Racemization of Ketorolac in Aqueous Solution

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Received May 11, 1995, from the *Pharmaceutical Research Syntex Research Palo Alto, CA 94304.* Accepted for publication June 23, 1995[⊗].

Abstract □ The racemization of ketorolac was studied in aqueous buffered solution at 25 and 80 °C and analyzed in detail with respect to the catalytic species in solution. The reaction has a U shaped pH rate profile at 80 °C with the pH of maximum stability occurring in the region of pH 3.0–7.5. A T_{90} value of 8 months was observed for a 1.5% (*R*)-ketorolac tromethamine solution at pH 7.4 and 25 °C. Additionally, the data shows that alternative salt forms are necessary in order to prepare a stable single isomer formulation. Alternative buffers, in particular phosphate buffer, provide formulations exhibiting a T_{90} greater than 2 years.

Introduction

Ketorolac tromethamine (Toradol) is a member of the pyrrolopyrrole group of nonsteroidal antiinflammatory drugs.[1] It is a prostaglandin synthesis inhibitor which exhibits potent analgesic, antiinflammatory, and antipyretic effects. Ketorolac tromethamine is an effective analgesic when administered intramuscularly and orally.[2–4] Ophthalmic solutions of ketorolac tromethamine have been effective in preventing postoperative inflammation following cataract extraction and intraocular lens therapy.[5]

Ketorolac tromethamine is marketed as a racemic mixture as are other non-steroidal anti-inflammatory drugs.[6] Studies evaluating the pharmacological properties of the *R* and *S* isomers of ketorolac tromethamine have shown that the majority of the activity resides with the *S* isomer.[7] Although ketorolac is chemically stable to degradation in aqueous solution,[8] it is susceptible to racemization.[9] In this study, the racemization rate of ketorolac was measured in aqueous solution to establish the feasibility of a single isomer formulation of this drug.

Experimental Section

Materials—Racemic ketorolac tromethamine and single isomers of ketorolac were obtained from the Institute of Organic Chemistry, Syntex Research.[7] The optical purity was ≥90% (optical rotation) for the *S* isomer and 98% (chiral HPLC) for the *R* isomer.

2-Octanol-*d* (99% optical purity) was obtained from Norse Laboratories. Water was purified using the Barnstead Nanopure System. Buffer salts were reagent grade. Spectral grade toluene (Mallinckrodt) was treated by washing with concentrated sulfuric acid and then washing with water, saturated sodium bicarbonate solutions followed by water until the pH was neutral. The toluene was then dried over sodium sulfate.

Determination of the Solubility of Ketorolac in 0.1 N HCl—The solubility of (*R*)-ketorolac and racemic ketorolac was measured by adding an excess of ketorolac to 3 mL of 0.1 N HCl in a 4 mL vial.

Table 1—Solubility of the Isomeric Forms of Ketorolac Free Acid

Solvent	Isomer	pH	Solubility (mg/mL)
0.1 N HCl	Racemate	1.0	0.050
0.1 N HCl	*R*	1.0	0.028

The vial was tightly sealed with a teflon-lined cap and the suspension was equilibrated for 3–8 days in a 25 °C water bath equipped with a sample holder that rotated at 25 rpm. The samples were filtered through 0.22 μ filters, diluted with the mobile phase, and analyzed by reverse phase HPLC method 1.

HPLC Methods—HPLC method 1 was an achiral method used to analyze solubility samples. It used a C-8 Ultrasphere column (4.6 mm × 250 cm) from Alltech and a mobile phase of acetonitrile/water/acetic acid (45:55:0.2). The flow rate was 1 mL/min with detection at 314 nm.[8]

Method 2 requires derivatization with 2-octanol-*d* and separation of the diasteriomers by normal phase HPLC. It was developed to analyze 80 °C kinetic studies. This method is a variation of a published method for naproxen[10] and was used prior to chiral column chromatography becoming routine. Samples (5 mL) were acidified with concentrated HCl, and the drug was extracted into two 5 mL aliquots of methylene chloride. The methylene chloride was allowed to evaporate and the residue was reacted with 1 mL of a mixture of 2-octanol-*d*/toluene/sulfuric acid (20:1000:1) at 40 °C for 19 h. The reaction was neutralized by the addition of 1 mL of 0.02 M sodium bicarbonate. The organic layer was separated and dried over anhydrous sodium sulfate. A 0.20 mL aliquot of the toluene solution was evaporated and diluted with 10 mL of HPLC mobile phase. The mobile phase was composed of hexane/ethyl acetate (96:4). Analysis was performed on an Altex Ultrasphere-SI, 5 μ, 4.6 mm × 25 cm silica column with a flow rate of 2 mL/min and a UV detection wavelength of 325 nm.

