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RESEARCH PAPER

Preformulation Studies for an Ultrashort-Acting Neuromuscular Blocking Agent GW280430A. I. Buffer and Cosolvent Effects on the Solution Stability

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ABSTRACT

GW280430A is an ultrashort-acting neuromuscular blocking agent targeted at muscle relaxation to facilitate surgical intubation. The objective of this work was to study the buffer and cosolvent effects on the solution stability of GW280430A. The buffer catalytic effect was examined in citrate, malate, tartrate, and glycine by measuring the rate of degradation of GW280430A (0.2 mg/mL) at constant pH (3), ionic strength (0.15 M), and various buffer concentrations (0.01–0.05 M). The temperature dependence of the buffer catalytic effect and the degradation of the GW280430A in cosolvent (ethanol, propylene glycol, polyethylene glycol 400, N,N-*dimethylacetamide)/water mixtures were studied at 40, 50, and 60°C. The loss of parent drug was monitored by reverse-phase high-performance liquid chromatography. The degradation of GW280430A followed first-order kinetics in all buffer solutions. Significant buffer-catalyzed hydrolysis of GW280430A was observed with citrate, tartrate, and malate buffers, but not in glycine-buffered solutions. The activation energies in all buffered drug solutions ranged from 70 to 80 kJ/mol and decreased with increasing buffer concentration. GW280430A degradation was primarily through ester hydrolysis and followed first-order kinetics in aqueous solutions. In cosolvent/water mixtures, new degradation products were observed, indicating a chemical reaction between GW280430A and cosolvents. The reaction activation energies in the cosolvent/water mixtures ranged from 75 to 85 kJ/mol, with the longest* $t_{0.9}$ *at 5°C equal to approximately 12 months and at 25°C equal to*

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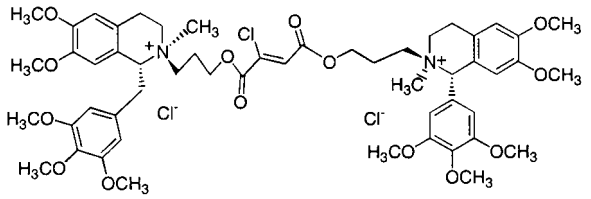
36 days. Consideration should be given to the incorporation of glycine or a low concentration of citrate, malate, or tartrate buffer in the parenteral formulation development of GW280430A. Cosolvents prolonged the predicted $t_{0.9}$ *for GW280430A in solution, but the enhancement was not significant enough to pursue a liquid formulation.*

Key Words: *Buffer catalysis; Cosolvent; Neuromuscular blocking agent; Solution stability*

INTRODUCTION

GW280430A is an ultrashort-acting, non-depolarizing neuromuscular blocking agent targeted at muscle relaxation as part of the surgical intubation procedure (1). The structure, chemical formula, and molecular weight of GW280430A are shown in Sch. 1. The most important physicochemical property of GW280430A is the hydrolytic instability of the ester carbonyl in aqueous solution (Fig. 1). This solution instability negatively impacts the ability to formulate the compound in water for injection (1).



Chemical formula: $C_{53}H_{69}Cl_3N_2O_{14}$

Molecular weight: 1064.49

Scheme 1. Molecular structure of GW280430A.

Since the drug is unstable in aqueous solution, two approaches were taken here in an attempt to stabilize GW280430A for injection: lyophilization and formulation in cosolvent mixtures. Lyophilization was considered because the primary route of degradation for GW280430A is ester hydrolysis. Lyophilized products often require maintaining the solution's pH upon reconstitution (2,3). Since solutions with a pH lower than 3 can be painful upon injection (4,5), and the rate of ester hydrolysis is at a minimum near pH 2–3 in pure aqueous solution, buffers with pK_a values near 3 were selected: citrate ($pK_{a1} = 3.13$), malate ($pK_{a1} = 3.40$), tartrate ($pK_{a1} = 2.93$), and glycine ($pK_{a1} = 2.34$). Because some buffers may catalyze a hydrolysis reaction (6,7), the first objective of this work was to study the buffer catalytic effect on the solution stability of GW280430A.

Cosolvents are frequently employed as a means of extending the shelf-life of parenteral formulations because they can inhibit degradation in purely aqueous media (8–10). The second objective of this work was to study the cosolvent effect on the solution stability of GW280430A. The cosolvents employed in this study include: ethanol (EtOH), propylene glycol (PG), polyethylene glycol 400 (PEG), and *N*,*N*-dimethylacetamide (DMA).

