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Solubilization of NSC-639829

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Abstract

Solubilization using pH combined with cosolvents, surfactants, and complexants are investigated for NSC-639829, an investigational anti-tumor agent. The intrinsic solubility of the drug is approximately 30 ng/ml and it has an ionizable dimethyl aniline group with an approximate base pK_a of 5. Samples buffered at pH 1.0, 2.0, and 7.0 with various concentrations of the solubilizing agents were used to study the solubilization of NSC-629829 when present as charged and uncharged species. The solubilization of NSC-639829 was found to be much more effective when the drug was present primarily in ionized form. At pH values 1.0 and 2.0 where the surfactant (SLS) and complexant (SBEβCD) carried a negative charge enhanced solubilities of more than a million-fold were observed for the drug.

Keywords: Solubilization; NSC-639829; Dimethyl aniline

1. Introduction

NSC-639829 is currently being investigated by the National Cancer Institute (NCI) for its anti-tumor activity. It is believed that benzoylphenylurea derivatives prevent the formation of the mitotic spindle during cell division by inhibiting tubline polymerization and causing microtubule depolymerization (Okada et al., 1999). However benzoylphenyl urea derivatives are poorly water-soluble and therefore, are difficult to formulate effectively.

Using the conventional solubilization methods of pH control, cosolvency, micellization or complexation, a stable solution of 15 mg/ml (the desired dose) was not possible. Li et al. (1998, 1999a,b) showed that the use of pH along with one of the three other methods could significantly enhance the solubility of poorly soluble drugs that are ionizable. The purpose of this paper was to investigate the solubilization of NSC-639829 using effects of pH combined with cosolvents, surfactants, or complexants. This study will also provide information regarding oral formulation of these compounds.

The chemical structure of NSC-639829 is shown below

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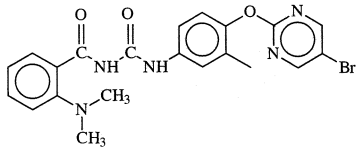

N-[4[(5-Bromo-2-pyrimidoxyl)-3-methylphenyl]-(2-dimethylamino)-benzoylurea

2. Background

2.1. Solubilization by pH

The exponential form of the well-known Henderson–Hasselbach equation can be used to determine the total aqueous solubility of an ionizable solute at a given pH. For a weak base such as NSC-639829 the equation is

$$S_{tot} = S_u(1 + 10^{(pK_a - pH)}) \quad (1)$$

2.2. Solubilization by the combined effect of pH and cosolvency

Cosolvents are commonly used in pharmaceutical formulations to enhance the solubility of non-polar drugs. They decrease the potential of the non-polar solute to be 'squeezed out' by reducing the hydrogen bond density of aqueous systems. The log linear model (Yalkowsky, 1999) is commonly used to quantify the total solubility, S^{cos}_{total} of a solute in a cosolvent system. Li et al. (1999a,b) described the combined effect of pH and cosolvents on the unionized and ionized solute in the cosolvent system as

$$S^{cos}_{total} = S_u 10^{\sigma_u f_c} + S_u 10^{(pK_a - pH)} 10^{\sigma_i f_c} \quad (2)$$

where σ_u is the cosolvent solubilizing power for the unionized solute, σ_i the cosolvent solubilization power for the ionized solute, and f_c is the fraction of the cosolvent used. It should be noted that in all cases σ_i is less than σ_u, that is, cosolvents are more effective in solubilizing the neutral drug than the ionized species. In the condition when $pKa - pH > \sigma_u - \sigma_i$ for a base such as NSC-639829, the total solubility of the charged species exceeds that of the neutral drug.

2.3. Solubilization by the combined effect of pH and micellization

Due to their amphiphilic nature surfactants have been widely used to solubilize drugs (Yalkowsky, 1999). The combined effect of the pH and the surfactant concentration has been described by Li et al. (1999a,b) to be

$$S^{surf}_{total} = S_u + S_u 10^{(pK_a - pH)} + \kappa_u S_u C_{mic} + \kappa_i S_u 10^{(pK_a - pH)} C_{mic} \quad (3)$$

where S^{surf}_{total} is the total solubility of the solute, κ_u the surfactant solubilizing power for the unionized form of the drug, κ_i the surfactant solubilizing power for the ionized drug, and C_{mic} is the concentration of the surfactant. Although κ_u is universally greater than κ_i, the solubility in the micelles will be greater for the ionized species than the unionized species if $10^{(pK_a - pH)} > \kappa_u/\kappa_i$.

