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Stacking complexation by nicotinamide: A useful way of enhancing drug solubility

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Abstract

The solubility enhancement of 11 poorly soluble drugs by complexation using nicotinamide has been studied. The solubilization efficiency of nicotinamide has been compared to that of hydroxypropyl-β-cyclodextrin and sulfobutylether-β-cyclodextrin. Solubility enhancements as high as 4000-fold are observed in 20% (w/v) nicotinamide solution. Furthermore, nicotinamide is more effective than cyclodextrins for solubilizing some of the drugs. The mechanism of drug solubilization by nicotinamide is investigated by studying the effects of nicotinamide concentration on the surface tension and the conductivity of water. A slight break in both, the surface tension and conductivity is noticed at around 10% (w/v), suggesting self-association at higher concentrations. Corresponding breaks in the solubility profiles of estrone and griseofulvin at similar concentrations support self-association. Based on this observation it appears that at low concentrations, one molecule of nicotinamide undergoes complexation with one drug molecule to form a 1:1 complex. At higher concentrations, two molecules of nicotinamide undergo complexation with one drug molecule forming a 1:2 complex. The complexation constants have been calculated for all the drugs and the data are well described by this model. Expectedly, increasing the temperature reduces the complexation constants.

Keywords: Nicotinamide; Solubilization; Stacking; Self-association

1. Introduction

The poor aqueous solubility of a drug may limit its efficacy and utility. Various techniques and solubilizing agents have been used to enhance the solubility of such drugs (Yalkowsky, 1999). The use of nicotinamide as a solubilizing agent has been widely reported (Rasool et al., 1991; Lim and Go, 2000; Lee et al., 2003; Agrawal et al., 2004). A combination of good solubilizing ability, low toxicity and economy makes nicotinamide a useful complexing agent. Although the efficiency of nicotinamide as a solubilizer has been widely reported, direct comparison with other complexing agents has not been undertaken extensively. One purpose of this study is to compare the solubilization strength of nicotinamide to that of hydroxypropyl-β-cyclodextrin (HP-β-CD) and sulfobutylether-β-cyclodextrin (SBE-β-CD) using a set of 11 structurally diverse drugs.

The mechanism of solubility enhancement using nicotinamide is of interest and various theories have been put forth to explain this. The term "hydrotropy" is sometimes used to describe the process. This term was originally proposed to define a non-stoichiometric solubilization of a solute by high concentrations of anionic aromatic compounds (Neuberg, 1916). Although, it may be practically useful, the term is often regarded as too general to explain the exact mechanism. Hydrotropic solubilization may be a result of stacking complexation; chaotropy, i.e., breakdown of water structure; or the formation of micellar aggregates.

The idea that nicotinamide undergoes stacking with drug molecules has been widely proposed (Hussain et al., 1993; Chen et al., 1994; Suzuki and Sunada, 1998; da Silva et al., 1999). A complex is formed by the interaction between the planer hydrophobic regions of the complexing agent and the drug. The stacked arrangement is one where the exposure of the hydrophobic regions to water is reduced. The driving force for this process is often passive. In other words, the drug and the complexing molecules may not have a direct affinity towards each other but interact in order to minimize their exposure to water. Stacking may occur between the molecules of same species

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(self-association) or different species (co-association). Stacking occurs primarily between planer molecules for which the exposure to water can be efficiently minimized. A simple 1:1 complex consists of one molecule each of the drug and complexing agent. A 1:2 sandwich complex may be formed where the central molecule is surrounded on two sides with the complexing agent.

Various theories and models have been proposed to explain the dependence of stacking complexation on the properties of the drug and the complexing agent. According to the maximum aromatic overlap model, the size of pi-electron system of the complexing agent is the single most important factor in determining the strength of complexation (Nakano and Igarashi, 1970). In a different study it was shown that the electrostatic force of the donor–acceptor type plays an important role in complexation (Badwan et al., 1983). The role of hydrogen bonding in stacking has also been studied although a clear relationship could not be established (Suzuki and Sunada, 1998; Kenley et al., 1986).