Room temperature samples were analyzed using a chiral HPLC column (HPLC method 3). Relative concentrations of the enantiomers were determined by peak area (response factor = 1.0). A ChromTech Chiral AGP column 5 μ, 4.6 mm × 10 cm from Richard Scientific (Novato, CA) was used at ambient temperature. The mobile phase was 0.04 mM potassium phosphate buffer (pH 7.0) and a flow rate of 0.6 mL/min was used. The eluent was monitored at 323 nm. An injection size of 2 μL was used.

Kinetic Methods—The kinetic studies at 80 °C were performed using the (*S*)-ketorolac in aqueous HCl, KOH, or buffer solutions from pH 1–11. The ionic strength was adjusted with KCl to $\mu = 0.56$ M. pH measurements of the solutions were performed at 80 °C and all concentrations were corrected for volume changes. Reactions were initiated by adding a methanol stock solution of (*S*)-ketorolac buffer solution equilibrated to 80 °C so that the final drug concentration was 0.02 mg/mL and 0.3% methanol. At selected time intervals samples were removed, cooled to 4 °C, derivatized and assayed immediately by HPLC method 2. Reactions taking more than 30 h were stored in glass ampules.

The kinetics at 25 °C were performed using the (*R*)-ketorolac in buffers containing 0.01% sodium azide as a preservative. Reactions were initiated by adding 0.25 mL of a concentrated solution of (*R*)-ketorolac to 10 mL of buffer solution so that the final drug concentration was approximately 0.8 mg/mL. The stock solution was prepared by dissolving (*R*)-ketorolac (94 mg) in 0.15 mL of DMSO and 3 mL of

[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1995.

0022-3549/95/3184-1045$09.00/0

Table 2—Observed Rate Constants for the Racemization of Ketorolac at 80 °C

pH	Buffer	$10^6 k_{obs}$ (s^{-1}) 0.2 M	0.1 M	0.05 M	0.02 M	$10^6 k_0$ (s^{-1})	$10^4 k_{buf}$ ($M^{-1} s^{-1}$)
0.96	HCl					27.7 ± 1.3	
2.11	HCl					4.6 ± 0.1	
2.74	Formate	17.2 ± 0.5	11.4 ± 0.1	7.3 ± 0.2	4.8 ± 0.2	3.9 ± 0.5	0.68 ± 0.05
3.23	Formate	31.7 ± 1.3	16.3 ± 0.6	10.0 ± 0.4	5.6 ± 0.3	2.6 ± 0.5	1.44 ± 0.04
3.73	Formate	39.6 ± 2.4	21.0 ± 2.0	11.3 ± 0.8	5.8 ± 0.4	2.0 ± 0.1	1.88 ± 0.01
4.04	Formate	29.2 ± 1.0	16.4 ± 0.6	9.0 ± 0.3	4.5 ± 0.2	2.1 ± 0.4	1.37 ± 0.04
4.14	Acetate	47.8 ± 1.3	26.4 ± 0.5	14.7 ± 0.5	6.6 ± 0.4	2.9 ± 0.8	2.27 ± 0.07
4.67	Acetate	35.3 ± 3.1	19.5 ± 1.5	12.1 ± 1.0	5.1 ± 0.2	2.9 ± 0.9	1.63 ± 0.08
5.19	Acetate	19.3 ± 1.3	10.5 ± 0.6	5.6 ± 0.4	2.9 ± 0.1	1.2 ± 0.2	0.90 ± 0.02
5.75	Acetate	7.9 ± 0.3	4.6 ± 0.3	2.9 ± 0.2	1.7 ± 0.1	1.1 ± 0.1	0.33 ± 0.01
6.06	Phosphate	18.3 ± 1.4	10.9 ± 0.7	5.9 ± 0.2	3.3 ± 0.3	1.9 ± 0.5	0.83 ± 0.04
6.62	Phosphate	13.3 ± 0.7	7.5 ± 0.3	4.8 ± 0.3	2.8 ± 0.2	1.8 ± 0.1	0.58 ± 0.01
7.17	Phosphate	8.6 ± 0.5	6.0 ± 0.5	3.9 ± 0.3	3.1 ± 0.2	2.5 ± 0.2	0.31 ± 0.02
8.64	Carbonate	51.0 ± 2.8	50.3 ± 3.1	56.0 ± 6.3	50.1 ± 5.8	51.8 ± 2.8	
9.43	Carbonate	291 ± 19	249 ± 15	249 ± 18	212 ± 10	215 ± 10	3.85 ± 9.7
10.0	KOH					704 ± 31	
10.5	KOH					2350 ± 170	
11.0	KOH					6200 ± 400	

