Stable Supersaturated Aqueous Solutions of Silatecan 7-t-Butyldimethylsilyl-10-Hydroxycamptothecin via Chemical Conversion in the Presence of a Chemically Modified β-Cyclodextrin

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Purpose. A method for obtaining clear supersaturated aqueous solutions for parenteral administration of the poorly soluble experimental anti-cancer drug silatecan 7-t-butyldimethylsilyl-10-hydroxycamptothecin (DB-67) has been developed.

Methods. Equilibrium solubilities of DB-67 were determined in various solvents and pH values, and in the presence of chemically modified water-soluble β-cyclodextrins. The stoichiometry and binding constants for complexes of the lactone form of DB-67 and its ring-opened carboxylate with sulfobutyl ether and 2-hydroxypropyl substituted β-cyclodextrins (SBE-CD and HP-CD) were obtained by solubility and circular dichroism spectroscopy, respectively. Kinetics for the reversible ring-opening of DB-67 in aqueous solution and for lactone precipitation were determined by HPLC with UV detection.

Results. Solubilities of DB-67 lactone in various injectable solvent systems were found to be at least one order of magnitude below the target concentration (2 mg/ml). DB-67 forms inclusion complexes with SBE-CD and HP-CD but the solubilization attainable is substantially less than the target concentration. Slow addition of DB-67/DMSO into 22.2% (w/v) SBE-CD failed to yield stable supersaturated solutions due to precipitation. Stable supersatured solutions were obtained, however, by mixing a concentrated alkaline aqueous solution of DB-67 carboxylate with an acidified 22.2% (w/v) SBE-CD solution. Ring-closure yielded supersaturated solutions that could be lyophilized and reconstituted to clear, stable, supersaturated solutions.

Conclusions. The method developed provides an alternative to colloidal dispersions (e.g., liposomal suspensions, emulsions, etc.) for parenteral administration of lipophilic camptothecin analogs.

KEY WORDS: cyclodextrin complexes; camptothecin analogs; parenteral formulation; lactone hydrolysis; solubilization; parenteral formulation.

INTRODUCTION

The low aqueous solubility and physical and chemical instability of many new drug candidates limits the options available for their formulation in parenteral dosage forms. While various solubilization approaches are available including salt formation, cosolvent solubilization, complexation, and the formation of mixed micelles, liposomes, emulsions, and micro/nanoparticles (1,2), these approaches often fail to provide the desired concentration for some very poorly water-soluble drugs. In such instances, supersaturated solutions may be one of the few alternatives, providing that drug precipitation can be prevented (3).

Silatecan 7-t-butyldimethylsilyl-10-hydroxycamptothecin (DB-67) is an experimental drug in the early stages of development as a cancer chemotherapeutic by the National Cancer Institute. DB-67, one of a class of A and B ring modified camptothecin analogs, displays superior binding to cellular and liposomal membranes and enhanced drug stability in the presence of human serum albumin when compared with clinically relevant more hydrophilic camptothecin analogs because of its increased lipophilicity and dual 7-alkylsilyl and 10-hydroxy substitution (4). DB-67 has comparable potency in *in vitro* cytotoxicity assays to other FDA approved camptothecin analogs (e.g., Camptosar and Hycamtin). As shown in Fig. 1, DB-67 can exist in an E-ring closed lactone (I) and an E-ring opened form (II) depending on solution pH and solvent properties. Structure-activity studies have shown that successful inhibition of DNA topoisomerase I by camptothecin analogs requires an intact lactone E-ring (5–8). Unfortunately, as reported in this study, the lactone form of DB-67 has very poor solubility in water. In the past, poor solubility has prevented the extensive use of highly lipophilic camptothecin analogs in the clinical treatment of cancer. Indeed, while a number of water soluble camptothecin derivatives including Camptosar, topotecan, 9-amino-camptothecin, 7-(4-methylpiperazino-methylene)-10,11-methylenedioxy camptothecin, 10,11-methylenedioxy-camptothecin and 10,11-ethylenedioxy-camptothecin have either been on the market, studied preclinically, or used in clinical trials to treat certain types of human cancer, few clinical studies have been conducted in human patients involving poorly water soluble, highly lipophilic camptothecin analogs (e.g., camptothecin, 10-hydroxy-7-ethyl camptothecin, DB-67, etc.) (9–13). Thus, a viable formulation of DB-67 for intravenous delivery should exhibit sufficient physical and chemical stability to maintain the desired concentration (2 mg/mL) exclusively in the lactone form.

This paper describes the development of a simple strategy for preparing a stable supersaturated parenteral formulation of DB-67. The solubility of DB-67 in various solvent systems (e.g., aqueous solutions varying in pH, cosolvents, emulsions, and liposomes), complexation with cyclodextrin derivatives (i.e., SBE-CD and HP-CD), hydrolysis kinetics at different pH values and in the presence and absence of cyclodextrins, and physical stability with respect to drug precipitation have been explored. Based on these experiments, a novel formulation involving chemical conversion in the presence of the chemically modified β-cyclodextrin, SBE-CD, has been developed.