2.4. Solubilization by the combined effect of pH and complexation

Recently, cyclodextrin derivatives have been widely used for enhancement of the aqueous solubility of drugs (Yalkowsky, 1999). This solubilization can approximated for a 1:1 complex by Li et al. (1998, 1999b).

$$S^{comp}_{total} = S_u + S_u 10^{(pK_a - pH)} + K_u S_u C_L + K_i S_u 10^{(pK_a - pH)} C_L \quad (4)$$

where C_L is the concentration of the ligand added, K_u the stability constant of the complex of the unionized solute, and K_i is the stability constant for the ionized solute. The ratio K_u to K_i is always larger than unity while the ratio of the slopes for the ionized-to-ionized solute is dependent on the pH, pK_a, and concentration of the ionized species. Therefore, by analogy to the case for surfactants the solubility of the ionized species will be larger than the unionized species if $10^{(pKa - pH)} > K_u/K_i$.

3. Methods

3.1. Materials

NSC-639829 (over 99% purity) was synthesized by the NCI and was used as received. Reagent grade solvents were purchased from either Aldrich (St. Louis, MO) or Sigma (St. Louis, MO). Hydroxypropyl-β-cyclodextrin (HPβCD) with an average molecular weight 1390 and an average degree of substitution of 4.4 was obtained from Cyclodextrin Technologies Development Inc. (Gainesville, FL). Sulfobutyl ether β-cyclodextrin (SBEβCD) with an average molecular weight of 2162 and an average degree of substitution of 7 was generously gifted by Cydex, L.C. (Overland Park, KS).

Buffers in the pH range of 3.0–8.0 were prepared with 0.01 M citric acid and 0.02 M disodium hydrogen phosphate while in the pH range of 8.0–11.0 with 0.01 M glycine and 0.01 N sodium hydroxide. Also 0.01 M glycine and 0.01 N hydrochloric acid were used to prepare buffers with pH values below 3.0. The ionic strength of all buffered solutions was adjusted to 0.2 M by adding sodium chloride.

3.2. Physical properties

The logarithm of the octanol–water partition coefficient for NSC-639829 was estimated by CLOGP® (Heekman, 1997). The melting point of the drug was determined by differential scanning calorimetery (TA Instruments, Model # 109, New Castles, DE-19720) using a heating rate of 10 °C/min.

3.3. Solubility determinations

An excess amount of NSC-639829 was added to 2 ml vials containing the following aqueous systems, buffers pH 1.2–11.5, various concentrations of ethanol (EtOH)/propylene glycol (PG)/dimethylsufoxide (DMSO)/polyethylene glycol-400 (PEG400) solutions with three different buffers of pH 1.0 ± 0.2, 2.0 ± 0.2 and 7.0 ± 0.2, respectively. Similarly, various concentrations of Tween 80, sodium lauryl sulfate (SLS), myristoyl carnitine (MC) hydrochloride were prepared with each of the following buffers of pH 1.0, 2.0, and 7.0. For the complexation study, different concentrations of both HPβCD and SBEβCD in buffers at pH 1.0, 2.0 or 7.0 were used. The sample vials were rotated for 3 days and then the pH of the solutions were measured and if needed, were readjusted. The samples were further rotated for 2 more days and then filtered and analyzed. All the samples were prepared in duplicates.

3.4. High performance liquid chromatography (HPLC) analysis

The HPLC assay for NSC-639829 was adapted from the method developed by the NCI Report 98, 2001. Briefly, the analysis included a 250×4.6 mm Lichosorb RP-18 column with particle size of 10 μm. The mobile phase comprised of 90% methanol and 10% water. A flow rate of 1.0 ml/min was maintained and the effluent was detected at a wavelength of 254 nm. None of the solubilizing species interfered with the assay.

4. Results and discussion

4.1. Aqueous solubility

The water solubility of NSC-639829 was determined to be approximately 30 ng/ml. The melting point and the logarithm of the octanol water partition coefficient for the drug were found to be 103–105 °C and 6.21, respectively. As these two properties contribute to the intrinsic solubility (Jain and Yalkowsky, 2001), it can be said that the non-polarity of this drug significantly contributes to the insolubility in water rather than the crystallinity. Therefore, the use of solubilizing agents to reduce the polarity of water can be expected to increase the solubility.