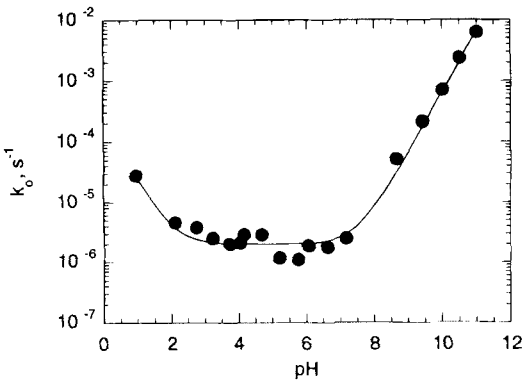


Figure 1—pH rate profile for the racemization of ketorolac at 80 °C. The k_0 values are extrapolated from the data in Table 2 using eq 1. The data was fitted using eq 2.

0.1 M KOH followed by filtration through a 0.45 μ PFTE filter. The drug solutions were stored in glass ampules protected from light until assayed by HPLC method 3. Model formulations in 0.04 M tris(hydroxymethyl)aminomethane buffer (tromethamine buffer) were prepared in a similar manner at a drug concentration of 1.0 and 10.0 mg/mL. The 10 mg/mL sample was diluted 10-fold prior to HPLC analysis.

First-order rate constants for the racemization of ketorolac, k_{obs}, were determined by fitting the enantiomeric excess (%R − %S or %S − %R) versus time to a first-order equation. The observed rate constants were plotted versus the buffer concentration to determine second-order rate constants for the buffer catalysis, k_{buf}, and the buffer independent rate constants, k_o. pK_a values for the catalytic species plots are literature values corrected for ionic strength and temperature.[11] The reported errors are the standard errors obtained from fitting the kinetic data.

Results and Discussion

Measurements of the Solubility of Ketorolac—Table 1 gives the solubility of the isomeric forms of ketorolac free acid in 0.1 N HCl. The solubility at pH 1 is a measure of the intrinsic solubility of the free acid. For ketorolac, the solubility of the pure isomer is approximately half that of the racemic free acid. This difference is not predictable and arises from differences in the crystal lattice energies of the single isomer and racemate crystals.

Reactivity at 80 °C—The effect of pH and buffer salts on the rate of racemization of ketorolac at 80 °C was monitored by HPLC. The results show that racemization (%S − %R) follows first-order kinetics for greater than three half-lives. The observed rate constants for the reaction in the various buffers at 80 °C and the second-order buffer rate constants obtained by fitting the data to eq 1 are shown in Table 2.

$$k_{obs} = k_{buf}[\text{buffer}] + k_0 \quad (1)$$

The buffer-independent rate constants, k_0 in eq 1, can be obtained by extrapolating the data in Table 2 to zero buffer concentration. The U-shaped pH-rate profile (Figure 1) can be fitted by a reaction mechanism that has specific acid, k_H, water, k_w, and specific base, k_{OH}, terms.

$$k_0 = k_H a_H + k_w + k_{OH} a_{OH} \quad (2)$$

In eq 2, a_H and a_{OH} are, respectively, the hydrogen ion and hydroxide ion activities at 80 °C. Fitting the data to eq 2 gives values of 2.3×10^{-4} $M^{-1} s^{-1}$, 2.0×10^{-6} s^{-1} and 0.26 $M^{-1} s^{-1}$ for k_H, k_w, and k_{OH}, respectively. Lack of significant curvature in the pH profile at the pK_a of ketorolac, pK_a=3.5,[8] suggests that the free acid and ionized form of ketorolac react at similar rates in the absence of buffer catalysis.