MATERIALS AND METHODS

Chemicals

DB-67 (NSC 708298) and Diluent-12 (NSC 614387; composed of 50% Cremophor and 50% ethanol) were supplied by the National Cancer Institute (Bethesda, MD, USA). β-cyclodextrin sulfobutyl ether, sodium salt, having an average degree of substitution of 7 sulfobutyl ether residues per cy-

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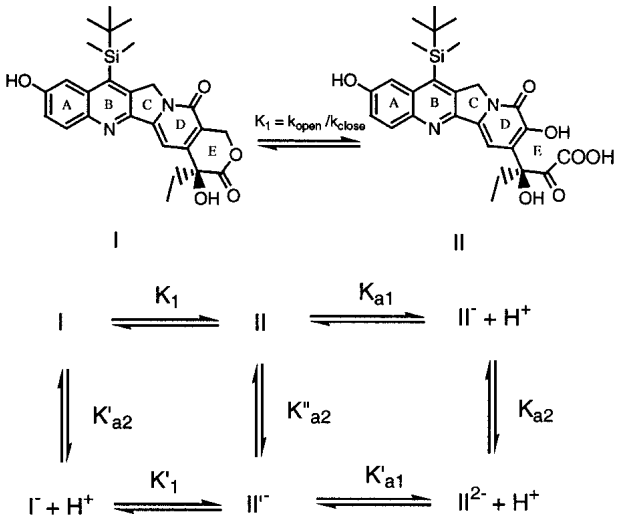

Fig. 1. The structures of the lactone ring intact DB-67 (species I) and its E-ring opened counterpart (species II) and the proposed reaction sequences for the E-ring opening hydrolysis and acid-base dissociations of DB-67.

clodextrin molecule, (SBE-CD, Captisol®) was a gift from CyDex, Inc. (Overland Park, KS, USA). 2-hydroxypropyl-β-cyclodextrin (HP-CD) with an average degree of substitution of 7.5 (ave. MW = 1540) was a gift from Pharmatec, Inc. PEG-400 and propylene glycol (99.5%) were obtained from Aldrich (Milwaukee, WI, USA). Camptothecin (CPT), dimethylsulfoxide (DMSO, ACS reagent), sucrose and cholesterol (+99%) were obtained from Sigma Chemical Inc. (St. Louis, MO, USA). 1,2-dimyristoyl-*sn*-glycerol-3-phosphocholine (DMPC) and 1,2-dimyristoyl-*sn*-glycerol-3-[phospho-rac-(1-glycerol)] (DMPG), sodium salt, were purchased from Avanti Polar Lipids (Pelham, AL, USA) and stored in a freezer upon arrival. All other compounds were reagent or HPLC grade from commercial sources and were used without further purification. Aqueous solutions were prepared using deionized water.

Solubility Determinations

Solubilities of DB-67 or camptothecin in a given solvent system (e.g., cosolvents, emulsions, and liposomes) were determined by adding an amount of drug well in excess of its estimated solubility to 1–2 mL of the solvent of interest in a 4-mL glass vial. Buffers employed for pH-solubility studies were 30 mM acetate (pH 5.2), 30 mM phosphate (pH 7.4), 23 mM carbonate (pH 9.5), and 500 mM carbonate (pH 9.8 & 10.2). Inclusion complex formation between the lactone of DB-67 and SBE-CD or HP-CD was determined by measuring DB-67 solubility at varying concentrations of cyclodextrin at a pH ≤4.6 maintained with a 30 mM citric acid buffer. The capped vials were rotated in a VWR2010 incubator (VWR Scientific Inc., San Francisco, CA, USA) set at 25°C for a period of 3 to 14 days. The samples were then filtered (0.45 μm, 4 mm Gelman Acrodisc, PN 4473 or 1.2 μm, 25 mm Gelman Acrodisc, PN 4190 for liposomal or emulsion samples) and the first 6 to 8 drops were discarded while the remaining 2 to 4 drops were collected in a 4-mL glass vial, weighed, diluted with methanol, and analyzed by HPLC. Liposome samples were prepared by dissolving DMPC, cholesterol, and DMPG (70:25:5 mole) in ethanol, evaporating under a stream of N_2 gas, vacuum-drying at 45°C overnight, rehydrating with a 10% (w/v) solution of sucrose in 30 mM acetate buffer at pH 5.1, and extruding the rehydrated suspensions through a 0.1 μm polycarbonate filter 15 times by a LiposoFast-Pneumatic Extruder (Avestin, Inc., Ottawa). The emulsions were prepared by weighing out egg PC, glycerin, and Tween 80, dispersing the weighed compounds with water, adding slowly a weighed amount of soybean oil into the aqueous solution with vigorous stirring, sonicating the mixture for about half an hour, and homogenizing (EmulsiFlex-C5, Avestin, Inc., Ottawa) the mixture at 10,000 psi for 15 cycles.

Circular Dichroism Measurements

Circular dichroism was used to examine inclusion complex formation between the DB-67 carboxylate and cyclodextrins (i.e., SBE-CD and HP-CD). The DB-67 concentration was set at 2.2×10^{-4} M and solution pH was maintained at 10.6 with 0.1 M Na_2CO_3. Circular dichroism measurements were conducted at ambient temperature (24°C) on a Jasco model J-710 Spectropolarimeter (Jasco Spectroscopic, Hachioji City, Japan) with computer-controlled data acquisition and analysis. The light source was a Xenon-arc lamp. The samples were transferred to a 0.5-cm path-length quartz cuvette aligned in a holder within a chamber under a constant flow of nitrogen gas. The scan speed, step resolution, response time, and accumulation were set at 50 nm/min, 1 nm, 3 seconds and 50, respectively.