EXPERIMENTAL

Materials

GW280430A was manufactured at GlaxoSmithKline Inc., Research Triangle Park, NC. All other materials were reagent grade or better and used without further purification.

Sample Preparation

For the buffer catalysis study, GW280430A was formulated at 0.2 mg/mL in aqueous solutions containing 0.01, 0.03, and 0.05 M of each of the following buffers: citrate, malate, tartrate, and glycine. The pH of the solutions was adjusted to 3 using NaOH or HCl, and the ionic strength of the solutions was adjusted to 0.15 M using NaCl. For comparison, an aqueous isotonic solution was formulated without buffer and adjusted to pH 3 with HCl. The formulations were sealed in glass ampules and stored at 40, 50, and 60°C. Additional samples were stored at −20°C as controls.

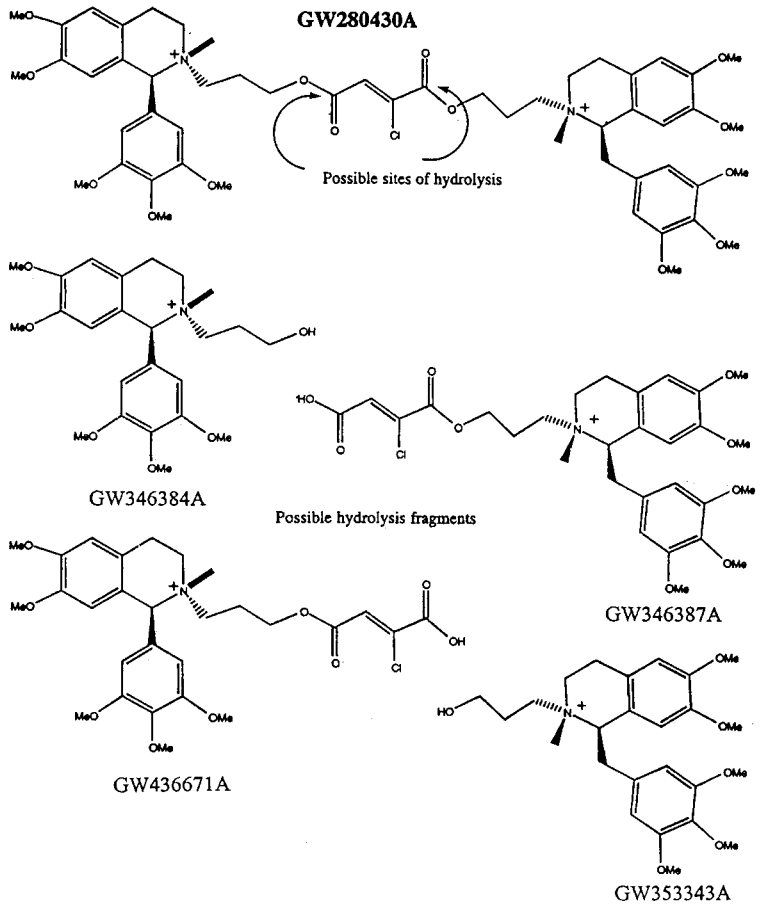


Figure 1. Possible hydrolysis sites and degradation products in aqueous solution.

Cosolvent mixtures employed in this solution kinetic study were prepared by mixing EtOH, PEG, PG, and DMA with saline adjusted to pH 3 using HCl (saline/pH 3) in the following ratios (v/v): 10% EtOH + 90% saline/pH 3; 10% EtOH + 40% PG + 50% saline/pH 3; 50% PEG + 50% saline/pH 3; 50% PG + 50% saline/pH 3; 50% DMA + 50% saline/pH 3. Solutions of 0.2 mg/mL GW280430A in the above five cosolvent mixtures were prepared. The formulations were sealed in glass vials and stored at 40, 50, and 60°C. Additional samples stored at −20°C were used as controls.

Sample Analysis

The buffer-formulated samples were analyzed by reverse-phase high-performance liquid chromatography (HPLC) to determine the peak area of non-hydrolyzed drug relative to a control at $t = 0$, 1, 2, 4, 6, 9, and 12 days, and the HCl samples were analyzed at $t = 0$, 1, 3, 5, 8, and 11 days. The final pH was measured for all samples on the last stability pull date and was relatively unchanged from the initial time point (remaining at pH 3.0–3.1).

