ELSEVIER

International Journal of Pharmaceutics 127 (1996) 229–237

international journal of pharmaceutics

# Preformulation studies to aid in the development of a ready-to-use injectable solution of the antitumor agent, topotecan

Albert S. Kearney*, Kamlesh Patel, Nagesh R. Palepu

*Pharmaceutical Development, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, UW2922, King of Prussia, PA 19406, USA*

Received 26 April 1995; revised 19 June 1995; accepted 10 July 1995

**Abstract**

Topotecan ((*S*)-9-dimethylaminomethyl-10-hydroxycamptothecin hydrochloride; SKF-S-104864-A) is a promising anticancer agent that is currently available as a lyophilized formulation. To evaluate the feasibility of formulating topotecan as a ready-to-use injectable solution, the pH-solubility profile was generated over a pH range of 2.5 to 4.5 at 25°C, and the pH-stability profile was generated over a pH range of 2 to 4, a temperature range of 60–80°C, and at an ionic strength of ~0.15 M. The former experiments revealed that desirable solubilities (i.e. 2.5 mg/ml in free base equivalents) are achievable at pH $< 4$, whereas extrapolation of the degradation kinetics to 25°C indicated that desirable stabilities (i.e. $\leq$ 2% degradation of topotecan over 2 years) are achievable at pH $< 3$. The stability results are consistent with a degradation mechanism involving deamination of topotecan proceeding through a reactive quinone methide intermediate. Subsequent attack of water on this intermediate gives the dihydroxylated product, 9-hydroxymethyl-10-hydroxycamptothecin, which may then lose formaldehyde to form the monohydroxylated product, 10-hydroxycamptothecin. The overall study results suggest that a solution pH of $\leq$ 2.5 is most appropriate for the formulation of a ready-to-use solution. Prototype formulations meeting these criteria have been placed on long-term stability.

*Keywords:* Topotecan; Anticancer; Solubility; Stability; Deamination

## 1. Introduction

Topotecan ((*S*)-9-dimethylaminomethyl-10-hydroxycamptothecin hydrochloride; SKF-S-104864-A; Fig. 1 (shown as the free base)) is a semi-synthetic, water-soluble analogue of the naturally occurring camptothecin, and it has demonstrated a broad spectrum of antitumor activity (e.g. Johnson et al., 1989; Kingsbury et al., 1991; Burris III et al., 1992; Houghton et al., 1992). Its cytotoxicity results from inhibition of DNA synthesis or, more specifically, the stabilization of the binding of topoisomerase I to DNA which ultimately results in fragmentation of DNA (Hsiang et al., 1985, 1989). The clinical significance of

* Corresponding author.

0378-5173/96/$15.00 © 1996 Elsevier Science B.V. All rights reserved
*SSDI* 0378-5173(95)04218-Y

topotecan and a few other camptothecin analogues has been recently reviewed (Slichenmyer et al., 1993; O'Reilly et al., 1995).

The current parenteral formulation of topotecan is lyophilized from a buffered aqueous solution. The pH of this solution is maintained at 3.0 with tartaric acid to ensure the adequate solubility of topotecan ( $\geq$ 2.5 mg/ml as the free base) and the adequate stability of topotecan (during lyophilization, storage, and reconstitution) as the pharmacologically active lactone form.

This work was undertaken to evaluate the feasibility of formulating a ready-to-use injectable solution dosage form of topotecan. To do this, a good understanding of the solubility characteristics and the kinetics and mechanisms of degradation under acidic conditions is essential. Although the lactone ring hydrolysis reaction has been well studied (Underberg et al., 1990; Fassberg and Stella, 1992; Akimoto et al., 1994), the same cannot be said of the degradation reaction(s) at pH less than 4, where lactone hydrolysis does not occur to any appreciable extent.

## 2. Experimental

### 2.1. Materials

Topotecan hydrochloride and 10-hydroxycamptothecin were synthesized by the Chemistry Department of SmithKline Beecham Pharmaceuticals (King of Prussia, PA). Dimethylamine hydrochloride and L-tartaric acid were obtained from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were of reagent grade, and the water was de-ionized then filtered through a Milli-Q Water Purification System prior to use.

### 2.2. Analysis

The stability-indicating HPLC analysis was conducted on a Shimadzu HPLC system equipped with either a Shimadzu SPD-6A or an Hitachi L-4000 UV spectrophotometric detector and a Cosmosil 5C18-AR column (4.6 $\times$ 250 mm, 5 $\mu$m). The mobile phase consisted of an 85:15:0.1 (% v/v/w) mixture of water, acetonitrile, and trifluoroacetic acid, respectively; the flow rate was 1.0 ml/min, the detection wavelength was 228 nm, the injection volume was 50 $\mu$l, and the run time was 60 min. Data acquisition and integration was performed with a Nelson System 6000 and Nelson Access*Chrom (version 1.8) software.