Analytical Methods

Concentrations of DB-67 or camptothecin in various samples were measured by HPLC in an isocratic mode. The HPLC system consisted of a Discovery C_{18} (5 μm) column (15 cm × 4.6 mm) and guard column (2 cm × 4.6 mm) (Supelco, Belleforte, PA, USA), a Beckman 110B solvent delivery module (Beckman Instruments, San Ramon, CA, USA), a Rheodyne M7125 injector with a 20 μl injection loop (Rainin Instrument, Woburn, MA, USA), a Waters M2487 Dual λ Absorbance detector (Water Associates, Milford, MA, USA) set at 254 nm, an HP 3392A integrator (Hewlett-Packard, Avondale, PA, USA), and mobile phases containing 25% and 41% (v/v) acetonitrile in 2% (w/v) triethylamine acetate for camptothecin and DB-67, respectively. The retention volumes for the lactone and ring-opened forms of DB-67 were approximately 4 and 10 mL, respectively. External standards prepared with 41% (v/v) acetonitrile/59% 2 mM HCl in water and 2 mM NaOH in water were used for the lactone and ring-opened species, respectively. The carry-over was less than 0.02% and the response factors (peak area/concentration) for both species were constant below 0.03 mg/mL. If necessary, the samples to be assayed were diluted with methanol before the HPLC run.

Forward and Reverse Hydrolysis Reactions

The kinetics for hydrolysis of the lactone and for the ring closure reaction were investigated in aqueous solutions at pH 7.4 and 4.0, respectively, maintained at 25 ± 0.1°C in a circulating water bath (Haake A81, Berlin, Germany). The reactions were initiated by adding 0.1 mL of reactant (DB-67 lactone in DMSO or DB-67 carboxylate in 2 mM NaOH) to

5 mL of a 30 mM phosphate (pH 7.41, 0.1 ionic strength) or acetate (pH 4.04, 0.1 ionic strength) buffer to obtain an initial reactant concentration of 2–4 × 10^{-6} M. After the addition and at different time intervals, aliquots of the reactant solutions were withdrawn and immediately assayed by HPLC. The concentrations of both the E-ring opened (II; cf., Fig. 1) and lactone forms of DB-67 (I) were monitored.

Precipitation Studies

Stability of supersaturated DB-67 solutions with respect to drug precipitation was monitored by measuring changes of DB-67 concentration in the supernatant after filtration. Initially, concentrated stock solutions of DB-67 lactone (20–25 mg/ml) in DMSO were added slowly (0.1 mL/min) to a continuously stirred solution of 22.2% (w/v) SBE-CD/water to a final concentration of either 1 or 2 mg/mL. Subsequently, a concentrated solution of DB-67 carboxylate (20 mg/mL) in an aqueous solution containing a molar excess of NaOH was filtered through a 0.2 μm filter (13 mm Gelman Acrodic, PN 4423T) and injected slowly (0.11 mL/min) into 22.2% (w/v) SBE-CD/water containing 2 mM acetic acid and a molar excess of HCl to neutralize the DB-67 carboxylate, induce ring closure, and achieve a final solution containing 2 mg/mL DB-67 lactone at a pH of approximately 4. After the dilutions, the samples were stored at 25°C. At certain time intervals, aliquots were filtered through a 0.45 μm (4 mm Gelman Acrodisc, PN4473) filter and after discarding the first 6 to 8 drops, the next 2 to 3 drops were collected, weighed, and diluted with methanol for subsequent HPLC assay.

Lyophilization

Solution formulations (3 mL) in glass vials were frozen in liquid nitrogen for 10 min, transferred to a freeze-dryer (FDC206, Savant Instruments Inc.) and lyophilized at <–50°C for one day. Secondary drying was performed for 4 h at room temperature.

RESULTS AND DISCUSSION

Solubilization of DB-67 in Various Solvent Systems

The effect of equilibration time on the solubility of DB-67 in a 50% Cremophor/50% EtOH (v/v) cosolvent, Diluent-12, was determined in triplicate after the samples were rotated for 3 and 14 days in an incubator controlled at 25°C. A *t* test analysis indicated no significant difference ($p < 0.025$) between apparent solubilities at 3 and 14 days. Thus, unless otherwise specified, the solubility experiments were conducted with an equilibration time of 3 days. The solubility of DB-67 in various solvent systems is reported in Table I. The intrinsic solubility of DB-67 in an aqueous solution at pH 5.2, wherein DB-67 exists predominantly as a lactone, is 0.11 μg/mL, well below the target concentration of 2 mg/mL needed to afford an appropriate administration time and volume. Various solubilization approaches such as cosolvents (e.g., mixtures of Diluent-12, PEG-400 or PG with water), complexation with water-soluble chemically modified cyclodextrins (e.g., SBE-CD and HP-CD), emulsions (95% Intralipid, 4% Tween 80, 1% eggPC), and liposomes (DMPC:Chol:DMPG, 70:25:5 (mole)) were explored to find an optimal solubilization method for the formulation. These vehicles are commonly considered for solubilizing highly lipophilic drug candidates and, in some cases (e.g., cyclodextrins, emulsions, and liposomes), it was anticipated that they might stabilize the lactone form of DB-67. As noted in Table I, however, these solvent systems were ineffective in achieving the desired concentration.

pH-Solubility Profiles for DB-67

Shown in Fig. 2 is the apparent solubility of DB-67 in aqueous solutions at different pH values. Based on rate constants reported previously (14), equilibration times of 3 days were more than sufficient to achieve equilibrium with respect to the ring opening-closing reactions of camptothecin analogues. In accordance with the fact that the E-ring opened form of DB-67 (II) has two ionizable groups (10-OH and the –COOH) in the pH range of 4 to 10, the reaction sequences described in Fig. 1 must be considered in interpreting the pH-solubility profile. The overall solubility (S) for DB-67 can then be written as:

$$S = [I] + [II] + [II^-] + [I^-] + [II^{2-}] + [II'^-] \quad (1)$$

or

$$S = S_o(1 + K_1 + K_1 10^{pH-pK_{a1}} + 10^{pH-pK'_{a2}} + K_1 10^{2pH-pK_{a1}-pK_{a2}} + K'_1 10^{pH-pK'_{a2}}) \quad (2)$$