4.2. Solubilization by pH

The experimental values of the total solubility in various buffers are represented as closed circles in Fig. 1. The solid line was generated using Eq. (1) by assuming an approximate basic pK_a value of 5.0 for the dimethyl aniline.

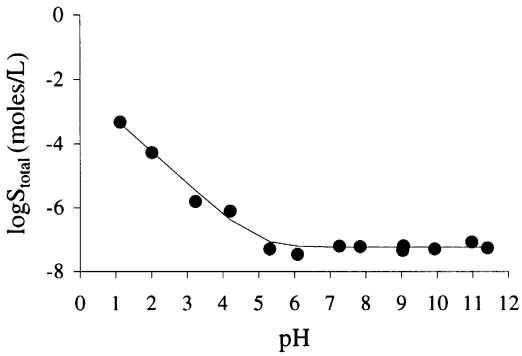

Fig. 1. pH-Solubilization plot for NSC-639829.

4.3. Solubilization by pH and cosolvents

Using the four cosolvents at least a hundred fold increase in solubility of NSC-639829 was observed at pH 1.0, 2.0, and 7.0. In the first two buffers, the drug is predominantly present in the ionized form while in the buffer at neutral pH it is unionized. As shown in Fig. 2, the solubility in all the cosolvents was highest at pH 1.0, then in the buffer at pH 2.0, and the least in pH 7.0 buffer. The solubilization slopes were calculated from Eq. (2) and are provided in Table 1.

Table 1
Solubilization power of cosolvents at pH 1, 2, and 7

Cosolvent	σ_u (pH 7)	σ_i (pH 2)	σ_i (pH 1)
EtOH	8.56	5.32	2.29
DMSO	5.21	2.79	0.75
PEG 400	5.07	3.80	1.12
PG	4.63	3.66	3.55

Although the slopes of the low pH are smaller than that of pH 7.0, the amount of drug solubilized is greater at the lower pH.

4.4. Solubilization by pH and surfactants

Three surfactants, tween 80, MC, and SLS represented non-ionic, cationic and anionic surfactants, respectively. The plots of the total solubility versus the percent surfactant at pH 1.0, 2.0, and

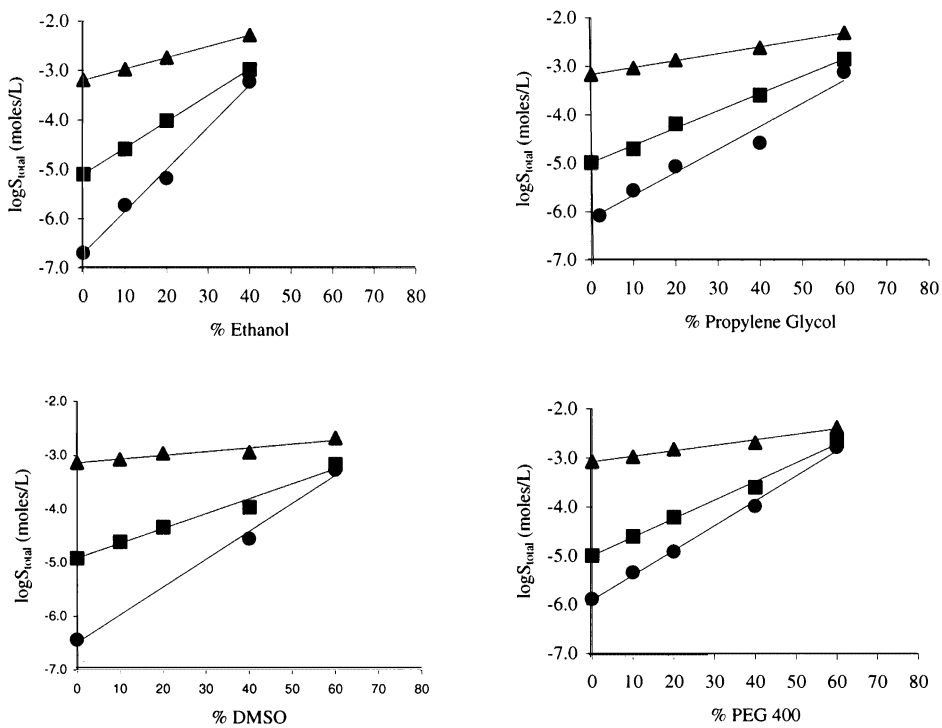

Fig. 2. Solubilization of NSC-639829 by EtOH, PG, Dimethyl Sulfoxide (DMSO), and PEG 400 at pH 1 ▲, 2 ■, and 7 ●.