The log *P* of drugs as a measure of their hydrophobicity has been correlated to the complexation constant with considerable success (Suzuki and Sunada, 1998; Kenley et al., 1986). It was postulated that a more non-polar drug molecule has a stronger driving force for undergoing complexation. This theory takes into account the overall non-polarity of the drug molecules. However, only a part of the molecule may be complexing. Considering the total non-polarity may therefore not be totally appropriate.

Higuchi and Kristiansen (1970) proposed a model according to which the compounds capable of undergoing stacking can be classified into two classes (classes A and B) based on their structure. The compounds in class A have higher affinity for compounds in class B than for those in class A and vice versa. Although, many exceptions to this theory have been cited, it generally gives a good indication about the relative complexation strengths.

Besides stacking, at least two other theories have been proposed to explain the mechanism of drug solubilization by nicotinamide. It has been postulated that nicotinamide acts like a chaotrope, i.e., breaks the self-associated structure of water. This results in higher drug solubility. Some researchers have also suggested that nicotinamide forms micellar aggregates in water, followed by the incorporation of drug into these aggregates. The term "critical hydrotrope concentration" has been proposed to refer to the concentration at which the aggregation starts (Balasubramanian et al., 1989). Whether these aggregates are micelles or higher order complex remains an issue of debate. In either case, formation of the aggregates was shown to be an important step in drug solubilization.

A variety of techniques have been used to study the mechanism of drug solubilization by nicotinamide. These include direct approaches such as spectral studies (UV, IR, NMR), thermal studies (DSC) microscopy (PXRD) and fluorescence quenching (Coffman and Kildsig, 1996; Oberoi et al., 2005). Indirect approaches such as solubility studies and measurement of thermodynamic parameters associated with the process (Suzuki and Sunada, 1998) have also been used. The results from the direct studies may sometimes be inconclusive due to several reasons. Often it is difficult to check the existence of the complex in a dissolved state. The interpretation based on a shift in the UV or IR spectra is confusing as these changes may occur either due to a direct interaction between the drug and the stacking agent or as a result of an indirect effect of the environment of drug molecules. The study of complexation in the solid state may not be possible if the complex is not stable upon evaporation of solvent from a drug-complexing agent solution. Therefore, the dried solid residue may not have the distinct characteristics of the complex. Nonetheless, this approach has been used successfully in some studies.

In this study, the effect of nicotinamide on the cohesiveness of water molecules is investigated by measuring the lowering of the surface tension of water as a function of nicotinamide concentration. A significant reduction in the surface tension of water will be indicative of its chaotropic effect. A break in the surface tension versus concentration profile followed by a reduction in slope will indicate micellar aggregation. The effect of nicotinamide on the conductivity of water is studied to check the possibility of self-association. Nicotinamide increases the conductivity of water. Self-association is expected to reduce the influence of nicotinamide concentration on the conductivity (Mukerjee, 1967). Thus, a break point followed by a negative deviation in the conductivity–concentration plot will suggest self-association at higher concentrations. The phase solubility data of 11 drugs are also used to study the mechanism and the strength of complexation. Griseofulvin and estrone are chosen for illustration purpose.

2. Materials

The following 11 drugs were used in the study: phenobarbitone, griseofulvin, phenytoin, ketoprofen, estrone, amiodarone (Sigma, St. Louis, MO); carbendazim, 2-phenoxy propionic acid (PPA) (Aldrich, Milwaukee, WI); XK-469, benzoylphenyl urea derivative (BPU) (NCI, Bathesda, MD); PG-300995 (Proctor & Gamble, Cincinnati, OH). These drugs vary widely in terms of their physicochemical properties. Their solubility ranges from 0.03 to 2951.21 μg/ml, while their log *P* ranges from 1.47 to 6.21. Five of these drugs are weak acids and four weak bases. Nicotinamide was obtained from Sigma–Aldrich, St. Louis, MO. HP-β-CD with an average molecular weight of 1380–1500 and an average degree of substitution of 0.6–0.9 was obtained from Wacker Biochem Corporation (Adrian, MI). SBE-β-CD with an average molecular weight of 2160 and an average degree of substitution of 6–7.1 was received as a gift from Cydex, L. C. (Overland Park, KS). All the other excipients were of reagent or HPLC grade and used without further purification. Water used in the study was double deionized using Milli-Q water system (Millipore Corporation, Billerica, MA) (Fig. 1).