### 2.3. Determination of an apparent ionization constant

An apparent ionization constant of topotecan was determined as a function of temperature by potentiometric titration using an Orion SA520 pH meter and an Orion semimicro-Ross combination glass electrode. Aqueous solutions of topotecan (0.001 M), having an ionic strength of 0.15 M with NaCl, were equilibrated at the temperature of interest then were titrated, in 0.5-ml increments, with 0.01 N NaOH having an ionic strength of 0.15 M with NaCl. The apparent ionization constant was obtained by a standard calculation method utilizing the pH values observed upon addition of the titrant (Albert and Serjeant, 1971).

### 2.4. Aqueous solubility

The solubility of topotecan under acidic conditions was determined, in duplicate, at 25°C. Excess solid topotecan (i.e. between 300 and 500 mg) was added to 10-ml glass centrifuge tubes having Teflon-lined screw caps; 4 ml of water were added to each tube and the tubes were placed on a VIBRO-Mixer (Chemap AG, Switzerland) which was set in a 25°C water bath. The VIBRO-Mixer

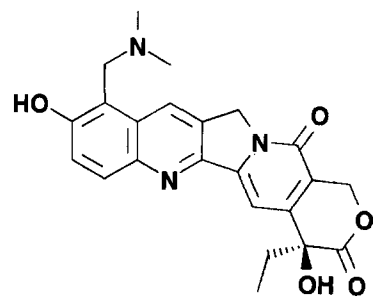


Fig. 1. The structure of topotecan (SKF-S-104864-A; (S)-9-dimethylaminomethyl-10-hydroxycamptothecin).

was run at a setting of '1' for at least 30 min, then small volumes of 2 N NaOH (50–250 μl) were added to each tube. The tubes were then mixed overnight on the VIBRO-Mixer. (Preliminary studies showed that this was ample time to attain the equilibrium solubility.) The next day, the samples were centrifuged, and the supernatants were filtered through 0.45 μm (Gelman Acrodisc 13 CR) PTFE syringe filters (which were previously shown not bind to topotecan). The first 0.5 ml of filtrate was discarded. The pH of the subsequent filtrate was taken, and then it was diluted accordingly and assayed by HPLC.

### 2.5. Kinetic experiments

The kinetics of degradation of dilute aqueous solutions of topotecan ( ~ 0.012 mg/ml or $2.8 \times 10^{-5}$ M in free base equivalents) were determined over a pH range of 2.0–4.0 and a temperature range of 60–80 ( ± 0.5)°C. The pH was maintained with varying concentrations of tartaric acid (i.e. 25, 50, 75, and 100 mM), and the ionic strength of the buffers was adjusted to 0.15 M with sodium chloride prior to pH adjustment with 2 N NaOH. Additionally, the degradation kinetics, at 80°C and in 100 mM tartaric acid of pH 3.0, were followed in the presence of varying concentrations of dimethylamine hydrochloride (ranging from 0 to 0.10 M) and in the presence of varying starting concentrations of topotecan (ranging from 0.05 to 2.57 mg/ml).

The kinetic experiments were conducted in one of two ways depending on the rate of the reaction. For the relatively fast reactions, the experiments were initiated by adding 100 μl of a stock solution of topotecan (dissolved in 0.1 M tartaric acid) to 10-ml glass volumetric flasks of the buffered media that were temperature equilibrated in a circulating water bath. At appropriate time intervals, aliquots were withdrawn, sealed into HPLC vials, and cooled in an ice-water bath (to quench the degradation reaction). For the slower reactions, the experiments were performed by flame-sealing 1-ml aliquots of the buffered media containing topotecan into precleaned 2-ml Flint glass ampoules which were then placed in a temperature-controlled oven. At appropriate time intervals, ampoules were withdrawn and cooled in an ice-water bath. For both sets of studies, the samples were stored in a 5°C cabinet until all of the samples were collected; then they were assayed for topotecan and degradant peaks by HPLC. The pH of the tartaric acid buffers was found to vary minimally with temperature; therefore, the pH of the reaction mixtures were determined at room temperature at the beginning and end of the experiment.

For the studies involving varying concentrations of topotecan, different volumes of a common stock solution of topotecan, containing 12.84 mg/ml in free base equivalents, were added to reaction media which were equilibrated at 80°C. At appropriate intervals, 3-ml aliquots were withdrawn and cooled in an ice-water bath. The samples were then diluted with an 85:15 (% v/v) mixture of 0.001 N HCl and acetonitrile; aliquots of these were then sealed in HPLC vials which were stored in a 5°C cabinet until all samples were collected. Then they were assayed by HPLC.