The rate constant for buffer catalysis, k_{buf}, is comprised of terms arising from the free base form and the free acid form of the buffer (eq 3).

$$k_{buf} = k_{fa}[HA] + k_{fb}[A^-] \quad (3)$$

The catalytic species in formate, acetate, phosphate, and carbonate buffers can be determined by plotting the buffer rate constants versus the fraction of buffer in the free acid form (Figure 2). The intercept on the left and right ordinates are the rate constants for catalysis by the base and acid components of the buffer, respectively (Table 3). For phosphate buffer only the ratio of $H_2PO_4^{1-}$:HPO_4^{2-} was considered to be important. The plot in Figure 2 shows that for phosphate and acetate buffer the free acid forms ($H_2PO_4^{1-}$ and CH_3COOH) are the most catalytically active forms. The plot for formate buffer shows curvature. Curvature in the plot can be interpreted as arising from changes in the catalytically active species of formate (HCOOH or $HCOO^-$) occurring due to the ionization state of ketorolac.

Reactivity in Model Formulations at 25 °C—The racemization of a prototype 1.0% formulation of ketorolac was

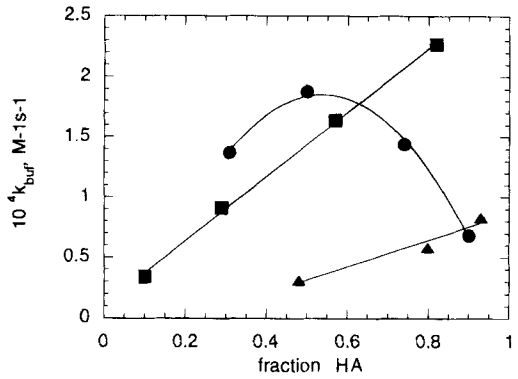


Figure 2—The second-order rate constants for the buffer-catalyzed racemization versus the fraction of acetate (■), formate (●), and phosphate (▲) in the free acid form at 80 °C. pK_a values are corrected for ionic strength and temperature effects.[11]

Table 3—Catalytic Rate Constants for the Racemization of Ketorolac in Buffer Solution

Buffer	T(°C)	10^6k_{fa} ($M^{-1}s^{-1}$))	10^6k_{fb} ($M^{-1}s^{-1}$)
Tromethamine	25	0.11 ± 0.01	0.77 ± 0.05
Phosphate	25	2.3 ± 0.2	
	80	110 ± 20	
Acetate	25	7.1 ± 0.9	
	80	267 ± 7	

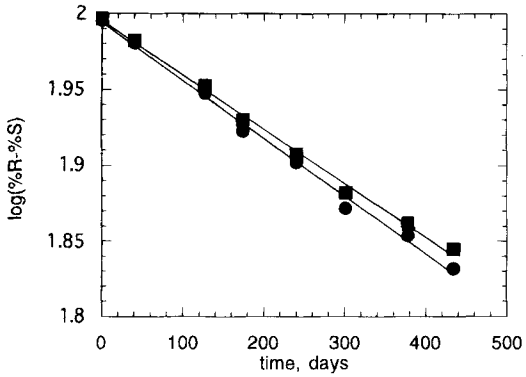


Figure 3—First-order plots for the racemization of (*R*)-ketorolac in 0.04 M tromethamine buffer (pH 7.4) at 25 °C. The ketorolac concentration was 1% (■) or 0.1% (●).

evaluated at room temperature in 0.04 M tromethamine buffer at pH 7.4. This model formulation is equimolar in both drug and tromethamine with the current 3.0% racemic product. The racemization reaction was fitted to a first-order equation (Figure 3) and can be interpolated to give a T_{90} (90% *R* isomer: 10% *S* isomer or racemization 20% complete) of 8 months. Similar data was also obtained at a drug concentration of 0.1% ketorolac showing that at pH 7.4 ketorolac does not catalyze its own racemization.

Additional buffers and buffer concentrations were studied to determine if an alternate salt form would give a stable formulation. The reaction was studied in 0.040, 0.020, and 0.004 M acetate, phosphate, and tromethamine buffers at room temperature in the range pH 5–8. Values for the observed rate constants, k_{obs}, in the three buffers are given in Table 4 and plotted in Figure 4. The horizontal line in Figure 4 predicts a T_{90} of 2 years. The second-order buffer rate constants obtained by fitting the data to eq 1 are shown in Table 4.