3. Methods

3.1. Phase solubility determination

Aqueous solutions containing increasing fractions (0–25%, w/v) of the three solubilizers, nicotinamide, HP-β-CD, SBE-

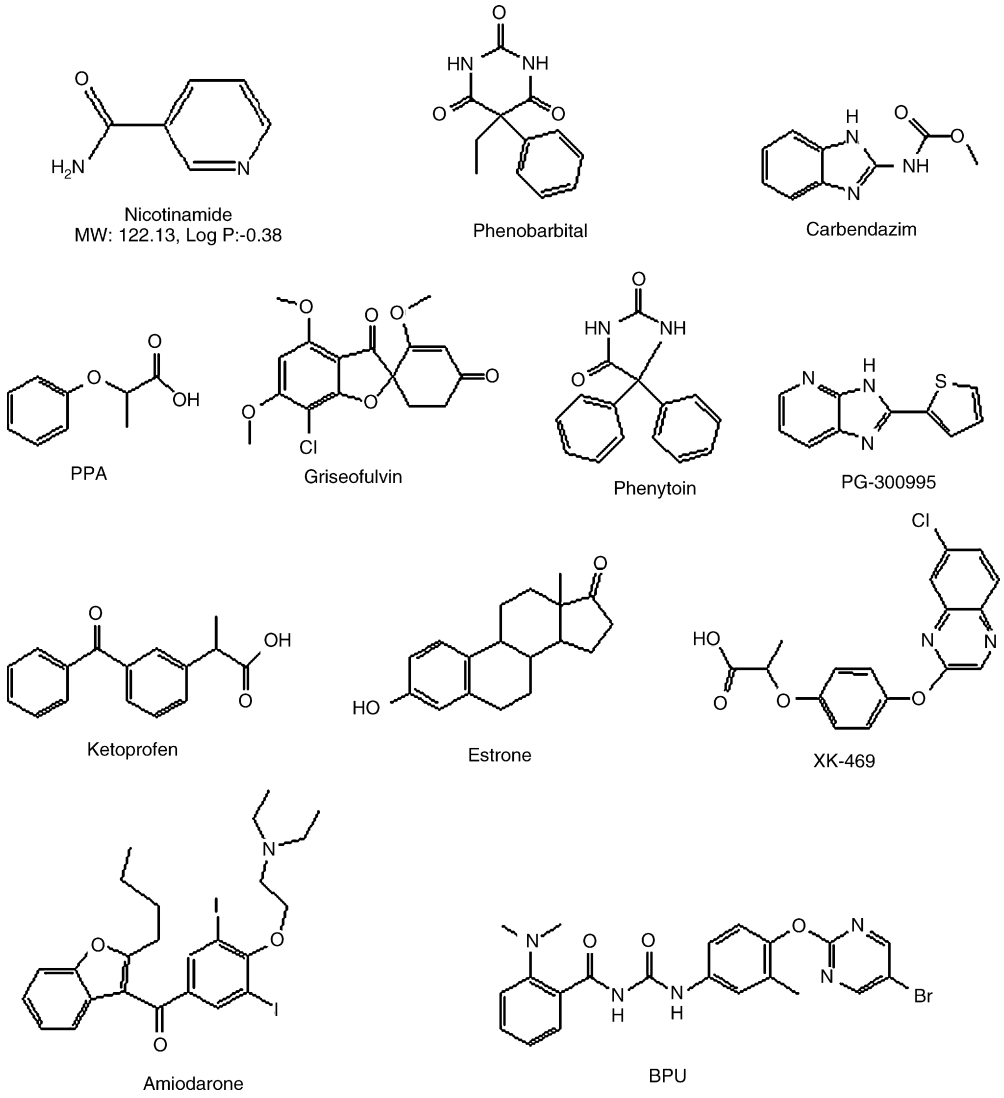

Fig. 1. Structures of nicotinamide and the 11 drugs used in the study.