The samples stored at 40 and 60°C were analyzed by reverse-phase HPLC to determine the peak area of non-degraded drug relative to a control at $t = 0$, 2, 5, 8, 14, and 18 days and at $t = 0$, 2, 4, 9, 13, and 17 days for samples stored at 50°C.

HPLC Method

Instrument: Waters™ 717 Plus Autosampler
Column: Inertsil ODS-2, 4.6 × 150 mm, 5 μm
Mobile phase: acetonitrile (0.05% TFA) : methanol : 0.1% aqueous TFA (22 : 12 : 66)

Flow rate: 1 mL/min
Wavelength: 244 nm
Column temperature: 35°C
Injection volume: 5 μL

Data Analysis

The peak area of non-hydrolyzed GW280430A relative to the control (−20°C) was plotted as a percent on a log scale as a function of time for each buffer system at each buffer concentration and for each cosolvent mixture. The hydrolysis rate constants were calculated from the slopes of the resulting best-fit lines. Activation energies were calculated from the slopes of the Arrhenius plots. The predicted $t_{0.9}$ values at 5 and 25°C were calculated using the Arrhenius equation.

RESULTS AND DISCUSSION

Figure 2 shows representative chromatograms for the 0.05 M citrate-buffered GW280430A solution at $t = 0$ (Fig. 2a) and $t = 12$ days (Fig. 2b). The chromatograms for the other buffered solutions appeared similar to those shown in Fig. 2. GW280430A has a retention time of 8.7 min and the degradation products have retention times of 2–5 min. It can be seen in Fig. 2b that the GW280430A peak has decreased significantly with a corresponding increase in degradant peaks.

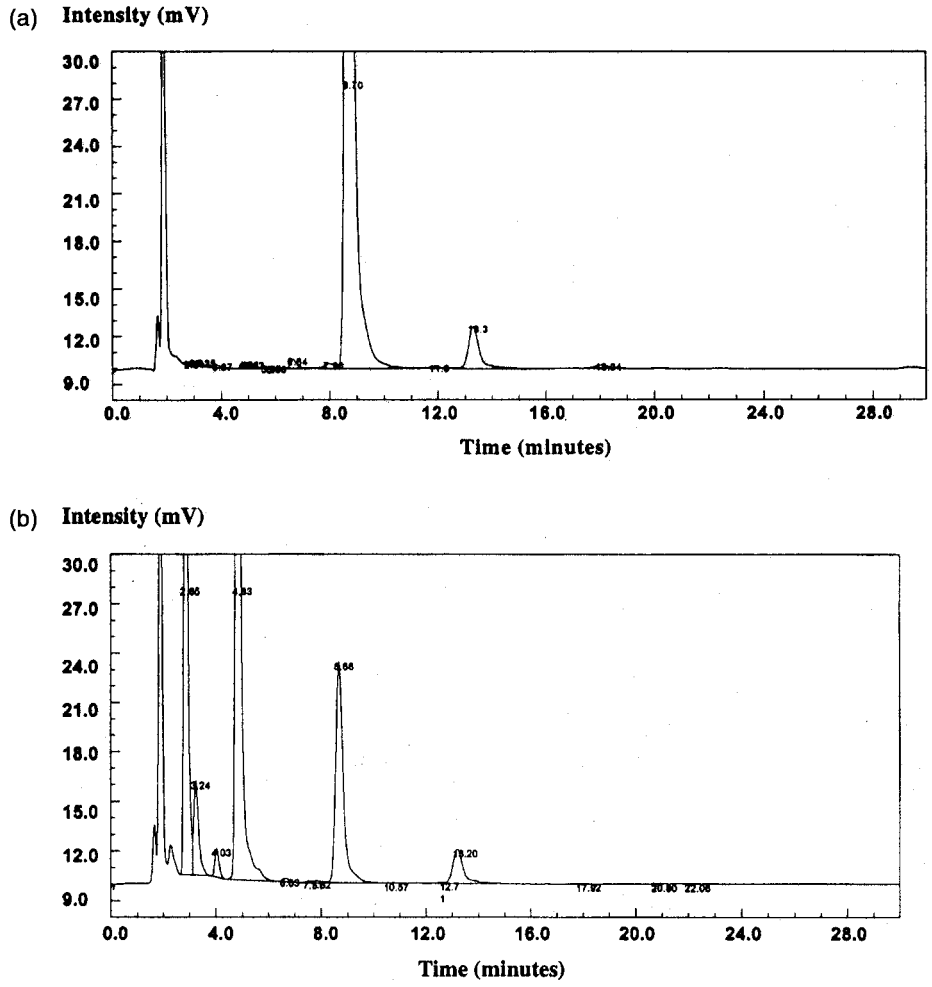


Figure 2. HPLC chromatogram of GW280430A (a) in 0.05 M citrate buffer at $t = 0$; (b) at 60°C for 12 days.