Figure 5 shows a plot of the second-order buffer rate constant obtained from the slopes of the plots in Figure 4 versus the fraction of the buffer in the protonated form for

Table 4—Observed Rate Constants for the Racemization of Ketorolac at 25 °C

pH	Buffer	10^9k_{obs} (s^{-1}) 0.040 M	0.02 M	0.004 M	10^9k_0 (s–1)	10^6k_{buf} ($M^{-1}s^{-1}$)
5.0	Acetate	13.5 ± 1.0	8.0 ± 1.0	4.0 ± 0.4	2.9 ± 0.2	26.4 ± 0.7
5.6	Acetate	5.1 ± 0.5	2.9 ± 0.3	1.6 ± 0.2	1.1 ± 0.2	9.8 ± 0.8
6.0	Acetate	2.2 ± 0.2	1.3 ± 0.2	0.7 ± 0.1	0.51 ± 0.06	4.2 ± 0.2
6.1	Phosphate	9.2 ± 0.3	4.6 ± 0.1	1.4 ± 0.1	0.4 ± 0.2	21.7 ± 0.9
7.2	Phosphate	5.0 ± 0.2	2.8 ± 0.1	1.3 ± 0.1	0.8 ± 0.1	10.3 ± 0.5
7.8	Phosphate	4.5 ± 0.1	3.5 ± 0.1	2.8 ± 0.2	2.59 ± 0.05	4.7 ± 0.2
6.6	Tromethamine	6.4 ± 0.2	3.8 ± 0.1	1.4 ± 0.1	0.9 ± 0.1	13.9 ± 0.6
7.3	Tromethamine	8.5 ± 0.2	4.8 ± 0.2	1.9 ± 0.1	1.15 ± 0.03	18.3 ± 0.1
7.7	Tromethamine	14.2 ± 0.4	8.2 ± 0.2	3.2 ± 0.2	2.0 ± 0.1	30.5 ± 0.4

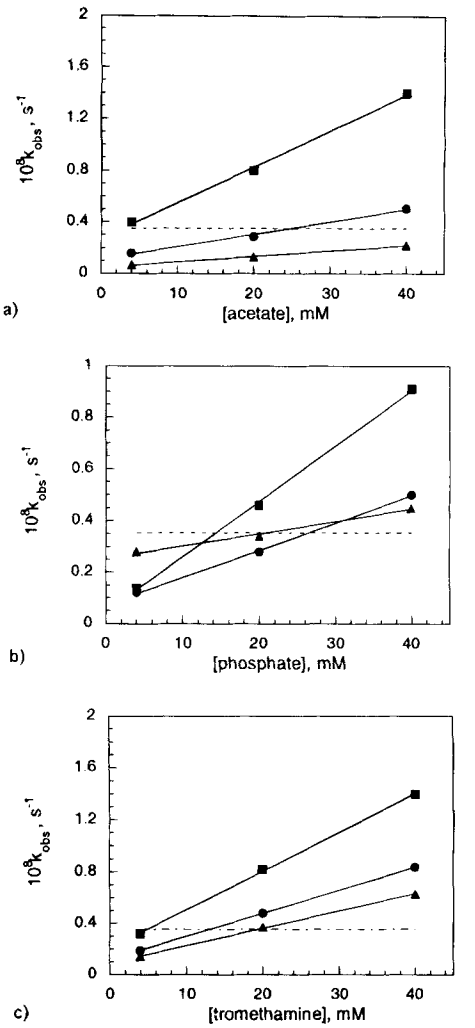


Figure 4—Plots of the observed first-order rate constants for the racemization at 25 °C versus buffer strength for (a) acetate buffer, pH 5.0 (■), 5.6 (●) and 6.0 (▲); (b) phosphate buffer, pH 6.1 (▲), 7.2 (●), and 7.8 (■); and (c) tromethamine buffer, pH 6.6 (▲), 7.3 (●), and 7.7 (■). The horizontal line represent a T_{90} of 2 years (90% *R* isomer:10% *S* isomer or racemization 20% complete).

acetate, phosphate, and tromethamine at 25 °C. The data for phosphate and acetate agree with the 80 °C results (Figure 2). Extrapolation of the data for tromethamine shows that although both forms are catalysts, the neutral form is approximately 7 times more catalytic than the protonated form (Table 3). The ability of tromethamine and not phosphate and acetate to act as a general base catalyst may result from the ionic nature of the species involved. Phosphate and acetate are anionic bases that cannot approach the α-hydrogen of ketorolac anion, whereas tromethamine is a neutral base and