β-CD were prepared. For ionizable drugs, buffers were used instead of water to make the solutions. The pH of the buffers was maintained at least 2 units away from the pK_a of the respective drug. This was done to ensure that the drug predominantly existed in an unionized form. For example, pH was maintained at 7.0 in the case of carbendazim which has a basic pK_a of ∼4.0. An excess amount of drug was added to the vials containing 1 ml of the aqueous solutions. The vials were placed in an end over end rotator at 20 rpm for sufficient length of time under room conditions. The samples were then filtered through a 0.45-μm filter followed by the analysis of the drug content using HPLC (Agilent 1100 HPLC with G1315B PDA detector, Agilent Technologies Palo Alto, CA with Chemstation software). Solubility studies at 40 °C were performed in temperature chambers.

3.2. Surface tension measurement

The Drop-Number method was used to measure the relative surface tension of nicotinamide–water mixtures (0–25%, w/v). A constant flow syringe pump was used at a flow rate of 0.04 ml/min to create the drops. The first three drops were sacrificed and the time required for the next five drops to form and fall was measured. The densities of the samples were measured using pycnometer. Water was used as the reference liquid. The surface tension was calculated using the following equation:

$$\gamma_{\text{sample}} = \gamma_{\text{water}} \times \frac{T_{\text{sample}}}{T_{\text{water}}} \times \frac{D_{\text{sample}}}{D_{\text{water}}} \quad (1)$$

where γ refers to the surface tension, T the time required for five drops to form and fall and D is the density.

3.3. Conductivity measurement

The conductivities of nicotinamide–water mixtures (0–25%, w/v) were measured at 20 °C using a conductivity meter (Hanna Instruments, Woonsocket, RI).

3.4. Solubilization efficiency

The ratio of the solubility obtained in the presence of 20% (w/v) solubilizer ($S_{0.2}$) to the intrinsic solubility (S_{int}) was calculated for each drug. These ratios were used to compare the relative solubilization efficiency of nicotinamide to that of the cyclodextrin derivatives. A 20% (w/v) was chosen as a reference since it is generally considered to be a practically acceptable concentration from the formulation and toxicity standpoint.

3.5. Calculation of complexation constants

The interaction between the drug and nicotinamide was studied by assuming that both 1:1 and 1:2 complexes are formed:

$$[D] + [N] = [DN] \quad (2)$$

$$[D] + 2[N] = [DN_2] \quad (3)$$

where D is the molar drug solubility in water, N the molar nicotinamide concentration, DN the concentration of 1:1 complex and DN_2 is the concentration of 1:2 complex. The complexation constants $K_{1:1}$ and $K_{1:2}$ are defined as:

$$K_{1:1} = \frac{[DN]}{[D][N]} \quad (4)$$

$$K_{1:2} = \frac{[DN_2]}{[D][N]^2} \quad (5)$$

Hussain et al. (1993) derived the following relationship to calculate $K_{1:1}$ and $K_{1:2}$ from the solubility data:

$$\frac{[D_{total}] - [D_{water}]}{[N_{total}] - 2([D_{total}]) - [D_{water}])} = A + B\{[N_{total}] - 2([D_{total}] - [D_{water}])\} \quad (6)$$

where $[D_{total}]$ and $[N_{total}]$ are the total molar concentration of drug and nicotinamide, respectively, and $[D_{water}]$ is the molar solubility of drug in water. Eq. (6) may be simplified as:

$$\frac{[D_{complex}]}{[N_{total}] - 2[D_{complex}]} = A + B\{[N_{total}] - 2[D_{complex}]\} \quad (7)$$

where $[D_{complex}]$ is the concentration of drug undergone complexation.

The values of A and B are given by:

$$A = \frac{K_{1:1}[D_{water}]}{1 - K_{1:1}[D_{water}]} \quad (8)$$

$$B = \frac{K_{1:2}[D_{water}]}{(1 - K_{1:2}[D_{water}])^2} \quad (9)$$

A and B were calculated from the slope and the intercept obtained from the plot of Eq. (7). The values of $K_{1:1}$ and $K_{1:2}$ were then calculated using Eqs. (8) and (9).