Table I. Solubilities of DB-67 in Various Solvent Systems at 25°C[a]

Solvent	S (mg/mL)
Water/30 mM acetate buffer at pH 5.23	$(1.11 \pm 0.00) \times 10^{-5}$
Water/0.5 M carbonate buffer at pH 10.20	1.78×10^{1}
40% (w/v) HPCD/water	$(4.9 \pm 0.2) \times 10^{-1}$
40% (w/v) SBE-CD/water/1mM HCl	$(2.09 \pm 0.04) \times 10^{-1}$
40% PG, 10% (v/v) EtOH/water	$(1.73 \pm 0.04) \times 10^{-1}$
10% (v/v) PEG-400/water	$(3.3 \pm 0.5) \times 10^{-2}$
50% (v/v) PEG-400/water	$(2.0 \pm 0.3) \times 10^{-1}$
Emulsion (20% soybean oil, 2% glycerin, 73% water, 4% Tween 80, 1% eggPC)	$(2.06 \pm 0.05) \times 10^{-1}$
Liposome (70:25:5 (mole) DMPC:Chol:DMPG)	$(7.4 \pm 0.3) \times 10^{-3}$
50% Cremophor, 50% (v/v) EtOH (Diluent-12)	$(7.5 \pm 0.2) \times 10^{0}$
5% Cremophor, 5% (v/v) in 5% (w/v) Dextrose/Water	2.00×10^{-1}

[a] Mean ± standard deviation from duplicate samples except in 10% (v/v) diluent-12 in 5% (w/v) dextrose/water and water/0.5 M carbonate buffer at pH 10.20 (single).

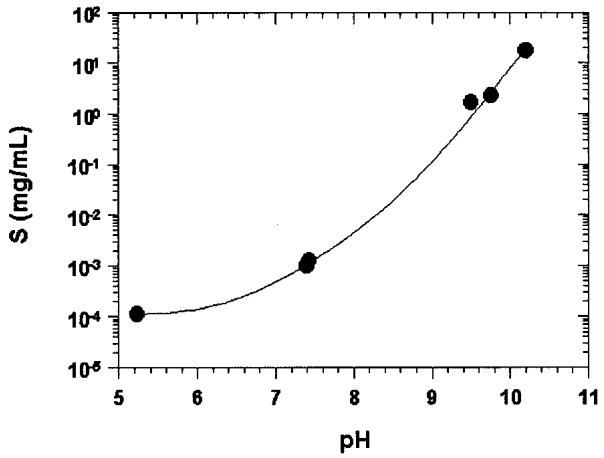

Fig. 2. Dependence of solubility, S (mg/ml), for DB-67 in aqueous solution at 25°C on solution pH values. The curve is a least-squares fit using Eq. (3).

where I and II denote the neutral lactone and E-ring opened species, respectively, and S_o is the intrinsic aqueous solubility of the lactone. The equilibrium constants corresponding to Fig. 1 and Eq. (2) are: $K_1 = [II]/[I]$, $K_{a1} = [II^-][H^+]/[II]$, $K'_{a2} = [I^-][H^+]/[I]$, $K''_{a2} = [II'^-][H^+]/[II]$, $K_{a2} = [II^{-2}][H^+]/[II^-]$, $K'_1 = [II'^-]/[II^-]$, and $K'_{a2} = [I^-][H^+]/[I]$, and $K'_{a1} = [II^{-2}][H^+]/[II'^-]$. Among these seven equilibrium equations, only five are independent. Because various A-ring substituents have only a minor effect on the kinetics and equilibrium of E-ring opening/closing and K_1 for unsubstituted camptothecin is small (2×10^{-3}) (14), several approximations can be made in Eq. (2) ($K_1 = K'_1$, $K_{a2} = K'_{a2} = K''_{a2}$, $K_{a1} = K'_{a1}$), resulting in the following simplified solubility equation:

$$S = S_o(1 + K_1K_{a1}10^{pH} + K_{a2}10^{pH} + K_1K_{a1}K_{a2}10^{2pH}) \quad (3)$$

where S_o, K_1K_{a1} and K_{a2} are adjustable parameters. A least-squares fit of the solubility data in Fig. 2 yielded K_1K_{a1} and pK_{a2} of $(3.4 \pm 0.3) \times 10^{-7}$ and 8.67 ± 0.20, respectively. For the ring-opened DB-67 (II), the carboxylic acid group may have a pK_a value around 3.8 since the pK_a values for structurally similar compounds, D-gluconic acid and α-hydroxybutyric acid, are 3.77 and 3.65, respectively, and a pK_a of 4.00 was assumed for 10-hydroxyl substituted camptothecin by Fassberg *et al.* (14). Assuming that $pK_{a1} = 3.80$, a value of $K_1 = 2.2 \times 10^{-3}$ can be estimated for K_1. This value is close to $K_1 = 2 \times 10^{-3}$ estimated for unsubstituted camptothecin by Fassberg *et al.* (14). The pK_{a2} value (8.67) obtained from the present regression analysis is close to the UV spectroscopic measurement of pK_{a2} (8.56) for the phenol group at position 10 in 10-hydroxyl-camptothecin (14), though the extremely low solubility for DB-67 would preclude the use of this technique for the determination of pK_{a2} for DB-67.

Referring to Fig. 2, at a pH below 7.4 but above the estimated pK_{a1} (3.8) for the carboxylic acid group in the ring-opened species, the solubility S increases by less than one order of magnitude over 3 pH units. This result suggests that the ring-opening reaction (I -> II) is unfavorable (i.e., K_1 is small). This is consistent with previous studies of the hydrolysis kinetics of camptothecin analogs (14) in which the K_1 constant was on the order of 2×10^{-3}.