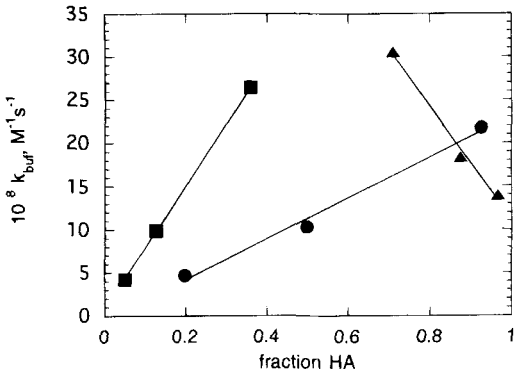
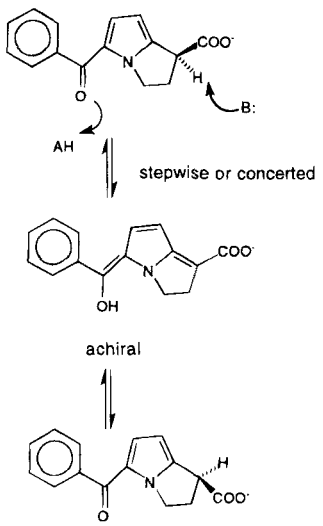


Figure 5—The second-order rate constants for the buffer catalyzed racemization versus the fraction of acetate (■), phosphate (●), and tromethamine (▲) in the free acid form at 25 °C. pK_a values for acetate, phosphate, and tromethamine are from ref 11.



Scheme 1—Possible mechanisms for the racemization of ketorolac.

can approach the α-hydrogen. Scheme 1 shows possible mechanisms for the general acid- and base-catalyzed reactions and the catalytic species involved.[12–14] It shows general acid and general base catalysis of the formation of an unstable enol occurring by protonation on the ketone carbonyl and abstraction of the α-hydrogen, respectively.

Formulation Implications—Since the data for the racemization of 1% ketorolac free acid in 0.040 mM ketorolac tromethamine at pH 7.4 suggested a T_{90} of 8 months, reformulation of a single isomer product is required.

Although many factors need to be considered in preparing a solution formulation, two of the most important are the stability and solubility of the drug. Examination of the pH rate profile at 80 °C shows that the pH of maximum stability would be in the region of pH 3.0 to 7.5 at 80 °C. This region would increase at 25 °C due to the increase in K_w from 25 °C to 80 °C. By using the intrinsic solubility of the single isomer of ketorolac and the dissociation constant of ketorolac, it is possible to predict (eq 4)[15] that a pH value greater than 6.1 would be required to prepare a 1% solution of ketorolac (Figure 6). Therefore, pH values between 6.1 and approximately 8.0 should be considered for a 1% single isomer formulation.

$$S = S_0 (1 + 10^{pH - pK_a}) \quad (4)$$

Catalysis of the racemization reaction by tromethamine buffer at pH 6.6, 7.3, and 7.7 (Figure 4) shows that a T_{90} of 2

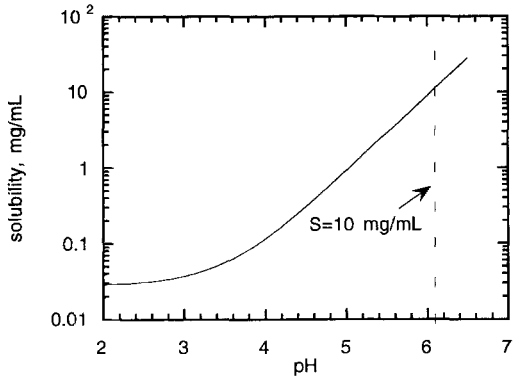


Figure 6—Predicted solubility of a single isomer of ketorolac as a function of pH at 25 °C. The curve was constructed using an intrinsic solubility of a single isomer of ketorolac of 0.028 mg/mL and a $pK_a = 3.5$.

years cannot be achieved in 0.040 M tromethamine buffer at any of the pH values studied. This is an important finding that shows that the tromethamine salt of ketorolac must be replaced by the free acid or other salt forms if a stable single isomer formulation is to be prepared. Stable single isomer formulations can be prepared in 0.020 M phosphate at pH 7.2. Acetate ($pK_a = 4.8$) does not exhibit sufficient buffer capacity[11] in the pH region required to solubilize 1% ketorolac and therefore is not a suitable buffer for a 1% formulation. The acetate data suggests that a formulation using another carboxylic acid buffer such as citrate buffer ($pK_a = 5.4$) may provide a stable formulation at pH values at the upper extreme of its buffering capacity (pH ≈ 6.4).

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Acknowledgments

The authors thank John Kern for developing chiral HPLC method 3.

JS950198Z