4. Results and discussion

4.1. Solubilization efficiency of nicotinamide

The solubility enhancements ($S_{0.2}/S_{int}$) obtained using the three solubilizers for the studied drugs are presented in Table 1. The highest enhancements have been highlighted. It is clear that nicotinamide is a potent solubility enhancer. Nicotinamide improves the solubility more than the cyclodextrin derivatives for carbendazim, griseofulvin and PG-300995. It can be seen that substantial improvement in the solubility is obtained for all the drugs. Solubility increase of more than 4000-fold is obtained for benzophenyl urea derivative in 20% (w/v) nicotinamide solution.

4.2. Mechanism of drug solubilization

In order to study the mechanism of drug solubilization by nicotinamide, the effect of its concentration on the surface tension and the conductivity of water as well as the drug solubility are measured.

4.2.1. The effect of nicotinamide concentration on the surface tension of water

Fig. 2 presents the effect of nicotinamide on the surface tension of water. It is clear that nicotinamide has a very small effect on the surface tension and reduces it by less than 10% even at the highest concentration.

Table 1
Solubility enhancements for the drugs using the three solubilizers

Drug	log P	log S_{int} (μg/ml)	$S_{0.2}/S_{int}$		
			HP-β-CD	SBE-β-CD	Nicotinamide
Phenobarbitone	1.47	2.99	**29.0**	17.2	1.3
Carbendazim	1.52	0.37	7.2	7.4	**17.8**
Phenoxy propionic acid	1.88	3.47	7.2	5.4	4.2
Griseofulvin	2.18	0.91	5.0	8.6	**32.7**
Phenytoin	2.47	1.03	**352.9**	213.2	22.6
PG-300995	2.59	1.59	10.8	17.5	**86.8**
Ketoprofen	3.12	1.89	**166.1**	57.5	12.0
Estrone	3.13	−0.20	**4400.1**	3426.7	587.2
XK-469	3.85	−0.64	**561.4**	558.4	27.2
Amiodarone	5.90	0.18	4441.8	**40034.4**	923.7
Benzophenyl urea derivative	6.21	−1.55	24343.0	**31401.0**	4018.2

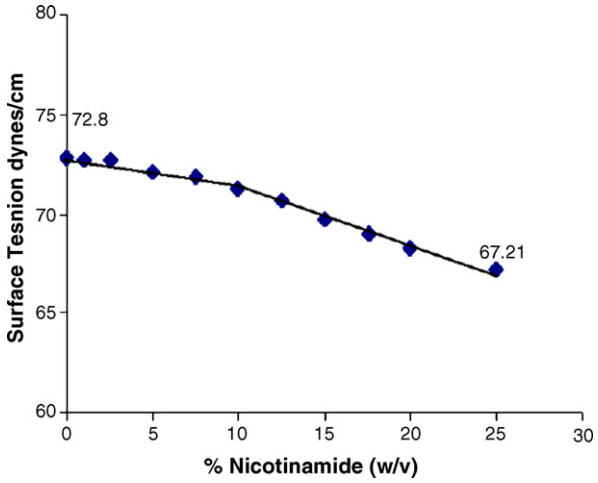

Fig. 2. The effect of nicotinamide concentration on the surface tension of water.

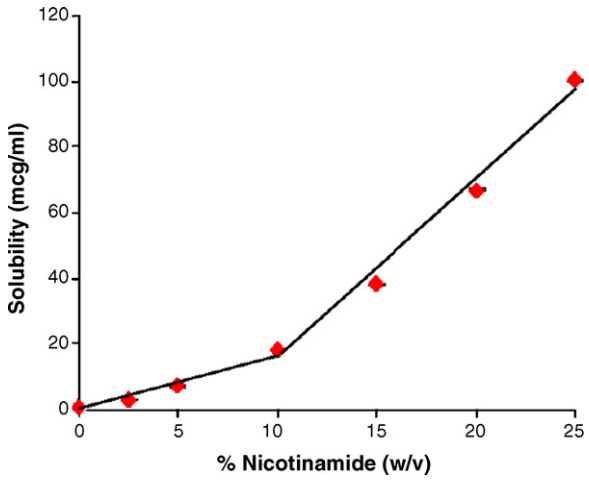

Fig. 4. Solubility profile for estrone.

