temperature dependence of the apparent self-association indicated a decrease in the extent of association with increasing temperature.

### Modeling of the Self-Association of Nicotinamide

The *in vitro* diffusion, osmometric, and partition coefficient studies indicated an apparent concentration-dependent self-association of nicotinamide in aqueous solution. To characterize further the association phenomena, various mathematical models of association were considered. General forms of molecular association for a variety of compounds have been studied comprehensively by many workers and include dimeric, trimeric, and numerous higher orders of association (21,24–26).

*Dimer Model.* The simplest form of self-association for an organic molecule is the formation of dimers. Equations (15) and (19) describe the relationship between concentration and the measured parameter for a drug undergoing dimerization as measured by osmotic molality and partition coefficients, respectively. In terms of the osmometric data, a plot of $(1 - \phi)/(2\phi - 1)^2$ vs molality would yield a straight-line relationship and the association constant, $K$, would be obtained from the slope of the relationship. However, as seen in Fig. 5, the plot of the osmometric data for nicotinamide was not linear over the studied concentration range, and the slope of the plot increased with increasing concentration, suggesting the potential involvement of higher orders of association (27).

The partition coefficient data were treated according to Eq. (19) and the profile of the data plotted as $(P_0/P)$ vs concentration of nicotinamide in the organic phase is presented in Fig. 6. This representation of the nicotinamide partition coefficient data, where the extent of the self-association is constrained to a dimer model, appears inadequate as indicated by the curvature in the profiles at the higher concentrations of nicotinamide. Furthermore, the curvature seen in Fig. 6 is most obvious at the lower temperatures, where the extent of the self-association process would be expected to be greater than at the higher temperatures.

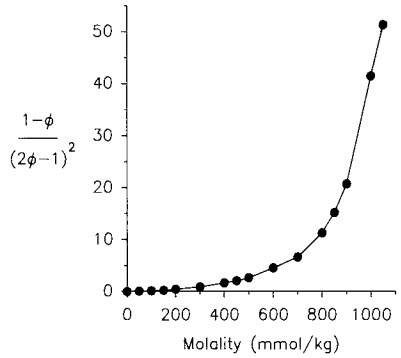


Fig. 5. Relationship between calculated values for $(1 - \phi)/(2\phi - 1)^2$ and the stoichiometric molality of nicotinamide in aqueous solution.

*Isodesmic Model.* The self-association of nicotinamide could not be adequately explained by constraining the size of the associated complex to a dimer. Careful and controlled variation of association models which included dimers and/or trimers, and combinations thereof, did not markedly improve the quality of the fit of the osmometric and partition coefficient data.

For nonelectrolytes that are weakly associated in aqueous solution, a model known as the isodesmic model of self-association has been developed for characterizing the effect of the association on the osmotic activity coefficients of weakly associated species. Models of this nature have found application in the characterization of the self-association of urea, pyridines, and various pyrimidines (11,13,14,19,27). The model, described in the theoretical section, assumes that the self-association proceeds via stepwise equilibria.

Figure 7 displays the osmometric data when the molal osmotic coefficients are plotted as a function of the stoichiometric molality of the nicotinamide solutions according to

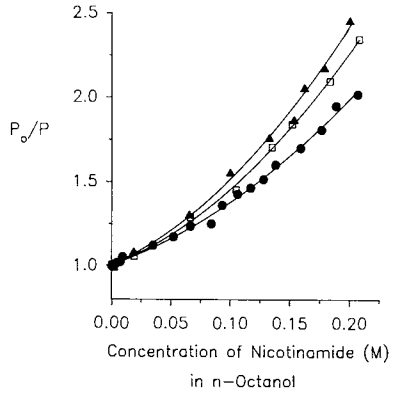


Fig. 6. Graphical representation of the calculated values for $(P_0/P)$ as a function of the concentration of nicotinamide in *n*-octanol and temperature. (●) 32°C; (□) 25°C; (▲) 15°C.

equation (14). The straight-line relationship between the parameters indicates that the isodesmic model adequately describes the association profile of nicotinamide. The association constant ($K$) obtained from the slope of the profile in Fig. 7 was 1.44 ± 0.01 $m^{-1}$ (1.59 ± 0.02 $M^{-1}$, $n$ = 15), which is in good agreement with a reported value of 1.41 $m^{-1}$ (11).