The observed degradation rate constants, $k_{obs}$, are obtained from the slopes of plots of the natural logarithm of the topotecan peak area versus time for reactions that were typically followed for two half-lives. The buffer-dependent rate constants, $k_{buff}$, are obtained from the slopes of plots of $k_{obs}$ versus the tartaric acid concentration, whereas the buffer-independent rate constants, $k_o$, are obtained from the $y$-intercepts of these plots. The $k'_z$ values were generated by non-linear regression analysis of Eq. (6) with the experimental data using the Nelder-Mead simplex algorithm in PCNONLIN® (SCI, Apex, NC).

## 3. Results and discussion

### 3.1. Ionization constants

Topotecan can exist in a cationic, uncharged, zwitterionic, and/or anionic form, depending on the pH of the solution and neglecting the ionization potential of the quinoline nitrogen ($K_{a1}$) that has been reported to have a $pK_a$ of about 0.6 at 25°C and an ionic strength of 0.5 M (Fassberg and Stella, 1992). The $pK_a$ values of the phenolic

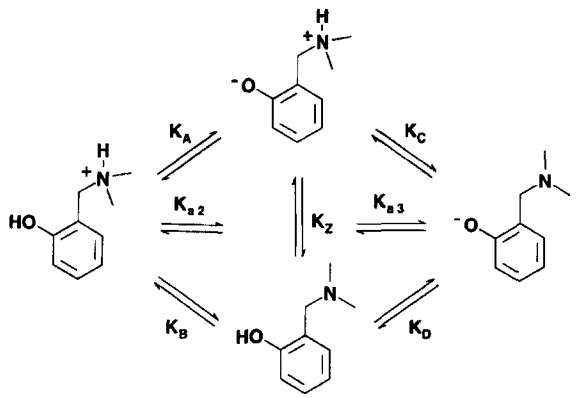


Fig. 2. A partial ionization scheme for topotecan. (Only the A ring of the topotecan structure is depicted.)

and benzyldimethylamine groups have been reported to be 6.99 and 10.50 at an ionic strength of 0.5 M and 25°C (Fassberg and Stella, 1992). A partial ionization scheme for topotecan is depicted in Fig. 2. The macroscopic equilibrium constants, $K_{a2}$ and $K_{a3}$, are related to the microscopic constants as follows (e.g. Teitel'baum et al., 1980),

$$K_{a2} = K_A + K_B \tag{1}$$

$$\frac{1}{K_{a3}} = \frac{1}{K_C} + \frac{1}{K_D} \tag{2}$$

Additionally, $K_Z$ represents the pH-independent equilibrium constant existing between the zwitterionic and uncharged species.

To aid in the mathematical assessment of the kinetic data, the apparent ionization constant of the phenolic group of topotecan was determined at an ionic strength of 0.15 M. The value (uncorrected for activity coefficients) was found to be 6.46, 6.02, 5.98, and 5.97 at 25°, 60°, 70°, and 80°C, respectively. Because precipitation was observed at higher pH values, the $K_a$ values were calculated based on the incremental addition of one-half an equivalent of titrant. The value of 6.46 at 25°C compares favorably with the previous spectrophotometrically generated value of 6.99 (Fassberg and Stella, 1992). This latter value was determined at an ionic strength of 0.5 M which could account for its higher value.

### 3.2. Aqueous solubility

As can be seen in Fig. 3, the apparent solubility of topotecan (expressed in free base equivalents) at 25°C decreases as the pH is increased. The solubility results are consistent with the ampholyte (i.e. the combination of the zwitterionic and neutral species) having a relatively poor aqueous solubility. In this narrow pH range, the solubility of topotecan can be attributed to the dicationic (not depicted in Fig. 2), monocationic, and ampholytic species. Therefore, the solubility can be expressed as a function of the intrinsic solubility of the ampholyte, $[A]$,

$$S = [A]\left(\frac{(a_H)^2}{K_{a1}K_{a2}} + \frac{a_H}{K_{a2}} + 1\right) \tag{3}$$

where $S$ is the apparent solubility of topotecan at a given hydrogen-ion activity, $a_H$, and $K_{a1}$ and $K_{a2}$ are the macroscopic ionization constants for the quinoline nitrogen and phenolic hydroxyl groups, $2.506 \times 10^{-1}$ and $1.023 \times 10^{-7}$ (Fassberg and Stella, 1992). Fitting the experimental data to Eq. (3) (PCNONLIN®) gives a solubility of 2.51 ( $\pm$ 0.08) $\times 10^{-3}$ mg/ml for the ampholyte. Based on the theoretical profile (the solid line in Fig. 3), the desired solubility of 2.5 mg/ml is achievable at pH $\leq$ 3.99.