The surface tension of a system is a measure of its molecular cohesiveness. Chaotropes reduce the surface tension of water by lowering its molecular cohesiveness. Surfactants on the other hand do so by selectively accumulating at the air–water interface. After saturating the interface, the surfactant molecules start aggregating as micelles in the aqueous phase. The concentration at which this occurs is referred as critical micelle concentration (CMC). Balasubramanian et al. (1989) coined an analogous term "critical hydrotrope concentration" to describe the aggregation of hydrotopes. The surface tension of water is reduced substantially with increasing the concentration below the CMC. At higher concentrations, it remains nearly constant. Generally, in the case of both, chaotropy or micellar aggregation, a substantial lowering in surface tension is noticed. The effect of nicotinamide on the surface tension of water is too small to justify the theory of it acting as a chaotrope or as a classic surfactant to accumulate on the air–water interface (in the studied concentration range). However, a small break in the profile at around 10% (w/v) suggests molecular aggregation.

4.2.2. The effect of nicotinamide concentration on the conductivity of water

The conductivity of water is dependent on the concentration of the conducting species present. Nicotinamide significantly increases the conductivity of water (Fig. 3). A break point in the plot at around 10% (w/v) indicates molecular aggregation (Mukerjee, 1967).

4.2.3. Complexation constants with nicotinamide

Figs. 4 and 5 present the solubility data for estrone and griseofulvin, respectively. A distinct break at ∼10% (w/v) nicotinamide concentration can be observed for both of these examples.

The complexation constants ($K_{1:1}$ and $K_{1:2}$) were calculated for each drug from the phase-solubility data using Eqs. (7)–(9). Table 2 presents these values. Eq. (7) describes the experimental data well with a $R^2 > 0.9$. This further supports the idea that at lower concentrations, nicotinamide undergoes formation of 1:1 complex while at higher concentrations 1:2 complexation occurs.

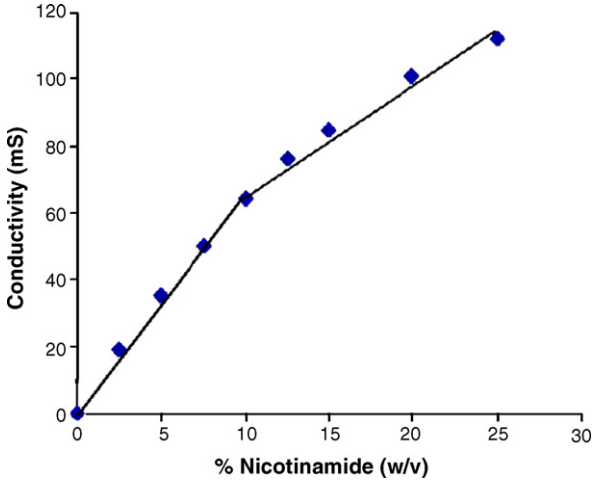

Fig. 3. Effect of nicotinamide concentration on the conductivity of water.

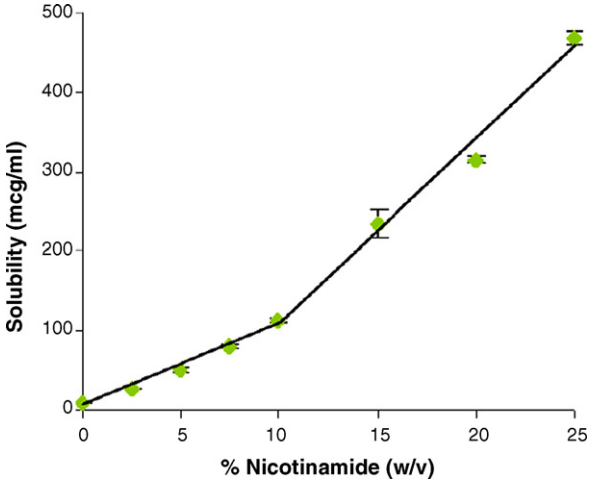

Fig. 5. Solubility profile for griseofulvin.