Figure 3 is a representative plot of the percent GW280430A on a log scale as a function of time in 0.05 M citrate buffer. First-order kinetics were observed for citrate-buffered drug solutions at all three temperatures. Similar plots for the other buffered solutions at the three concentrations were analyzed (not shown) and were also linear.

Buffer catalysis is often identified by an increase in the rate of hydrolysis with an increase in buffer concentration. Figure 4 shows that at 60°C glycine does not exhibit buffer catalysis, whereas citrate, malate, and tartrate all exhibit buffer concentration-dependent rates of hydrolysis. The trends for samples stored at 40 and 50°C (not shown) appear similar to the trends for samples stored at 60°C.

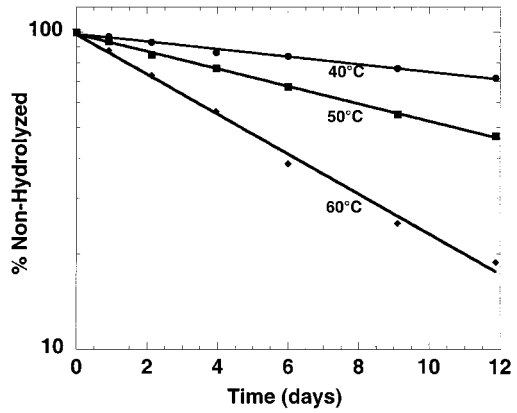


Figure 3. Percent non-degraded GW280430A (0.2 mg/mL) as a function of time in 0.05 M citrate buffer.

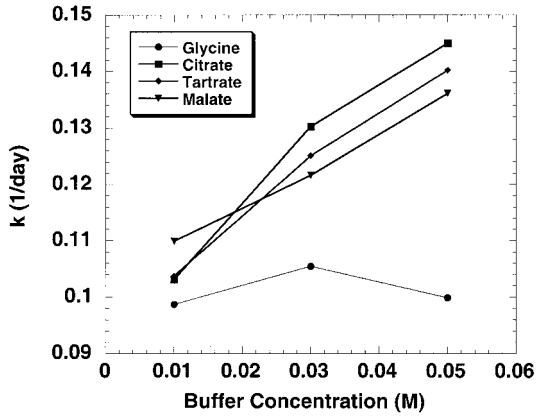


Figure 4. Rate of hydrolysis of GW280430A (0.2 mg/mL) as a function of buffer concentration at 60°C.

The activation energies for each buffer at each concentration were calculated from the slopes of the Arrhenius plots and are recorded in Table 1. The activation energy range for the hydrolysis of GW280430A is 70–80 kJ/mol, a normal range for a hydrolysis reaction (3). There appeared to be a correlation between buffer concentration and activation energy for the buffers exhibiting buffer catalysis. The activation energy decreased with increasing buffer concentration, which may explain the higher hydrolysis rate for citrate, tartrate, and malate at the higher buffer concentrations. While one would expect the activation energy for the glycine-buffered solution to be higher, in comparison with the other three (Table 1), the energies may not be directly comparable due to the possibility that different mechanisms of action exist between the mono- and dicarboxylic acids. Further experimentation would be required to fully elucidate the mechanisms involved.

The predicted $t_{0.9}$ values at 5 and 25°C were calculated and plotted for each buffer at each concentration in Figs. 5 and 6. These plots show that the $t_{0.9}$ for the glycine-buffered GW280430A solution is not significantly affected by buffer concentration, whereas the $t_{0.9}$ for citrate-, malate-, and tartrate-buffered solutions decreased as the buffer concentrations increased. These plots also show that at 5°C, 0.01 M citrate and tartrate solutions had the longest predicted $t_{0.9}$, while at 25°C, 0.01 M tartrate solution had the longest predicted $t_{0.9}$.