Kopecky *et al.* (11) analyzed osmometric data of nicotinamide as a function of a number of different empirically derived association models. These workers found that a model which was more specific and constrained than the isodesmic model (where the dimerization constant was regarded as independent of the other equilibria, and all higher partial association constants for the stepwise equilibria are equal to each other) was able to improve marginally the fit of the data. When the osmometric data from the present study were subjected to a similar analysis, it was found that with specifically constrained models, a marginally better fit to the data set could be obtained. However, in terms of the descriptive appeal and reasonably good fit of the data by the isodesmic model, and poor discriminatory power of the measurements, it was decided to employ the more simple isodesmic model.

Equation (24), which was derived according to the isodesmic model of step-association, predicts a linear relationship between $(P/P_0)^{0.5}$ and the concentration of nicotinamide in the $n$-octanol. The data in Fig. 4 were analyzed according to Eq. (24) and the profile is presented in Fig. 8. The linear relationship indicates that the step-association model is an adequate description of the partitioning character of nicotinamide between the two solvents. The underlying assumption, supported by the SSF data (Fig. 2) and NMR data (23), in the derivation of Eq. (24) is that only monomeric nicotinamide is present in the organic phase. The calculated association constants ($M^{-1}$, mean ± SD) for the isodesmic model based upon the partition coefficient data are 0.65 ± 0.01 ($n$ = 10), 0.60 ± 0.01 ($n$ = 9), and 0.48 ± 0.01 ($n$ = 19) at 15, 25, and 32°C, respectively. A similar effect of temperature on the association constant (isodesmic model) was observed when studying the self-association of nicotinamide at the freezing point (1.41 $m^{-1}$) and at 40°C (0.88 $m^{-1}$) by vapor pressure osmometry (11).

Thermodynamic parameters (expressed per mole of monomer) for the self-association of nicotinamide were estimated from a van't Hoff plot of the temperature dependence of the association constants calculated for the isodesmic model determined from the partition coefficient data. The values (mean ± SD, $n$ = 3) obtained by regression analysis from the intercept and slope of the van't Hoff plot were $\Delta H = -2.98 \pm 0.59$ kcal · mol$^{-1}$ and $\Delta S = -11.8 \pm 2.0$ cal · K$^{-1}$ · mol$^{-1}$. The calculated enthalpy of association is in the range which reflects the potential involvement of hydrogen bonding in the formation of the associated species (28,29), and the large change in the entropy is a reflection of the increased order as a consequence of the self-association. Similar estimates of entropy loss following self-association have been observed with some alkane alcohols (28) and a variety of pyrimidines (14).

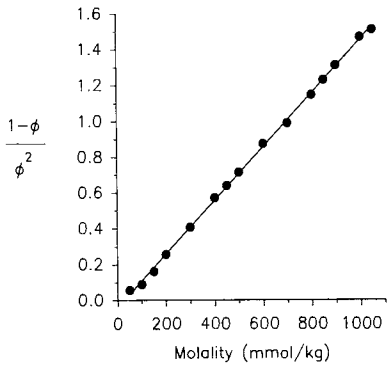


Fig. 7. Plot of $(1 - \phi)/\phi^2$ as a function of nicotinamide concentration (molality). The points were calculated according to Eq. (14) and the line was determined by linear regression.

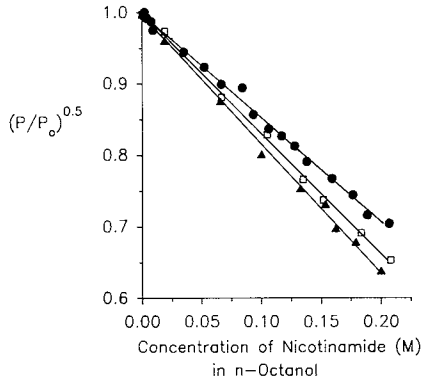


Fig. 8. Relationship between calculated values for $(P/P_0)^{0.5}$ and the monomeric concentration of nicotinamide in $n$-octanol. (●) 32°C; (□) 25°C; (▲) 15°C. The data were fitted to Eq. (24) and the line was determined by linear regression.