Above pH 7.4, the solubility increases dramatically with a slope of approximately 2 near pH 10, due to ionization of the carboxylic group in the E-ring opened species and the hydroxyl group at position 10 in DB-67. At pH 10.20, the solubility reaches approximately 18 mg/mL, well above the target DB-67 concentration of 2 mg/mL, but the E-ring opened species of a camptothecin analog is therapeutically inactive, has a significantly shorter plasma half-life, and exhibits greater toxicity than the lactone. This is supported by pharmacologic evidence from clinical studies in humans and other mammalian species receiving sodium camptothecin, 9-amino camptothecin and Topotecan (15,16). Thus, an alternative strategy to solubilize DB-67 is needed.

Inclusion Complexation between DB-67 and Cyclodextrins

The results in Table I indicate that DB-67 can be solubilized by the water-soluble chemically modified β-cyclodextrins (e.g., SBE-CD and HP-CD). To understand the complexation mechanisms behind this solubilization effect, two methods were employed to determine the complex formation constants for the neutral lactone and ionized carboxylate forms of DB-67 with two water-soluble β-cyclodextrins, SBE-CD and HP-CD, since no single method was suitable for both species of DB-67. A solubility method was employed for DB-67 lactone as the intrinsic solubility for the neutral species in aqueous solution is very low and sensitive to cyclodextrin concentration, [CD], as shown in Fig. 3(A). The linear solubility profile for DB-67 with SBE-CD in Fig. 3 indicates that 1:1 complexes predominate. In contrast, a quadratic dependence of DB-67 solubility on [CD] was observed for complexation of the lactone with HP-CD, suggesting the formation of 1:2 complexes at a higher HP-CD concentration.

In the limit of [CD] >> S_o, the relevant formation constants can be evaluated as,

$$S = S_o(1 + K_f^{1:1}[CD] + K_f^{1:2}[CD]^2) \quad (4)$$

where S_o is the solubility in the absence of cyclodextrin. A linear least-squares fit of the data for SBE-CD in Fig. 3(A) yielded $K_f^{1:1} = 8.5 \pm 0.2 \times 10^3\ M^{-1}$ ($r^2 = 0.999$). The inability for SBE-CD to form 1:2 complexes with DB-67 apparently arises from the presence of an average of seven negatively charged sulfobutyl ether groups in the molecule preventing the approach of two SBE-CDs to each other to form 1:2 complexes. A least-squares fit of the data for HP-CD in Fig. 3(A) according to Eq. (4) yielded of $K_f^{1:1} = 5.8 \pm 0.2 \times 10^3\ M^{-1}$ and $K_f^{1:2} = 3.8 \pm 0.2 \times 10^4\ M^{-2}$ ($r^2 = 1.000$).

The ability of HP-CD to form 1:2 complexes with the lactone form of DB-67 suggests that there are at least two binding sites in the lactone. The influence of the 7-t-butyldimethylsilyl substituent on the complexation strength can be evaluated by comparing the complexation of DB-67 and unsubstituted camptothecin (CPT) using the same solubility method. The results for CPT are presented in Fig. 3 (B) and fitted with a linear model. The 1:1 complex formation constants for CPT with SBE-CD and HP-CD are presented in Table II along with those for DB-67. Interestingly, the 1:1 complex formation constants for CPT with SBE-CD and HP-CD are about 30 times smaller than those for the lactone form of DB-67 with the corresponding cyclodextrin. Since the 10-OH is hydrophilic and thereby less inclined to enter into the CD cavity, the present results suggest that the 7-t-butyldi-

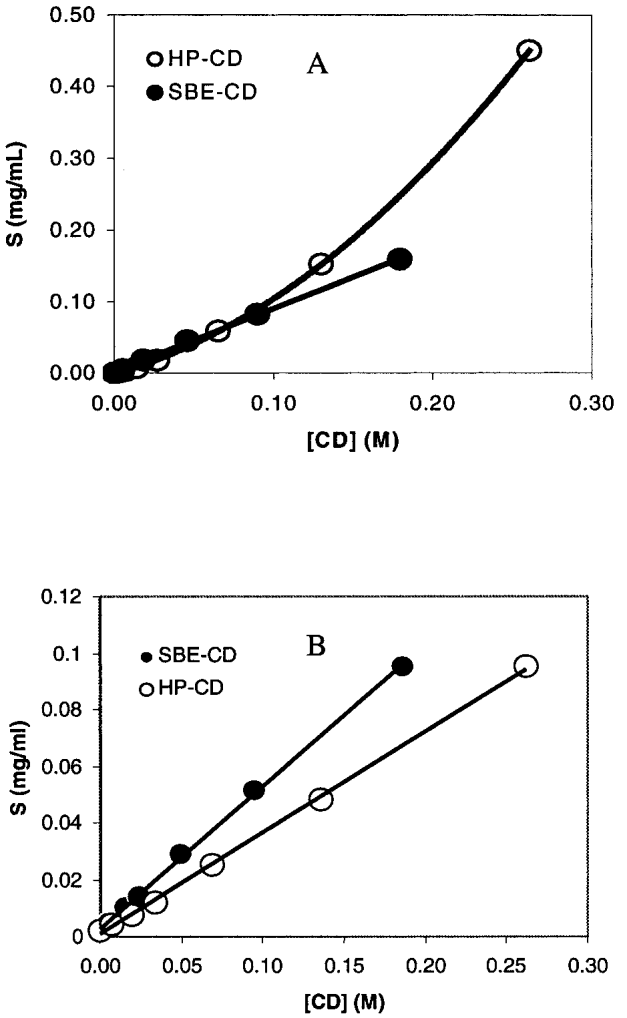

Fig. 3. Solubility for the neutral lactone form of DB-67 (A) and camptothecin (B) at 25°C as a function of cyclodextrin concentrations (SBE-CD and HP-CD) in aqueous solution. The curves are least-squares fits using Eq. (4) or its linear version.