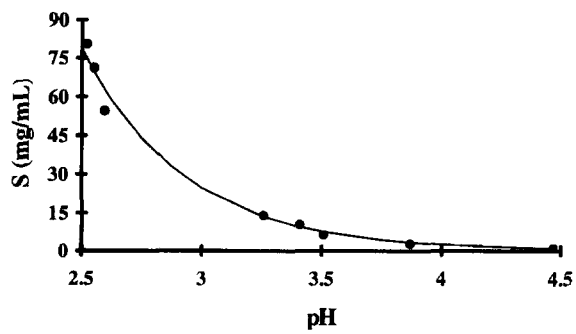


Fig. 3. The effect of pH on the apparent solubility of topotecan, expressed in free base equivalents, at 25°C. The line represents the theoretical profile generated as described in the text.

Table 1
The effect of temperature and pH on the buffer-independent, $k_o$, and buffer-dependent, $k_{buff}$, rate constants for the degradation of topotecan at $\mu \sim 0.15$ M[a]

| Temp. (°C) | pH | $k_o$ ($h^{-1}$) | $k_{buff}$ ($mM^{-1}$ $h^{-1}$)b |
|---|---|---|---|
| 80 | 2.0 | 2.21 (± 0.05) × $10^{-3}$ | nc |
| | 2.5 | 6.26 (± 0.15) × $10^{-3}$ | nc |
| | 3.0 | 1.90 (± 0.05) × $10^{-2}$ | 3.52 (± 0.97) × $10^{-5}$ |
| | 3.5 | 5.20 (± 0.08) × $10^{-2}$ | 1.08(± 0.15) × $10^{-4}$ |
| | 4.0 | 1.25 (± 0.03) × $10^{-1}$ | 3.64 (± 0.52) × $10^{-4}$ |
| 70 | 2.0 | 4.84 (± 0.12) × $10^{-4}$ | nc |
| | 2.5 | 1.85 (± 0.20) × $10^{-3}$ | nc |
| | 3.0 | 5.39 (± 0.02) × $10^{-3}$ | nc |
| | 3.5 | 1.20 (± 0.02) × $10^{-2}$ | 1.68 (± 0.40) × $10^{-5}$ |
| | 4.0 | 3.07 (± 0.12) × $10^{-2}$ | 5.96 (± 2.08) × $10^{-5}$ |
| 60 | 2.0 | 1.09 (± 0.02) × $10^{-4}$ | nc |
| | 2.5 | 2.85 (± 0.03) × $10^{-4}$ | nc |
| | 3.0 | 8.95 (± 0.05) × $10^{-4}$ | nc |
| | 3.5 | 2.65 (± 0.05) × $10^{-3}$ | nc |
| | 4.0 | 8.06 (± 0.34) × $10^{-3}$ | 1.42 (± 0.61) × $10^{-5}$ |

[a]The values in parentheses are the standard errors.[b]nc indicates that no apparent buffer catalysis was observed.

### 3.3. Examination of the pH-rate constant profiles

Under all conditions studied, the degradation of topotecan was well described as a first-order process. As can be seen in Table 1, the degradation of topotecan was catalyzed by tartaric acid under the following conditions: pH $\geq$ 3.0 at 80°C; pH $\geq$ 3.5 at 70°C; and pH = 4.0 at 60°C. The data show that as the temperature decreased so too did the significance of buffer catalysis and that at 70° and 80°C, the larger $k_{buff}$ values occur at higher pH consistent with general-base catalysis.

The dependence of the buffer-independent rate constant, $k_o$, on pH and temperature is shown in Fig. 4 and Table 1. The interpretation of the degradation kinetics is complicated by the fact that topotecan can exist in several ionic forms (Fig. 2); however, the rate of degradation of topotecan over the pH range investigated can be described by the following relationship:

$$k_o = k_z f_z \tag{4}$$

or in its expanded form,

$$k_o = k_z \left( \frac{a_H K_{a2}}{(a_H)^2 + a_H K_{a2} + K_{a2} K_{a3}} \right) \left( \frac{K_z}{1 + K_z} \right) \tag{5}$$

where the zwitterion is assumed to be the reactive ionic species and where $k_z$ is the rate constant for degradation of the zwitterion, $f_z$ is the fraction of topotecan present in the zwitterionic form, and $K_{a2}$, $K_{a3}$, and $K_z$ are the equilibrium constants defined previously. Since the $K_{a2}K_{a3}$ term becomes insignificant in the pH range studied and since $K_z$ was not determined (the difficulties inherent in such a determination can be gleaned from the work of Teitel'baum et al. (1980)), Eq. (5) can be simplified as shown below:

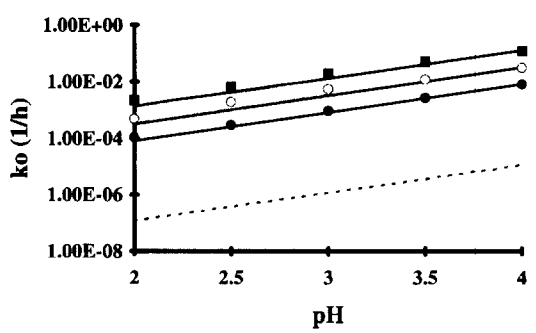


Fig. 4. The pH-$k_o$ profiles for the degradation of topotecan at $\mu \sim 0.15$ M and at 80 (●) 70 (○), and 60°C (■). The solid lines represent the respective theoretical profiles, and the dashed line is the theoretical profile at 25°C.