Table 2
Complexation constants for the studied drugs

Drug	Complexation constants (M^{-1})	
	$K_{1:1}$	$K_{1:2}$
Phenobarbitone	0.02	0.09
Carbendazim	6.57	3.67
Phenoxy propionic acid	2.88	0.80
Griseofulvin	2.64	2.76
Phenytoin	4.39	6.42
PG-300995	25.10	24.50
Ketoprofen	4.92	2.15
Estrone	10.78	31.32
XK-469	3.02	14.29
Amiodarone	51.96	318.01
Benzophenyl urea derivative	172.40	724.00

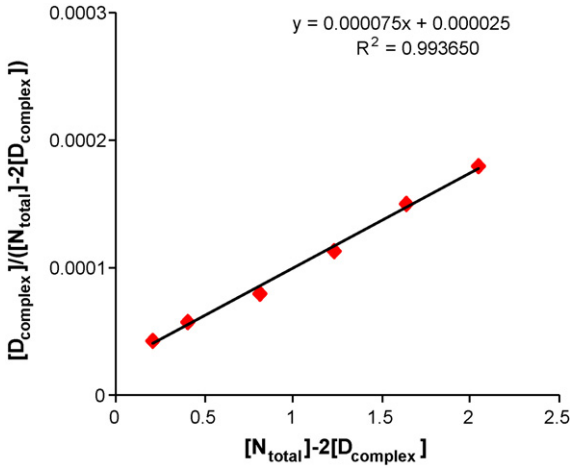

Fig. 6. Solubility profile for estrone.

The application of Eq. (7) on the solubility data of estrone and griseofulvin are demonstrated in Figs. 6 and 7. It can be seen that the experimental data are described reasonably well.

Attempts to correlate $K_{1:1}$ and $K_{1:2}$ with drug properties such as log P, planarity or aromaticity resulted only in a little success. As mentioned previously, log P is a measure of the overall non-

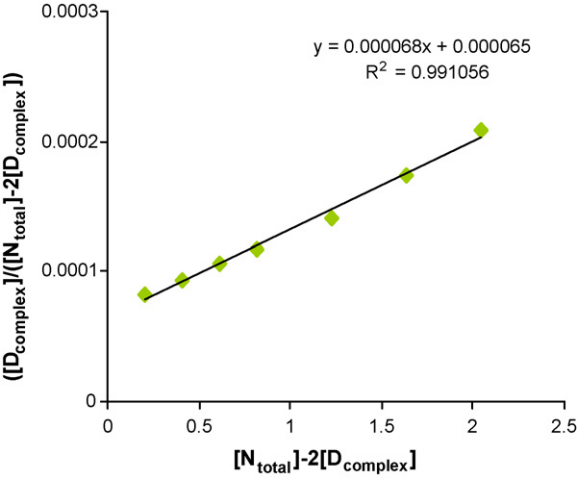

Fig. 7. Solubility profile for griseofulvin.

Table 3
Effect of temperature on complexation constants of estrone and griseofulvin

Drug	Temperature (°C)	log S_{int} (μg/ml)	$K_{1:1}$ (M^{-1})	$K_{1:2}$ (M^{-1})
Estrone	20	−0.20	10.78	31.32
	40	0.15	6.00	25.19
Griseofulvin	20	0.91	2.64	2.76
	40	1.19	1.46	1.54

polarity of the molecule while only a part of the molecule may be undergoing complexation. On the other hand, quantitating molecular planarity or aromaticity is difficult.

4.2.4. The effect of temperature on the complexation constants

Solubility studies using estrone and griseofulvin were performed at 40 °C and the values of $K_{1:1}$ and $K_{1:2}$ were calculated using Eqs. (7)–(9) (Table 3). Although, increasing the temperature increased the intrinsic solubility of the drugs, it decreased the complexation constants. A lot of factors may be responsible for this. Increasing the temperature may indirectly affect complexation by reducing the squeezing out effect of water. This would reduce the enthalpic contribution towards complexation. Higher temperature may also directly reduce complexation by increasing the effect of entropy.

5. Conclusion

The solubilization strength and mechanism of nicotinamide has been demonstrated on a set of 11 structurally diverse drugs. The solubility enhancement is an outcome of stacking complexation between drug and nicotinamide and not due to cosolvency or micellar mechanisms. At low nicotinamide concentrations, 1:1 complex is formed while at higher concentrations 1:2 complexation occurs. The higher order complexation appears to start at around 10% (w/v). Nicotinamide is more efficient than the cyclodextrin derivatives for three of the drugs, which is due to stronger stacking interactions as compared to the inclusion interactions.