methylsilyl substituent enhances complex formation. The failure of CPT to form 1:2 complexes with HP-CD also implies that the absence of the 7-t-butyldimethylsilyl in CPT depletes one binding site to HP-CD. Based on these results, it is concluded that the predominant 1:1 complex for DB-67 involves inclusion of the 7-t-butyldimethylsilyl residue. The DB-67 E-ring may be involved in 1:2 complex formation with HP-CD as steric hindrance may prevent the binding of two cyclodextrins near the A and B rings. Further evidence for this structure arises from the fact that cyclodextrins stabilize DB-67 toward hydrolysis as discussed in the next section.

Table II. Formation Constants, $K_f^{1:1}$ (M^{-1}) and $K_f^{1:2}$ (M^{-2}), for 1:1 and 1:2 Inclusion Complexes of DB-67 and CPT with SBE-CD and HP-CD

Solute	SBE-CD	HP-CD
Neutral lactone (DB-67)	$8.5 \pm 0.2 \times 10^3$ (1:1)	$5.8 \pm 0.2 \times 10^3$ (1:1) $3.8 \pm 0.1 \times 10^4$ (1:2)
Carboxylate (DB-67)	$2.5 \pm 1.2 \times 10^2$ (1:1)	$1.7 \pm 0.3 \times 10^3$ (1:1)
Neutral lactone (CPT)	$2.5 \pm 0.1 \times 10^2$ (1:1)	$1.8 \pm 0.0 \times 10^2$ (1:1)
Carboxylate (CPT)	—	—

Inclusion complex formation between DB-67 carboxylate and SBE-CD or HP-CD was examined by circular dichroism. The inverse of the ellipticity difference, $\Delta\theta$, between the complexed and free DB-67 (II^{2-}) at 280 nm (the wavelength at which the differences were maximized) was plotted vs. 1/[CD] as shown in Fig. 4. The complex formation constant K_f was obtained from a linear least-squares fit according to the Benesi-Hildebrand equation (17),

$$\frac{1}{\Delta\theta} = \frac{1}{\Delta\theta_{AB} K_f [CD]} + \frac{1}{\Delta\theta_{AB}} \quad (5)$$

where $\Delta\theta_{AB}$ is the difference in molar extinction coefficients for right and left circularly polarized light between the free and bound DB-67. The regression analyses yielded $K_f = 2.5 \pm 1.2 \times 10^2\ M^{-1}$ ($r^2 = 0.994$) and $\Delta\theta_{AB} = -15.5 \pm 6.6$ mdeg ($r^2 = 0.994$) for complexes involving the ionized E-ring opened form of DB-67 with SBE-CD and $K_f = 1.7 \pm 0.3 \times 10^3\ M^{-1}$ and $\Delta\theta_{AB} = -11.7 \pm 1.3$ mdeg ($r^2 = 0.996$) for complexes involving the ionized E-ring opened form of DB-67 with HP-CD, respectively. As shown in Table II, E-ring opening and ionization of DB-67 carboxylate decrease its binding constant with SBE-CD by about one order of magnitude, though these effects are less dramatic for complexation with HP-CD. This difference may be due to charge repulsion between the DB-67 carboxylate and the negatively charged SBE-CD.

Kinetics of DB-67 E-Ring Opening/Closing

Figure 5(A) shows the kinetic profiles for the appearance of DB-67 lactone (I) and the disappearance of the E-ring opened DB-67 (II) along with the overall DB-67 concentration vs. time at pH 4. At this pH, the reaction involves primarily the neutral lactone (I) and unionized ring-opened form of DB-67 (II) (Fig. 1). The equilibrium constant, K_1, for this reversible hydrolysis reaction determined from the pH-solubility profile is 2×10^{-3}. Thus, the reverse reaction, lactone hydrolysis, can be neglected. Assuming ring closure to be first-order, the concentration profiles should obey the following relations:

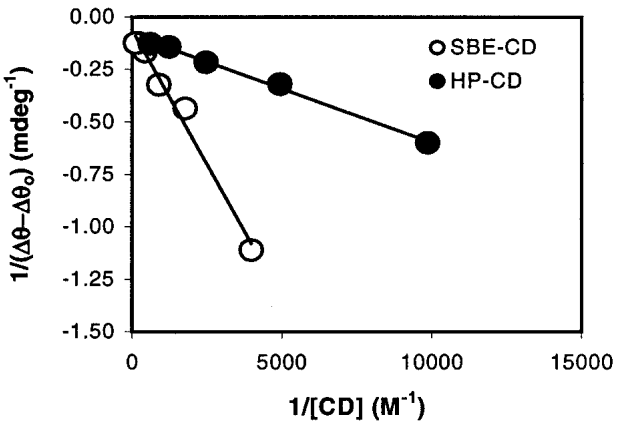

Fig. 4. $1/\Delta\theta$ vs. 1/[CD], where $\Delta\theta$ is the ellipticity difference between the bound and free species of the ring opened form of DB-67 (II^{2-}) and [CD] is the cyclodextrin concentration. The lines are least-squares fits using Eq. (5).