Esters are susceptible to attack by nucleophilic agents. The pK_{a1} values of the buffers under study increased in the order: glycine (2.34) < tartaric acid (2.93) < citric acid (3.15) < malic acid (3.40), suggesting glycine is the strongest acid. Stronger acids have weaker conjugate bases, which are lower energy

Table 1

Activation Energies (kJ/mol) of GW280430A in Buffered Solutions

	Buffer Concentration		
Buffer	0.01 M	0.03 M	0.05 M
Citrate	75.7	73.9	71.9
Malate	78.9	73.5	71.6
Tartrate	77.8	71.2	69.8
Glycine	70.5	70.9	70.6

The HCl sample has an activation energy of 70.8 kJ/mol.

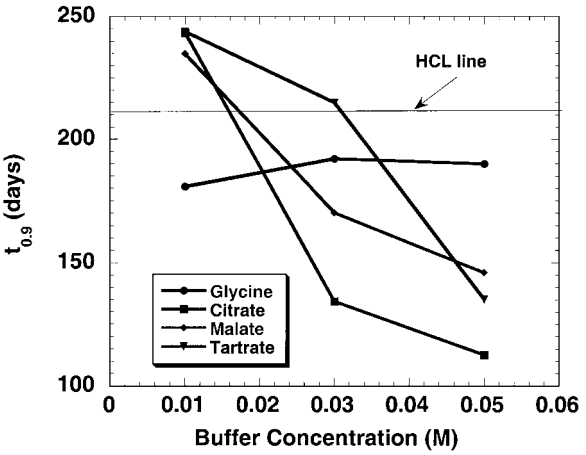


Figure 5. Predicted $t_{0.9}$ at 5°C as a function of buffer concentration.

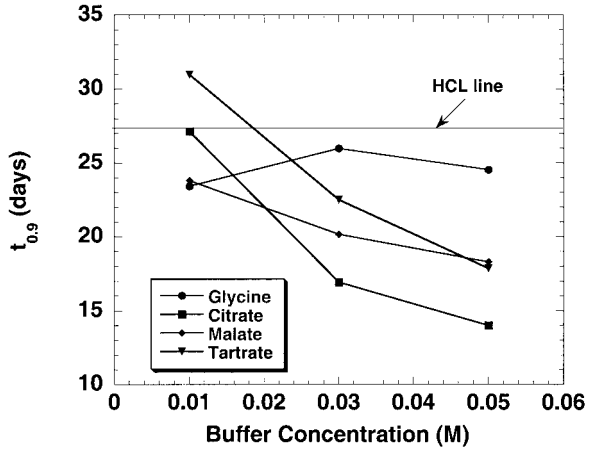


Figure 6. Predicted $t_{0.9}$ at 25°C as a function of buffer concentration.

Table 2

The Retention Time of the Additional Degradation Peaks of GW280430A in Cosolvent Mixtures

Cosolvent Mixture	Retention Time (min)
10% EtOH + 90% saline/pH 3	20.36
50% PG + 50% saline/pH 3	8.13
10% EtOH + 40% PG + 50% saline/pH 3	8.12, 20.35
50% PEG + 50% saline/pH 3	4–16 (multiple peaks)
50% DMA + 50% saline/pH 3	20.85

species and thus less reactive. Hence, glycine is less likely to enter into a nucleophilic reaction than the other three buffer agents. Supporting data is given in Fig. 4, where at the buffer concentration studied, GW280430A hydrolysis is slower in glycine-buffered solutions compared with the other three. Additionally, glycine is a monocarboxylic acid, whereas the other three buffers are di- or tricarboxylic acids, and offer the possibility of an intramolecular general base catalysis.

The retention times of each additional peak in the cosolvent mixtures are summarized in Table 2. GW280430A had a retention time of 8.7 min. New chemical species were produced in cosolvent mixtures, as indicated by the additional peaks present in chromatograms compared with those in saline/pH 3. These peaks suggest different degradation mechanisms other than hydrolysis in the GW280430A cosolvent mixtures. One possible mechanism is transesterification. Transesterification is the conversion of an ester into another ester by heating it with an excess of either an alcohol or a carboxylic acid in the presence of an acidic or basic catalyst. One possible transesterification reaction is presented in Fig. 7 using EtOH as an example.