Table 2
Predicted pH dependency of the $k_o$ and $t_{98}$ values at 25°C and at an initial topotecan concentration of ~0.012 mg/ml[a]

| pH | $k_o$ ($h^{-1}$) | $t_{98}$ (years) |
|---|---|---|
| 2.0 | $1.20 \times 10^{-7}$ | 19.2 |
| 2.5 | $3.80 \times 10^{-7}$ | 6.1 |
| 3.0 | $1.20 \times 10^{-6}$ | 1.9 |
| 3.5 | $3.80 \times 10^{-6}$ | 0.6 |
| 4.0 | $1.20 \times 10^{-5}$ | 0.2 |

[a]The $k_o$ and $t_{98}$ (i.e. the time required for the degradative loss of 2% of topotecan) values were calculated using the established Arrhenius relationship and Eq. (6).

$$k_o = k'_z\left(\frac{K_{a2}}{a_H + K_{a2}}\right) \quad (6)$$

where

$$k'_z = k_z\left(\frac{K_z}{1 + K_z}\right) \quad (7)$$

The theoretical profiles in Fig. 4 were constructed with Eq. (6), the experimentally determined $K_{a2}$ values, and the following $k'_z$ values: 12.19 ( ± 0.60) $h^{-1}$ at 80°C; 3.04 ( ± 0.15) $h^{-1}$ at 70°C; 0.86 ( ± 0.01) $h^{-1}$ at 60°C.

The apparent activation parameters were determined from the effect of temperature on $k'_z$. The energy of activation ($E_a$) is 31.0 ( ± 1.4) kcal/mol; the Arrhenius frequency factor ($A$) is $1.729 \times 10^{20}$ $h^{-1}$; the enthalpy of activation ($\Delta H^{\ddagger}$) is 30.3 ( ± 1.4) kcal/mol; the entropy of activation ($\Delta S^{\ddagger}$) is 15.5 ( ± 4.0) eu. Using the extrapolated $k'_z$ value at 25°C of $3.4 \times 10^{-3}$ $h^{-1}$ and the experimentally determined $K_{a2}$ value of $3.47 \times 10^{-7}$, the $k_o$ and apparent $t_{98}$ (i.e. the time required for the degradative loss of 2% of topotecan) values can be predicted at 25°C. The results are listed in Table 2 and show that to achieve a $t_{98}$ value showing ≤ 2.0% loss of topotecan, a ready-to-use solution has to be maintained at a pH of less than 3.0.

### 3.4. Effect of topotecan concentration on the rate

Since the results shown in Table 2 were generated using an initial topotecan concentration well below the desired formulation concentration (i.e. 0.012 versus 2.5 mg/ml), the effect of the starting concentration of topotecan on the degradation rate was studied at 80°C and in 100 mM tartaric acid, pH 3.0; the results are shown in Fig. 5. For the reaction mixtures initially containing between 0.5 and 2.6 mg/ml of topotecan, precipitation was observed within the first sampling interval (between 0 and 8 h); the precipitation appeared to have no effect on the kinetics of degradation of topotecan which were well described by first-order processes. The precipitation was found to be 10-hydroxycamptothecin by HPLC analysis.

Fig. 5 shows that the rate of degradation of topotecan has an apparent linear dependence on the starting concentration of topotecan in solution. The degradation rate constant increases about 1.3-fold as the concentration of topotecan increases from 0.05 to 2.6 mg/ml. The increase in the degradation rate with increasing topotecan concentration may be due, in part, to the occurrence of a dimerization reaction. This reaction appears to occur to a small extent under acidic conditions and appears to be most favored under basic conditions. Another factor which may play a role in the observed rate changes is the increase in the ionic strength that accompanies the increasing topotecan concentration.