The modeling of the osmometric and partition coefficient data in terms of the isodesmic model is a simplification of the complex association process, although the approach has the advantage that the model is not constrained to a single associated species. By the nature of the equilibria invoked, the model is weighted toward the formation of the lower (e.g., $n$ = 2 to 4) associated species. The model oversimplifies the actual association process, which no doubt exhibits greater specificity and diversity than is tacitly assumed in the isodesmic model. The association process, which appears to represent a continuum rather than specific forms, could theoretically be characterized by distinct equilibrium constants for each associated form. However, the osmometric and partition coefficient data are not sufficiently accurate, nor do they provide sufficient discrimination to permit further insight into the mechanisms of the association. NMR spectroscopy has been employed to investigate the molecular basis of the association of nicotinamide in the context of the associated complex (23).

The utility of the isodesmic model for the association of nicotinamide is that it allows an explanation and characterization of the observed physical chemical properties of aqueous solutions of nicotinamide. It is possible to use the isodesmic model to predict the concentration-dependent changes in the SSF of nicotinamide from an aqueous donor phase across a model membrane by "correcting" the donor phase concentration to reflect the concentration of monomeric nicotinamide in the donor phase. Mikkelson *et al.* (9) performed an analogous correction on the SSF data for the permeation of phenol (which also self-associates) across a model membrane.

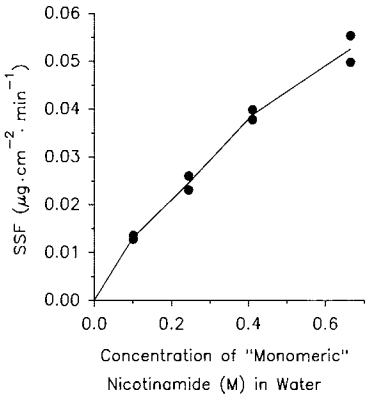


**Fig. 9.** Relationship between observed SSF and the calculated concentration of "monomeric" nicotinamide in the aqueous donor phase. See text for the method used in the calculation of the monomeric concentration of nicotinamide.

Figure 9 represents the profile of the SSF of nicotinamide when corrected for the effective monomer concentration of drug present in the aqueous donor phase. The calculation for the monomer concentration of nicotinamide in the aqueous donor phase was based upon the substitution of the association constant calculated from the isodesmic model of the partition coefficient data at 32°C in Eq. (23).

The profile of the SSF in Fig. 9 is close to linearity, indicating that the observed SSF is dependent upon the monomeric concentration of nicotinamide present in the aqueous donor phase. Therefore, the concentration-dependent SSF of nicotinamide across a model membrane can be explained by considering self-association of drug in the aqueous donor phase, and it is not necessary to invoke a membrane-related phenomenon.

## ACKNOWLEDGMENT

C.S.C.L. gratefully acknowledges support from a Cyril Tonkin Scholarship awarded by The Pharmaceutical Society of Australia (Victorian Branch).