Figure 8 is a representative plot of the percent GW280430A on a log scale as a function of time for GW280430A in 10% EtOH + 90% saline/pH 3. First-order kinetics were observed for all three temperatures. Similar plots for the other cosolvent mixtures (10% EtOH + 40% PG + 50% saline/pH 3, 50% PG + 50% saline/pH 3, 50% PEG + 50% saline/pH 3, and 50% DMA + 50% saline/pH 3) were made (not shown) and were also linear. However, first-order kinetics were not observed for 50% PEG + 50% saline/pH 3 at 40 and 50°C (Fig. 9). Since PEG 400 is a mixture of numerous alcohols with different molecular weights, it is reasonable that more than one transesterification reaction could occur simultaneously. Thus, the activation energy and predicted $t_{0.9}$ at 5 and 25°C of GW280430A in 50% PEG were not calculated.

The activation energies for the cosolvent mixtures were calculated from the slopes of the Arrhenius plots and are recorded in Table 3. Figure 10 is a representative Arrhenius plot for GW280430A in 10% EtOH + 90% saline/pH 3 cosolvent mixture. Similar plots for the 10% EtOH + 40% PG + 50% saline/pH 3, 50% PG + 50% saline/pH 3, and 50% DMA + 50% saline/pH 3 cosolvent mixtures were made (not shown). The activation energy range for

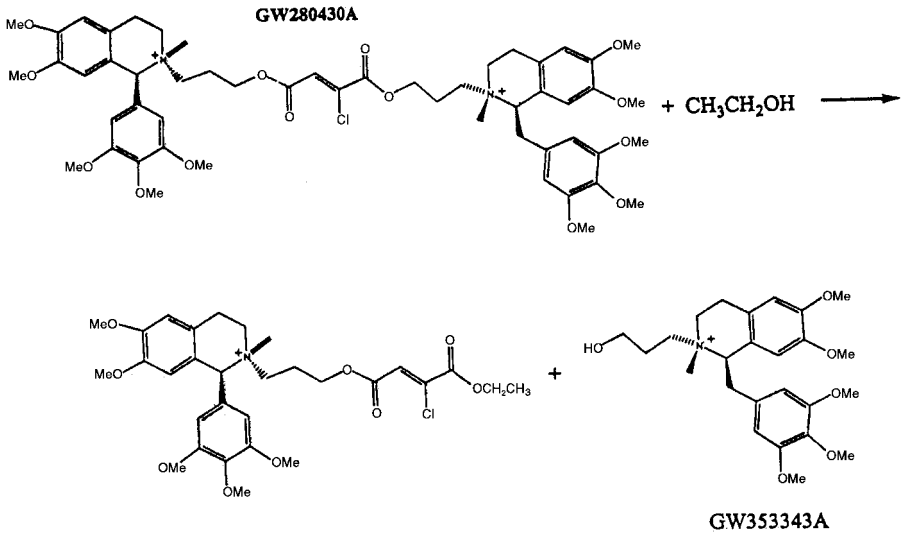


Figure 7. Possible transesterification reaction of GW280430A with EtOH.

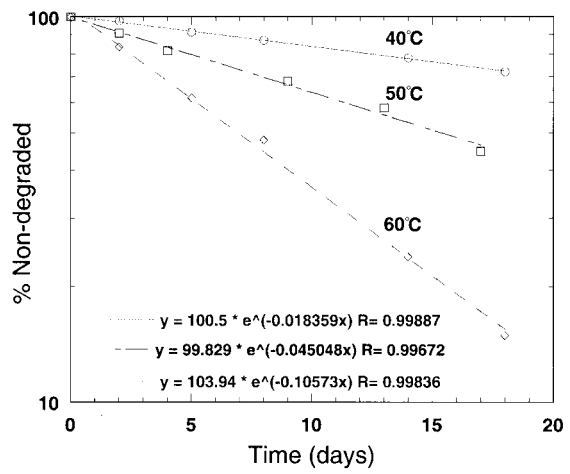


Figure 8. Percent non-degraded GW280430A as a function of time in 10% EtOH + 90% saline/pH 3 cosolvent mixture showing first-order kinetics.