From a formulation standpoint, the significance of the concentration-dependent rate change is that dilute solution kinetics will underestimate the rate (and therefore extent) of topotecan degradation under more concentrated conditions. The faster rate of degradation observed at the desired dosing concentration (2.5 mg/ml as the free base) can be

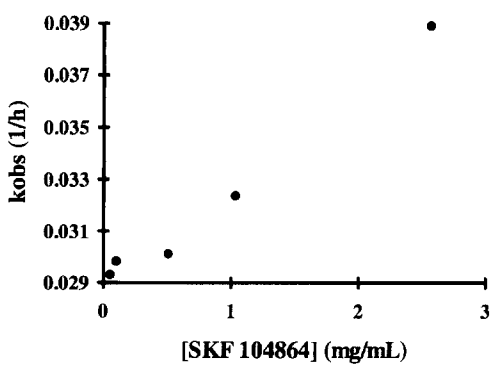


Fig. 5. Effect of the initial topotecan concentration on $k_{obs}$ at 80°C and in 100 mM tartaric acid solutions, pH 3.01.

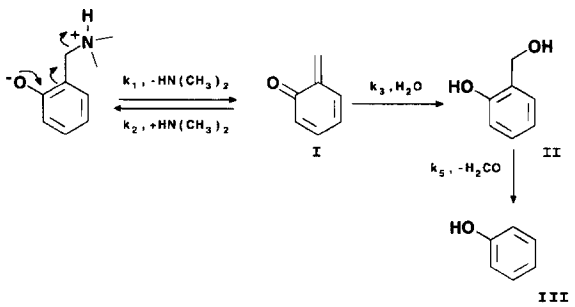


Fig. 6. Postulated degradation mechanism for the zwitterionic species of topotecan under acidic conditions (pH < 4). (Only the A ring of the topotecan structure is depicted).

nullified by lowering the formulation pH. The predicted $t_{98}$ value increases about 3-fold for each 0.5 unit drop in pH (Table 2). Hence, a pH of ≤ 2.5 would be more appropriate than 3.0 for the formulation of a ready-to-use solution. An unknown factor at this point (due to the unavailability of reasonable quantities of 10-hydroxycamptothecin) is whether precipitation of 10-hydroxycamptothecin will adversely impact the development of a ready-to-use solution.

### 3.5. Products and mechanism(s) of degradation

A degradation mechanism consistent with the experimental data generated is shown in Fig. 6. (The dimerization reaction mentioned previously has not been included in the figure since it appears to be a minor degradative pathway under the conditions studied.) Deamination is shown to proceed through a reactive quinone methide intermediate (**I**) which, upon attack of water at the terminal methylene group, forms the dihydroxylated product, 9-hydroxymethyl-10-hydroxycamptothecin (**II**). This reaction scheme has been previously proposed for structurally similar compounds (e.g. Gardner et al., 1959; Ivanov et al., 1968; Andrisano et al., 1970), and the zwitterionic and anionic species have been postulated to be the reactive entities (Teitel'baum et al., 1982). In a subsequent step, the formation of the monohydroxylated product, 10-hydroxycamptothecin (**III**), is shown to occur via the loss of formaldehyde from **II**. Electron-impact mass spectroscopic evaluation of the two predominant HPLC peaks formed during the degradation of topotecan support the proposed mechanism. The earlier eluting peak is consistent with **II** based on the presence of an $(M + H)^+$ peak at $m/z$ 395 and on the coelution of an authentic sample, whereas the later eluting peak is consistent with **III** based on an $(M + H)^+$ peak at $m/z$ 365 and on the coelution of an authentic sample. The substantially positive $\Delta S^{\ddagger}$ of 15.5 eu suggests that the formation of the quinone methide intermediate (i.e. a unimolecular step) is the rate-determining step (Schaleger and Long, 1963).

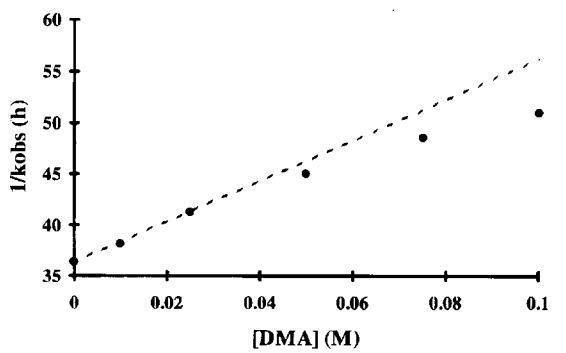


Fig. 7. Effect of dimethylamine concentration on the reciprocal of $k_{obs}$ at pH 3.01 and 80°C.

Table 3
The effect of added dimethylamine (DMA) on the $k_{obs}$ value and on the relative percent of topotecan and 9-hydroxymethyl-10-hydroxycamptothecin (**II**) present after 71 h at pH 3.01 and 80°C[a]

| [DMA] (mM) | $k_{obs}$ ($\times 10^{-2}$ $h^{-1}$) | % of Topotecan remaining | % of **II** present |
|---|---|---|---|
| 0 | 2.75 | 14.1 | 16.4 |
| 10 | 2.62 | 15.2 | 14.5 |
| 25 | 2.42 | 16.8 | 12.6 |
| 50 | 2.22 | 19.3 | 10.3 |
| 75 | 2.06 | 21.3 | 8.6 |
| 100 | 1.96 | 23.0 | 7.4 |

[a]The relative percentages represent the peak area of a given compound after 71 h at a given condition relative to the initial peak area of topotecan (i.e. the peak area after 0.1 h) under the same conditions.