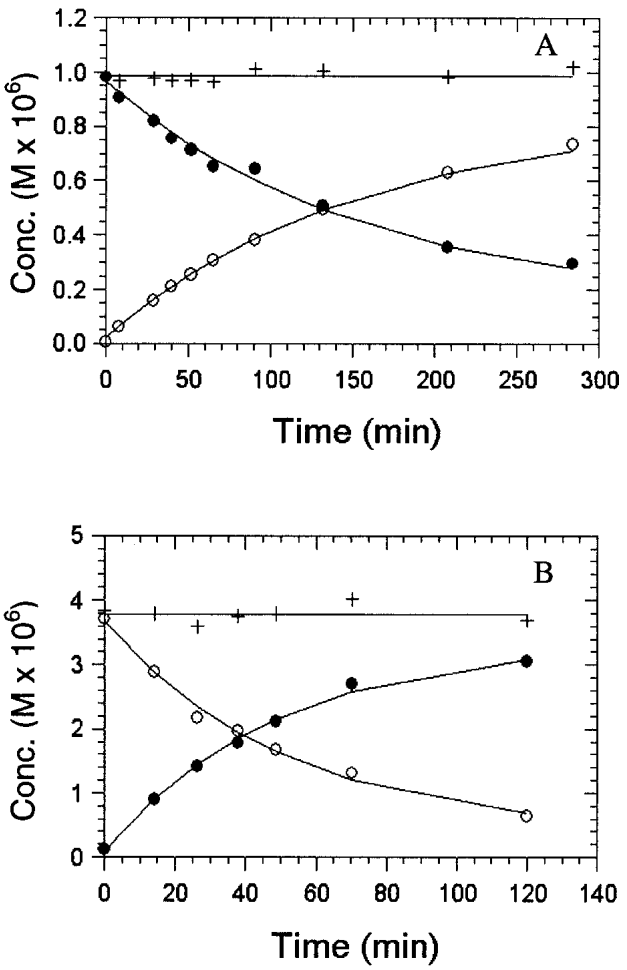

Fig. 5. (A) Kinetic profiles for the appearance of the lactone (I) and the disappearance of the E-ring opened DB-67 carboxylate (II) along with the overall DB-67 concentration vs. time in pH 4.04 buffer (30 mM acetate, I = 0.1). (B) Kinetic profiles for the appearance of species II and the disappearance of species I along with the overall DB-67 concentration vs. time in pH 7.41 buffer (30 mM phosphate, I = 0.1). The curves are least-squares fits using Eqs. (6–7).

$$[I]_t = [I]_{eq}(1 - e^{-k_{close}t}) + [I]_o \quad (6)$$

$$[II]_t = ([II]_o - [II]_{eq})e^{-k_{close}t} + [II]_{eq} \quad (7)$$

where the subscripts t, o, and eq stand for time t, initial time, and at equilibrium, respectively. Simultaneous non-linear least-squares fits of the kinetic profiles in Fig. 5 (A) according to Eqs. (6) and (7) yielded the first-order rate constant, $k_{close} = 3.3 \pm 0.3 \times 10^{-4}\ s^{-1}$, which is close to the interpolated value of $3.0 \times 10^{-4}\ s^{-1}$ at pH 4.04 for unsubstituted camptothecin (14), suggesting that substitution on the A and B rings has no significant effect on the E-ring closure kinetics. The mass balance results, also shown in Fig. 5(A), indicate that no other reactions occur under these conditions.

Fig. 5(B) displays profiles for the appearance of the E-ring opened form of DB-67 (II) and the disappearance of the lactone (I) along with the overall DB-67 concentration vs. time at pH 7.4. At this pH, the ionized carboxylate, II^-, dominates. Thus, the reverse reaction from II to I can be neglected and Eqs. (6) and (7) are applicable for species I and II + II^-. Simultaneous non-linear least-squares fits of the profiles in Fig. 5(B) yielded the first-order rate constant, $k_{open} = 1.1 \pm 0.1 \times 10^{-4}\ s^{-1}$, which is close to the interpolated value of $1.3 \times 10^{-4}\ s^{-1}$ at pH 7.40 for unsubstituted camptothecin (14), suggesting that substitution at the A and B rings has no significant effect on the E-ring closure kinetics. The mass balance results in Fig. 5(B) indicate that no other reactions contribute significantly under these conditions.

The hydrolysis of the lactone was also investigated at pH 7.4 in the presence of 20% (w/v) SBE-CD and HP-CD, respectively. The temporal profiles for the disappearance of the lactone and the appearance of the E-ring opened form of DB-67 were found to follow first-order kinetic models similar to those in Eqs. (6) and (7). Values for k_{open} were $(7.1 \pm 0.1) \times 10^{-5} s^{-1}$ and $(6.2 \pm 0.1) \times 10^{-5} s^{-1}$ in 20% (w/v) SBE-CD and HP-CD, respectively. The presence of 20% (w/v) SBE-CD or HP-CD reduces the rate of E-ring opening by 1.6- and 1.8-fold, respectively. The mechanisms responsible for this improved stability of the therapeutically viable DB-67 may differ in the cases of SBE-CD and HP-CD. In 20% (w/v) HP-CD, >99.9% of DB-67 is included in the CD cavity, and roughly 46% of the complexes are in the form of 1:2 complexes. If the hydrolysis site on the E-ring is included in the CD cavity in the 1:2 complexes with HP-CD but not in the 1:1 complexes, about 46% of the E-rings should be protected from attack by OH^- ions. This may explain the ca. 44% reduction in the observed rate constant. On the other hand, in 20% (w/v) SBE-CD, >99.8% of DB-67 is bound to SBE-CD in 1:1 complexes but perhaps only a small fraction of the 1:1 complexes involve those in which the E-ring is included in the CD cavity. However, the negative charged sulfobutyl ether groups in SBE-CD bound to the lactone may repel OH^- ions in the vicinity and reduce the rate of the OH^- catalyzed hydrolysis reaction.