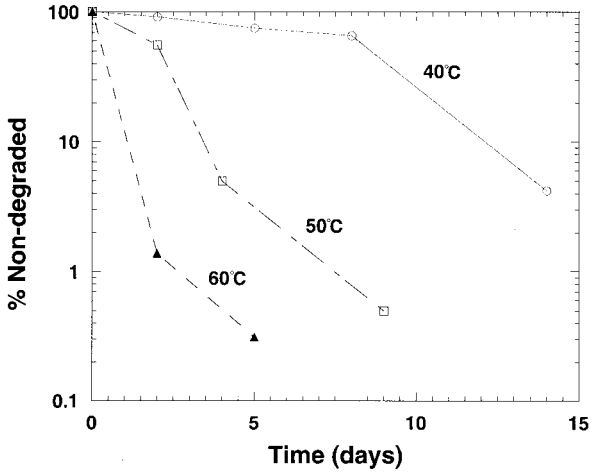


Figure 9. Percent non-degraded GW280430A as a function of time in 50% PEG + 50% saline/pH 3 cosolvent mixture showing non-first-order kinetics.

Table 3

Degradation Rate Constants, Activation Energies, and Predicted $t_{0.9}$ *of GW280430A in Cosolvent Mixtures*

Cosolvent Mixture	E_a (kJ/mol)	k (day^{-1}) (5°C)	$t_{0.9}$ (5°C) (month)	k (day^{-1}) (25°C)	$t_{0.9}$ (25°C) (day)
10% EtOH + 90% saline/pH 3	75.8	0.000328	10	0.00318	35
10% EtOH + 40% PG + 50% saline/pH 3	76.3	0.000316	11	0.00290	36
50% PG + 50% saline/pH 3	79.0	0.00029	12	0.00287	37
50% DMA + 50% saline/pH 3	79.8	0.00029	12	0.00317	33
Saline/pH 3	74.0	0.000342	9	0.0039*	27

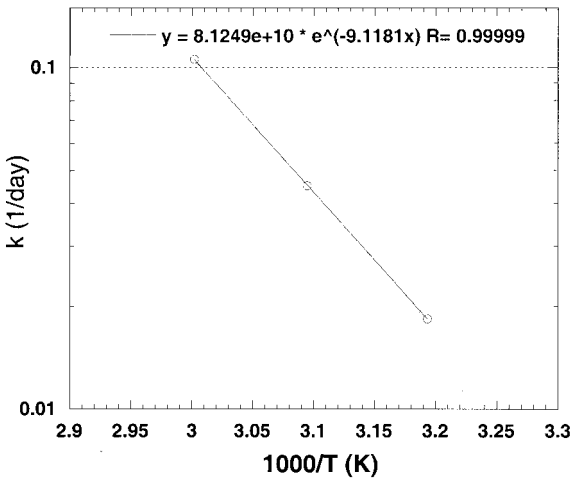


Figure 10. Arrhenius plot of GW280430A in 10% EtOH + 90% saline/pH 3 cosolvent mixture.

the degradation of GW280430A in various cosolvent mixtures was 75–85 kJ/mol. The predicted $t_{0.9}$ values at 5 and 25°C were calculated and are also recorded in Table 3. These data indicate that cosolvent mixtures prolonged the predicted $t_{0.9}$ for GW280430A in solution, but the enhancement was not significant enough to pursue a liquid formulation.

CONCLUSIONS

The ester hydrolysis reaction of GW280430A was catalyzed by citrate, malate, and tartrate buffers, but not by glycine. Glycine did not produce a concentration-dependent degradation rate and the drug formulated in 0.01 M glycine had a shorter predicted $t_{0.9}$ at both 5 and 25°C than that formulated in 0.01 M citrate, malate, or tartrate. However, 0.05 M glycine-buffered drug clearly has a longer predicted $t_{0.9}$ than the 0.05 M citrate-, malate- or tartrate-buffered drug at 0.05 M. The data show that all buffers provided adequate 24 hr stability for a reconstituted product ($t_{0.9}$ at 25°C >13 days). Since glycine does not cause buffer-catalyzed hydrolysis, it could be used throughout the development with more flexibility than the other buffers. Formulation optimization with a glycine-buffered system may be more predictable, since it does not cause concentration-dependent hydrolysis. Glycine or a low concentration of citrate, malate, or tartrate will be considered in a lyophilized parenteral formulation development of GW280430A.

Since new chemical species were produced in each cosolvent mixture, it is assumed that all solvents employed influenced the kinetics of GW280430A in solution. Though cosolvents prolonged the predicted $t_{0.9}$ for GW280430A in aqueous solution, the enhancement was not significant enough to pursue a partly non-aqueous formulation.

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