7.0 are shown in Fig. 3. At neutral pH, the drug is primarily uncharged and the solubilization by all the surfactants are similar, yielding a solubility of approximately 10^5 times that of the intrinsic solubility value. Since the drug is positively charged at pH 1.0 and 2.0, the non-ionic tween 80 and the cationic MC hydrochloride are less effective than the anionic SLS as solubilizing agents.

Table 2
Solubilization capacities of the surfactants used to solubilize NSC-639829

Surfactant	κ_u (pH 7)	κ_i (pH 2)	κ_i (pH 1)
Tween 80	0.040	0.025	0.026
MC	0.009	0.025	0.038
SLS	0.043	0.263	0.163

The final concentrations obtained with SLS is 10–14 mg/ml. The solubilization capacities of the three surfactants are given in Table 2.

Interestingly, at pH 2.0 SLS resulted in higher solubility than pH 1.0. This can be attributed to the fact that at pH 1.0 the percent ionized SLS will be less than that at pH 2.0 as the pK_a of SLS is one. At pH 2.0 the concentration of the anionic

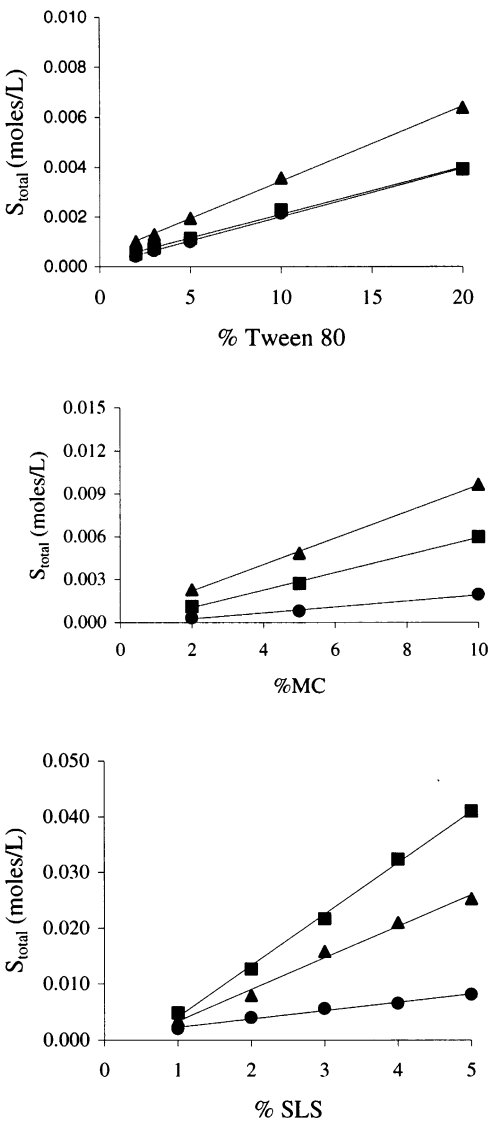

Fig. 3. Solubilization of NSC-639829 by Tween 80, Myristoyl Carnitine hydrochloride (MC), and Sodium Lauryl Sulfate (SLS) at pH 1 ▲, 2 ■, and 7 ●.