Based on its high solubilization efficiency, low toxicity and economic considerations, nicotinamide warrants consideration as a solubility enhancer in pharmaceutical formulations.

References

Agrawal, S., Pancholi, S.S., Jain, N.K., Agrawal, G.P., 2004. Hydrotropic solubilization of nimesulide for parenteral administration. Int. J. Pharm. 274, 149–155.

Badwan, A.A., El-Khordagui, L.K., Saleh, A.M., Khalil, S.A., 1983. The solubility of benzodiazepines in sodium salicylate solution and a proposed mechanism for hydrotropic solubilization. Int. J. Pharm. 13, 67–74.

Balasubramanian, D., Srinivas, V., Gaikar, V.G., Sharma, M.M., 1989. Aggregation behavior of hydrotropic compounds in aqueous solution. J. Phys. Chem. 93, 3865–3870.

Chen, A.X., Zito, S.W., Nash, R.A., 1994. Solubility enhancement of nucleosides and structurally related compounds by complex formation. Pharm. Res. 11, 398–401.

Coffman, R.E., Kildsig, D.O., 1996. Hydrotropic solubilization-mechanistic studies. Pharm. Res. 13, 1460–1463.

da Silva, R.C., Spitzer, M., da Silva, L.H.M., Loh, W., 1999. Investigation on the mechanism of aqueous solubility increase caused by some hydrotropes. Thermochim. Acta 328, 161–167.

Higuchi, T., Kristiansen, H., 1970. Binding specificities between small organic solutes in aqueous solutions: classification of some solutes into two groups according to binding tendencies. J. Pharm. Sci. 59, 1601–1608.

Hussain, M.A., Diluccio, R.C., Maurin, M.B., 1993. Complexation of moricizine with nicotinamide and evaluation of complexation constants by various methods. J. Pharm. Sci. 82, 77–79.

Kenley, R.A., Jackson, S.E., Winterle, J.S., Shunko, Y., Visor, G., 1986. Water soluble complexes of the antiviral drugs, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine and acyclovir: the role of hydrophobicity in complex formation. J. Pharm. Sci. 75, 648–653.

Lee, J., Lee, S.C., Acharya, G., Chang, C., Park, K., 2003. Hydrotropic solubilization of paclitaxil: analysis of chemical structures for hydrotropic property. Pharm. Res. 20, 1022–1030.

Lim, L., Go, M., 2000. Caffeine and nicotinamide enhances the aqueous solubility of the antimalarial agent halofantrine. Eur. J. Pharm. Sci. 10, 17–28.

Mukerjee, A.T., 1967. The nature of the association equilibriums and hydrophobic bonding in aqueous solutions of association colloids. Adv. Colloid Interface Sci. 1, 241–275.

Nakano, N.I., Igarashi, S.J., 1970. Molecular interactions of pyrimidines, purines, and some other heteroatomic compounds in aqueous media. Biochemistry 9, 577–583.

Neuberg, H., 1916. Hydrotropy. Biochem. Z. 76, 107–176.

Oberoi, L.M., Alexender, K.S., Riga, A.T., 2005. Study of interaction between Ibuprofen and Nicotinamide using differential scanning calorimetry, spectroscopy, and microscopy and formulation of a fast-acting and possibly better ibuprofen suspension for osteoarthritis patients. J. Pharm. Sci. 94, 93–101.

Rasool, A.A., Hussain, A.A., Dittert, L.W., 1991. Solubility enhancement of some water-insoluble drugs in the presence of nicotinamide and related compounds. J. Pharm. Sci. 80, 387–393.

Suzuki, H., Sunada, H., 1998. Mechanistic studies on hydrotropic solubilization of nifedipine in nicotinamide solution. Chem. Pharm. Bull. 46, 125–130.

Yalkowsky, S.H., 1999. Solubility and Solubilization in Aqueous Media. Oxford University Press, Cambridge.