Table 4
Stability assessment of prototype ready-to-use solutions of topotecan stored in glass vials in the upright position[a]

| pH adjusted with | Storage temp (°C) | Storage interval (months) | Assay (mg/ml) | % Degradation products/impurities[b] | pH | Turbidity[c] |
|---|---|---|---|---|---|---|
| HCl | 5 | Initial | 2.55 | 0.84 | 2.20 | 0.20 |
| | | 3 | 2.53 | 0.96 | 2.17 | 0.30 |
| | | 6 | 2.52 | 0.99 | 2.20 | 0.29 |
| | 30 | Initial | 2.55 | 0.84 | 2.20 | 0.20 |
| | | 3 | 2.52 | 1.22 | 2.17 | 0.32 |
| | | 6 | 2.52 | 1.47 | 2.17 | 0.27 |
| | 40 | Initial | 2.55 | 0.84 | 2.20 | 0.20 |
| | | 3 | 2.53 | 2.36 | 2.21 | 0.31 |
| | | 6 | 2.46 | 3.83 | 2.15 | 0.90 |
| Tartaric acid | 5 | Initial | 2.72 | 1.32 | 2.24 | 0.30 |
| | | 3 | 2.71 | —[d] | 2.15 | 0.28 |
| | | 6 | 2.67 | 1.04 | 2.15 | 0.32 |
| | 30 | Initial | 2.72 | 1.32 | 2.24 | 0.30 |
| | | 3 | 2.71 | 1.08 | 2.17 | 0.35 |
| | | 6 | 2.71 | 1.68 | 2.17 | 0.42 |
| | 40 | Initial | 2.72 | 1.32 | 2.24 | 0.30 |
| | | 3 | 2.65 | 2.42 | 2.22 | 0.61 |
| | | 6 | 2.61 | 4.17 | 2.15 | 1.55 |

[a]Following filtration of each formulation through a 0.2 μm nylon filter, 4-ml aliquots were filled into 5 ml Type 1, Flint glass vials which were then capped with a 20 mm Soteflon gray rubber septum and an aluminum seal.
[b]The calculated percentage is based on peak area responses.
[c]The turbidity measurements were performed on a Hach Ratio Turbidimeter.
[d]A problem with the chromatographic baseline made this number inaccessible.

### 3.6. *Effect of added dimethylamine on the rate*

To probe the degradation mechanism further, the effect of added dimethylamine hydrochloride (DMA) on the degradation rate was studied at 80°C in 100 mM tartaric acid, pH 3.0. Based on the mechanism depicted in Fig. 6 and applying the steady-state assumption to the reactive quinone methide intermediate (**I**), the observed rate constant can be defined as its component microscopic rate constants,

$$k_{obs} = \left( \frac{k_1 k_3 [H_2O]}{k_2[DMA] + k_3[H_2O]} \right) \quad (8)$$

or through rearrangement,

$$\frac{1}{k_{obs}} = \frac{1}{k_1} + \frac{k_2[DMA]}{k_1 k_3 [H_2O]} \quad (9)$$

Fig. 7 shows the data plotted according to this latter relationship. Upon performing a least-squares linear-regression analysis of the initial linear portion of the plot (i.e. the first three data points), the resulting slope reveals that the $k_3[H_2O]$ term is about 100 times greater than the $k_2$ term. The negative deviation from linearity suggests that a change in the degradation mechanism may be occurring as the DMA concentration is increased. The existence of the quinone methide intermediate as well as the reversibility of the first step of the reaction (Fig. 6) is confirmed by the decrease in the rate of loss of topotecan and by the resultant decrease in the rate of formation of **II** as the concentration of DMA is increased (Table 3).

## 4. Conclusions

The aqueous solubility and degradation kinetics of topotecan, under acidic conditions, were determined to evaluate the feasibility of developing a ready-to-use injectable solution dosage form. The results suggest that topotecan is sufficiently soluble and stable at pH $\leq$ 2.5 to formulate as a

ready-to-use solution. The physiologic acceptability of a formulation having such a low pH should not be an issue because the formulation will be diluted prior to infusion into a patient. Prototype formulations meeting this criteria have been prepared and placed on long-term stability, and Table 4 shows a summary of the stability data generated to date.

## Acknowledgements

The authors wish to thank Mr. Uday Bhatt for his technical assistance in the stability testing of the prototype topotecan formulations.