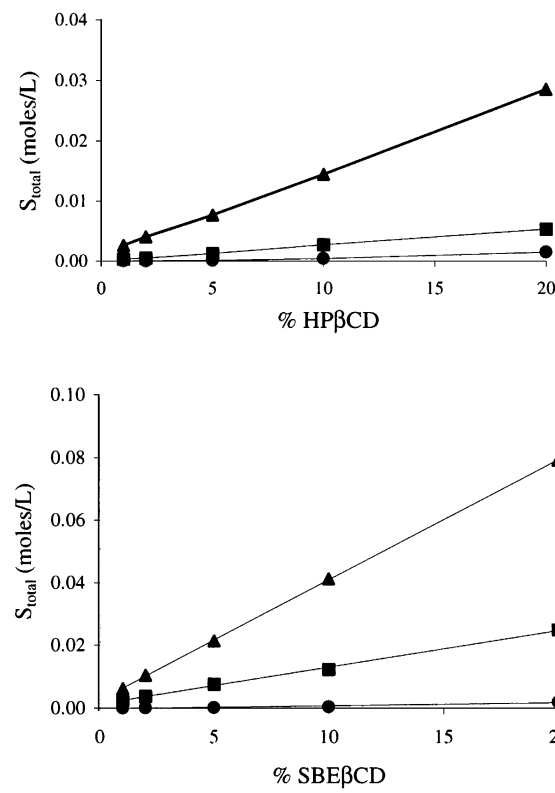

Fig. 4. Solubilization of NSC-639829 by Hydroxypropyl-β-Cyclodextrin (HPβCD) and Sulfabutylether-β-Cyclodextrin (SBEβCD) at pH 1 ▲, 2 ■, and 7 ●.

Table 3
Solubilization slopes and formation constants of the complexants used to solubilize NSC-639829

Complexant	pH 7		pH 2		pH 1	
	Slope	K_u	Slope	K_i	Slope	K_i
HPβCD	0.01	189732	0.04	443	0.19	280
SBEβCD	0.01	207509	0.25	3855	0.83	5823

surfactant is much larger than that at pH 1.0 thereby, more of the cationic drug is solubilized.

4.5. Solubilization by pH and complexation

The solubility of NSC-639829 in various concentrations of HPβCD and SBEβCD are shown in Fig. 4. At pH 7.0, 10% of both the complexing agents were able to solubilize 20000 times the intrinsic solubility to result in approximately 1.0 mg/ml solutions. Clearly, SBEβCD was more efficient than HPβCD at solubilizing the cationic drug. This can be attributed to the fact that the former carries a negative charge at low pH while the latter is neutral. The added electrostatic attraction in the case of SBEβCD leads to a powerful means of solubilization for the cationic drug and at 20% concentration yields approximately 36 mg/ml solution. Thus, SBEβCD and pH 1.0 produces over a million-fold increase in the solubility over the intrinsic solubility of NSC-639829.

Using Eq. (4), the solubilization in all the buffers were used to calculate the formation constants for HPβCD and SBEβCD and are included in Table 3 along with the observed solubilization slopes.

5. Conclusions

Solubilization of NSC-639829 by combining pH control with cosolvents, surfactants, and complexants was found to be more effective than use of any of the techniques alone. Table 4 summarizes the results of these methods at usable concentrations of the solubilizing agents in three buffers at pH 1.0, 2.0, and 7.0. The bold numbers represent solutions of greater than 1.0 mg/ml solutions that is, three million fold increase in the intrinsic solubility. At pH 1.0 and 2.0, the drug is primarily charged leading to a higher solubility. When these buffers are combined with cosolvents, surfactants and complexants, the solubility is further enhanced producing solubility increases much higher than the uncharged compound. Thus in site of the solubilization powers of the cosolvents, surfactants, and the complexants are much higher for the uncharged species than the ionized species the latter are solubilized to a greater extent.

The surfactant SLS, and the complexant SBEβCD were the most powerful in solubilizing NSC-639829 and it can be attributed to the fact that both are present as anions at pH 1.0 and 2.0. This illustrates the role of electrostatic attraction in both micellization and complexation. Therefore, use of either a complexant or an anionic

Table 4
Summary of solubilization of NSC-639829 using combined approach of pH with cosolvents, surfactants, and complexants

System	pH 7 (mg/ml)	pH 2 (mg/ml)	pH 1 (mg/ml)
Buffer	0.00003	0.024	0.210
20% Ethanol	0.003	0.047	0.848
40% Propylene Glycol	0.017	0.119	1.114
20% DMSO	0.002	0.021	0.511
20% PEG 400	0.006	0.028	0.702
20% Tween 80	1.800	1.817	2.948
5% SLS	3.746	14.625	10.521
10% MC	0.895	2.762	4.457
20% HPβCD	0.681	2.461	13.165
20% SBEβCD	0.879	11.514	36.508

surfactant at low pH can be expected to increase the oral bioavailability of the drug.

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