Stability with Respect to Drug Precipitation

A successful supersaturated parenteral formulation requires physical stability with respect to drug precipitation. While supersaturated solutions prepared by dilution of concentration stock solutions of DB-67 lactone in DMSO provided clear solutions at 1 mg/mL with no signs of precipitation within 3 days, precipitation was evident even during the dilution process at 2 mg/mL and continued after the dilution phase. Since the poorly water-soluble lactone was employed in the DMSO additions, the local drug concentration near the injection tip may have been sufficient to induce rapid nucleation and precipitation. Proper control of the release pattern (local vs. uniform) and rate of DB-67 incorporation into the dilution medium appeared to be critical to the success of this type of formulation approach.

The desired slow and uniform release of DB-67 into the dilution medium could be accomplished by employing a chemical approach, namely, by converting the highly water-soluble carboxylate (II) to the lactone (I) in the presence of a chemically modified cyclodextrin. Studies by Fassberg and Stella (14) for other camptothecin analogs suggest that the rate of this chemical reaction can be regulated by solution pH. Consequently, a concentrated alkaline solution of DB-67 carboxylate was diluted with an acidic buffer to facilitate a timely conversion from the E-ring opened DB-67 to its lactone. The target final drug concentration was 2 mg/mL. Fig. 6 (A) shows

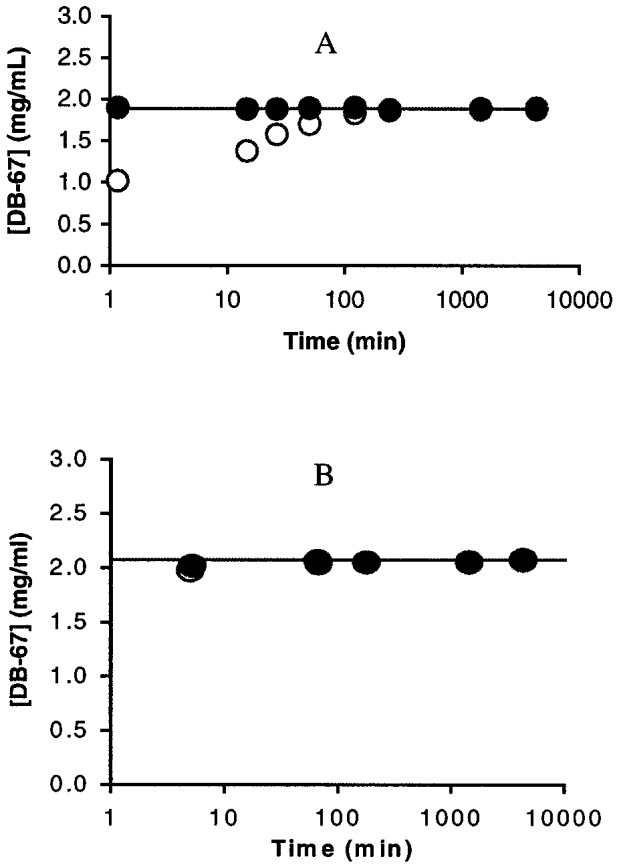

Fig. 6. (A) Concentration of the neutral DB-67 lactone (open circles) and overall DB-67 concentration (solid circles) in filtered solutions after 1 + 9 dilution from a high-pH aqueous solution into 20% (w/v) SBE-CD as a function of time after the completion of mixing. (B) DB-67 concentration in the filtered solutions of duplicate samples after reconstitution of the lyophilized formulation. The horizontal line indicates the averaged overall DB-67 concentrations in the unfiltered solutions.

the overall DB-67 concentration and the concentration of converted neutral DB-67 in the solution containing 20% (w/v) SBE-CD at different time intervals. The lactone slowly built up in the final solution in a uniform manner, attaining completion within 100 m to yield a clear solution at pH 4.39. The overall DB-67 concentration remained constant over an extended period of three days, well beyond the target stability of one day. This slow and uniform formation of the lactone may explain the superior physical stability of this formulation approach in comparison to supersaturated solutions prepared from a concentrated stock solution of the lactone in DMSO. In the absence of cyclodextrins, however, the precipitation was found to occur in less than one minute after dilution from a concentrated DB-67 solution at a high pH (10.50). Thus, it appears that only a combined approach of chemical conversion via pH adjustment and complexation with cyclodextrins would provide a stable formulation with the desired drug concentration.

For long-term storage, the 2 mg/mL solution of the lactone in 20% (w/v) SBE-CD prepared according to the procedure described above was lyophilized after the chemical conversion process was complete. Lyophilized samples were reconstituted and the stability of the reconstituted solution was examined following the same procedure as described above. As shown in Fig. 6 (B), the reconstituted solution remained clear for more than seven days, suggesting that no significant nucleation/precipitation occurred during or after lyophilization.

SUMMARY

The present solubility studies indicate that traditional solubilization approaches failed to achieve an equilibrium target concentration of 2 mg/mL for the lactone form of DB-67. To this end, a clear, supersaturated solution was prepared via pH induced chemical conversion of the ring-opened form of DB-67 to the lactone in the presence of a chemically modified water soluble β-cyclodextrin, SBE-CD. Solutions both before and after lyophilization and reconstitution remained clear and stable for at least three days.

This formulation method can potentially be utilized for the development of clear injectable solutions for other lipophilic and poorly water-soluble camptothecin analogs.

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