## References

Albert, A. and Serjeant, E.P., *Ionization Constants of Acids and Bases*, 2nd Edn., T. and A. Constable, Edinburgh, 1971, pp. 9–22.

Akimoto, K., Kawai, A. and Ohya, K., Kinetic studies of the hydrolysis and lactonization of camptothecin and its derivatives, CPT-11 and SN-38, in aqueous solution. *Chem. Pharm. Bull.*, 42 (1994) 2135–2138.

Andrisano, R., Della Casa, C. and Tramontini, M., Reactivity of mannich bases. Part XIII. Mechanism of the reaction between aminomethylnaphthols and benzenethiols. *J. Chem. Soc. (C)*, (1970) 1866–1869.

Burris III, H.A., Hanauske, A.-R., Johnson, R.K., Marshall, M.H., Kuhn, J.G., Hilsenbeck, S.G. and Von Hoff, D.D., Activity of topotecan, a new topoisomerase I inhibitor, against human tumor colony-forming units in vitro. *J. Natl. Cancer Inst.*, 84 (1992) 1816–1820.

Fassberg, J. and Stella, V.J., A kinetic and mechanistic study of the hydrolysis of camptothecin and some analogues. *J. Pharm. Sci.*, 81 (1992) 676–684.

Gardner, P.D., Rafsanjani, H.S. and Rand, L., Reaction of phenolic mannich base methiodides and oxides with various nucleophiles. *J. Am. Chem. Soc.*, 81 (1959) 3364–3367.

Houghton, P.J., Cheshire, P.J., Myers, L., Stewart, C.F., Synold, T.W. and Houghton, J.A., Evaluation of 9-dimethylaminomethyl-10-hydroxycamptothecin against xenografts derived from adult and childhood solid tumors. *Cancer Chemother. Pharmacol.*, 31 (1992) 229–239.

Hsiang, Y.-H., Hertzberg, R., Hecht, S., Liu, L.F., Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J. Biol. Chem.*, 260 (1985) 14873–14878.

Hsiang, Y.-H., Lihou, M.G. and Liu, L.F., Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. *Cancer Res.*, 49 (1989) 5077–5082.

Ivanov, B.E., Valitova, L.A. and Vavilova, T.G., Kinetics of the interaction of phenolic mannich bases with triethyl phosphite. *Izv. Akad. Nauk SSSR, Ser. Khim.* (1968) 768–772.

Johnson, R.K., McCabe, F.L., Faucette, L.F., Hertzberg, R.P., Kingsbury, W.D., Boehm, J.C., Caranfa, M.J. and Holden, K.G., SK and F 104864, a water-soluble analog of camptothecin with broad-spectrum activity in preclinical tumor models. *Proc. Am. Assoc. Cancer Res.*, 30 (1989) 623.

Kingsbury, W.D., Boehm, J.C., Jakas, D.R., Holden, K.G., Hecht, S.M., Gallagher, G., Caranfa, M.J., McCabe, F.L., Faucette, L.F., Johnson, R.K. and Hertzberg, R.P., Synthesis of water-soluble (aminoalkyl)captothecin analogues: Inhibition of topoisomerase I and antitumor activity. *J. Med. Chem.*, 34 (1991) 98–107.

O'Reilly, S., Kennedy, M.J., Rowinsky, E.K. and Donehower, R.C., Vinorelbine and the topoisomerase-1 inhibitors — Current and potential roles in breast-cancer chemotherapy. *Breast Cancer Res. Treatment*, 33 (1995) 1–17.

Schaleger, L.L. and Long, F.A., Entropies of activation and mechanisms of reactions in solution. *Adv. Phys. Org. Chem.*, 1 (1963) 1–33.

Slichenmyer, W.J., Rowinsky, E.K., Donehower, R.C. and Kaufmann, S.H., The current status of camptothecin analogues as antitumor agents. *J. Natl. Cancer Inst.*, 85 (1993) 271–291.

Teitel'baum, A.B., Derstuganova, K.A., Shishkina, N.A., Kudryavtseva, L.A., Bel'skii, V.E. and Ivanov, B.E., Tautomerism in the ortho-aminomethylphenols. *Izv. Akad. Nauk SSSR, Ser. Khim.* (1980) 803–808.

Teitel'baum, A.B., Kudryavtseva, L.A., Bel'skii, V.E. and Ivanov, V.E., Kinetics of hydrolysis of *o*-aminomethylphenols and their ammonium salts. *Izv. Akad. Nauk SSSR, Ser. Khim.* (1982) 81–85.

Underberg, W.J.M., Goossen, R.M.J., Smith, B.R. and Beijnen, J.H., Equilibrium kinetics of the new experimental anti-tumour compound SK and F 104864-A in aqueous solution. *J. Pharm. Biomed. Anal.*, 8 (1990) 681–683.