## REFERENCES

1. G. P. Mathur. Skin lightening method and composition. U.S. Patent 4096240 (1978).
2. J. E. Bernstein. Composition of treating acne vulgaris. European Patent 52705 (1982).
3. A. G. Gilman, L. S. Goodman, and A. Gilman. *Pharmacological Basis of Therapeutics,* Macmillan, New York, 1985, pp. 834–835.
4. R. H. Guy, R. C. Wester, E. Tur, and H. I. Maibach. Noninvasive assessments of the percutaneous absorption of methyl nicotinate in humans. *J. Pharm. Sci.* 72:1077–1079 (1983).
5. R. H. Guy, E. M. Carlstrom, D. A. W. Bucks, R. S. Hinz, and H. I. Maibach. Percutaneous penetration of nicotinates: In vivo and in vitro measurements. *J. Pharm. Sci.* 75:968–972 (1986).
6. R. Kohli, W. I. Archer, and A. W. P. Li. Laser velocimetry for the non-invasive assessment of the percutaneous adsorption of nicotinates. *Int. J. Pharm.* 36:91–98 (1987).
7. D. F. Evered, F. Sadoogh-Abasian, and P. D. Patel. Absorption of nicotinic acid and nicotinamide across human buccal mucosa in vivo. *Life Sci.* 27:1649–1651 (1980).
8. T. Higuchi. Pro-drug, molecular structure and percutaneous delivery. In E. B. Roche (ed), *Design of Biopharmaceutical Properties Through Prodrugs and Analogs,* American Pharmaceutical Association, Washington, DC, 1977, pp. 409–421.
9. T. J. Mikkelson, S. Watanabe, J. H. Rytting, and T. Higuchi. Effects of self-association of phenol on its transport across polyethylene film. *J. Pharm. Sci.* 69:133–137 (1980).
10. B. W. Barry. *Drugs and the Pharmaceutical Sciences, Vol. 18,* Marcel Dekker, New York, 1983.
11. F. Kopecky, M. Vojtekova, and M. Bednarova-Hyttnerova. Osmometric study of self-association of nicotinamide and isonicotinic acid hydrazide in aqueous solution at 40°C and the freezing point. *Collect. Czech. Chem. Commun.* 43:37–46 (1978).
12. P. Rohdewald and G. Elmahrouk. Osmometric determination of the osmotic coefficients of the sodium and hydrochloride salts of aromatic amino acids, the sodium salt of N'-(2,6-dimethoxypyrimidin-4-yl)-sulphanilamide, and nicotinamide. *J. Chem. Res. (S)*:54–55 (1981).
13. P. O. P. Ts'o, I. S. Melvin, and A. C. Olson. Interaction and association of bases of nucleosides in aqueous solution. *J. Am. Chem. Soc.* 85:1289–1296 (1963).
14. E. Plesiewicz, E. Stepien, K. Bolewska, and K. L. Wierzchowski. Osmometric studies on self-association of pyrimidines in aqueous solution: Evidence for involvement of hydrophobic interactions. *Biophys. Chem.* 4:131–141 (1976).
15. J. L. Czeisler and D. P. Hollis. Studies of the interaction of adenine and nicotinamide ring systems in aqueous solution by high resolution nuclear magnetic resonance. *Biochemistry* 14:2781–2785 (1975).
16. E. R. Cooper and B. Berner. Skin permeability. In D. Skerrow and C. J. Skerrow (eds), *Methods in Skin Research,* John Wiley, New York, 1985, pp. 407–432.
17. S. A. Akhter, S. L. Bennett, I. L. Waller, and B. W. Barry. An automated diffusion apparatus for studying skin penetration. *Int. J. Pharm.* 21:17–26 (1984).
18. K. Yamaoka, Y. Tanigawara, T. Nakagawa, and T. Uno. A pharmacokinetic analysis program (Multi) for microcomputer. *J. Pharmacobio. Dyn.* 4:879–885 (1981).
19. J. Schellman. The thermodynamics of urea solutions and the heat of formation of the peptide hydrogen bonds. *Compt.-Rend. Lab. Carlsberg Ser. Chim.* 29:233–239 (1956).
20. G. N. Lewis, M. Randall, K. S. Pitzer, and L. Brewer. *Thermodynamics,* 2nd ed., McGraw-Hill, New York, 1961.
21. K. A. Connors. *Binding Constants.* Wiley-Interscience, New York, 1987.
22. R. C. Weast (ed.), *CRC Handbook of Chemistry and Physics,* 62nd ed., CRC Press, Boca Raton, FL, 1981.
23. W. N. Charman, C. S. C. Lai, D. J. Craik, B. C. Finnin, and B. L. Reed. Manuscript in preparation.
24. D. Guttman and T. Higuchi. Reversible association of caffeine and some caffeine analogs in aqueous solution. *J. Am. Pharm. Assoc. Sci. Ed.* 46:4–10 (1957).
25. B. D. Anderson, R. A. Conradi, and K. Johnson. Influence of pre-micellar and micellar association on the reactivity of methylprednisolone 21-hemiesters in aqueous solution. *J. Pharm. Sci.* 72:448–454 (1983).
26. Y. Chang and J. R. Cardinal. Light scattering studies on bile acids. I. Pattern of self-association of sodium cholate, sodium glycolate and sodium taurocholate in aqueous electrolyte solutions. *J. Pharm. Sci.* 67:174–181 (1978).
27. P. O. P. Ts'o and S. I. Chan. Interaction and association of bases and nucleosides in aqueous solution. II. Association of 6-methylpurine and 5-bromouridine and treatment of multiple equilibria. *J. Am. Chem. Soc.* 86:4176–4181 (1964).
28. J. H. Rytting, B. D. Anderson, and T. Higuchi. Vapor pressure studies of the self-association of alcohols in isooctane 2. The effect of chain branching. *J. Phys. Chem.* 82:2240–2245 (1978).
29. M. D. Joesten and L. J. Schaad. *Hydrogen Bonding,* Marcel Dekker, New York, 1974.