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Technical Notes

Determination of the Optimum pH for Isolation of a Compound with Multiple p*K*a's from Aqueous−Organic Solvent Mixtures

Louis S. Crocker,* Yaling Wang, and James A. McCauley

Merck Research Laboratories, RY818-A112, P.O. Box 2000, Rahway, New Jersey 07065, U.S.A.

Abstract:

Potentiometric titration of (4*R*,5*S*,6*S*,8*R*,2′*S*,4′*S*)-3-[[2-[[(3-carboxyphenyl)amino]carbonyl]pyrrolidin-4-yl]thio]-4-methyl-6-(1-hydroxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-en-2-carboxylic acid, a broad-spectrum antibiotic, in several solvent systems was used to generate values for the three dissociation constants of the compound. Simultaneous solution for the constants was employed. The proportions of the various species in solution were calculated from the dissociation constants; differences were observed between the optimum pH for isolation of the monoanion salt in aqueous−organic systems and the value calculated from aqueous titration results. Changes in the organic content of the solvent influenced the three dissociation constants to different extents.

Introduction

Adjustment of pH is frequently required for the successful isolation of an organic compound with dissociation constants within the accessible range. For compounds with a single ionizable group, proper adjustment may be as simple as adding acid or base to the solution until the pH is below or above the level necessary to make the desired species the predominant one in solution.[1] In the case of multiple dissociation constants, the required range in pH may be much more restricted, and a knowledge of the dissociation constants as determined, for example, by potentiometric titration can be useful for the rational design of an isolation. Further complexities arise when mixtures of water and organic solvents are used in the isolation, due to both changes in the response of the electrode in use and changes in the dissociation constants in different solvent systems.[2,3]

This work gives a practical example in which pK_a measurements are used to facilitate the isolation of an organic species from solution. The broad-spectrum antibiotic **1** (Figure 1) contains three ionizable groups and therefore three pK_a's. The compound may exist in solution as four species, the protonated form, the neutral (or zwitterionic) form, the monoanion, and the dianion. The proportion of each of these species depends on the pH and the dissociation constants at the conditions used. The isolation of the solid as the monosodium salt was selected as a useful process due to the superior crystallinity of the phase produced (as shown by X-ray powder diffraction) which resulted in desirable rejection of impurities during the isolation. Measurements of the pK_a's in water and aqueous alcohol solutions are presented, as well as the results of calculations of the proportions of the various species present under the isolation conditions.

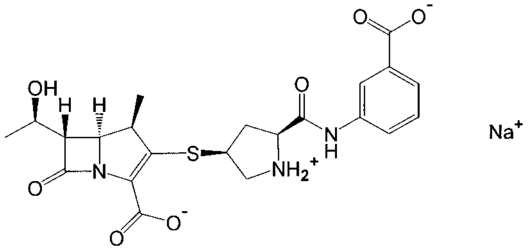

Figure 1. **Structure of antibiotic 1.**

Experimental Section

Solid **1** was characterized by Karl Fischer titration, flame atomic absorption (for sodium content), and gas chromatography (for residual solvents). Dynamic potentiometric titrations were carried out at room temperature in distilled/deionized water and in mixtures of water with methanol and *n*-propanol (Fisher), which were prepared by combining measured volumes of the pure solvents. Weighed quantities of **1** (weights corrected for water and residual solvents) were dissolved in 20.00 mL solvent and titrated with 0.1 M aqueous HCl using a Metrohm 736 GP Titrino with a 1 mL buret. Titrations were performed in duplicate. An Orion Ross semimicro combination glass electrode which had been calibrated using aqueous buffers (pH 4.00, 7.00, and 10.00; Fisher) was used. The titrant was standardized with tris(hydroxymethyl)-aminomethane (NIST 723b). Titrant volume/pH data were collected automatically.

Response of the electrode was measured in various solvent mixtures by measuring the pH of solutions containing known amounts of HCl.

Results and Discussion

Titration in water of a mixture of the monosodium and disodium salts of **1**, isolated under basic conditions, resulted in the pH/titrant volume curve shown in Figure 2. Evaluation of the first endpoint indicated 28.9 wt % monosodium salt (expressed on a dry basis). This result was used as a correction in subsequent calculations. The titration results allowed calculation of the sodium content of the solid (7.4 wt %) which agreed with the flame atomic absorption result of 7 wt % sodium. Figure 2 also shows the pH/titrant volume curves obtained in mixtures of water, methanol and *n*-propanol.

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* To whom correspondence should be addressed.

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Table 1. **Measured pK_a's of antibiotic 1 in aqueous−organic solvent mixtures**

solvent (volume ratio)	pKa_1[a]	pKa_2[a]	pKa_2[b]	pKa_3[b]
H_2O	2.72 (±0.01)	3.94 (±0.01)	3.98 (±0.02)	7.06 (±0.03)
H_2O/*n*-PrOH (9/2)	3.10 (±0.03)	4.30 (±0.02)	4.35 (±0.03)	6.87 (±0.04)
H_2O/MeOH/*n*-PrOH (9/5/5)	3.81 (±0.03)	5.16 (±0.01)	5.23 (±0.02)	6.99 (±0.04)
H_2O/MeOH/*n*-PrOH (9/10/10)	4.12 (±0.04)	5.57 (±0.02)	5.65 (±0.02)	7.17 (±0.01)

[a] Calculated using the data around the second endpoint. [b] Calculated using the data around the first endpoint.

Calculation of the dissociation constant for a species with a single ionizable group from titration data may be carried out in the traditional way, taking into account the ionic equilibria which are present in solution. This technique may be applied to systems with multiple dissociation constants if the resulting pK_a values are more than approximately 2.7 units apart.[1] With closer dissociation constants the presence of appreciable amounts of more than two species must be taken into account. Below, the simultaneous solution of K_a(2) and K_a(3), neglecting K_a(1), is given. Assuming that $[OH^-]$ is negligible and that only dianion was present at the start of a titration with HCl

$$K_a(2) = (X_1Y_2 - X_2Y_1)/(Z_2Y_1 - Z_1Y_2)$$

and

$$K_a(3) = (X_1Z_2 - X_2Z_1)/(X_2Y_1 - X_1Y_2)$$

where

$$X = [H^+]([H^+] + 2C - [Cl^-])$$

$$Y = 1 - [Cl^-]/[H^+]$$

and

$$Z = [H^+] + C - [Cl^-]$$

In these expressions $[Cl^-]$ equals chloride concentration (assuming complete ionization of HCl) and C equals the total concentration of compound without regard to ionization state. The subscripts refer to two different points from the pH/titrant volume curve, one before and one after the monoanion endpoint. These equations were derived using a method similar to that described for titration of a diacid with sodium hydroxide.[1,4] Correction of these calculations for the presence of monosodium salt was carried out by adjusting the chloride concentration accordingly.

Simultaneous solution for K_a(1) and K_a(2) was also carried out using similar equations, neglecting K_a(3).

Values for the dissociation constants (expressed as pK_a) calculated from the titration data in several solvent systems are shown in Table 1. The values shown are based on the averages of K_a values calculated from 22 pairs of pH/titrant volume data (11 pairs from each of two replicate titrations in each solvent system). The imprecisions equal the standard deviations of the distributions of individual pK_a values. Two calculated values are shown for pK_a(2) in each solvent system, one from the simultaneous solution of pK_a(1) and pK_a(2) and the other from the simultaneous solution of pK_a(2) and pK_a(3). The excellent agreement between the pK_a(2) values shows that the simultaneous solution of all three dissociation constants is unnecessary.

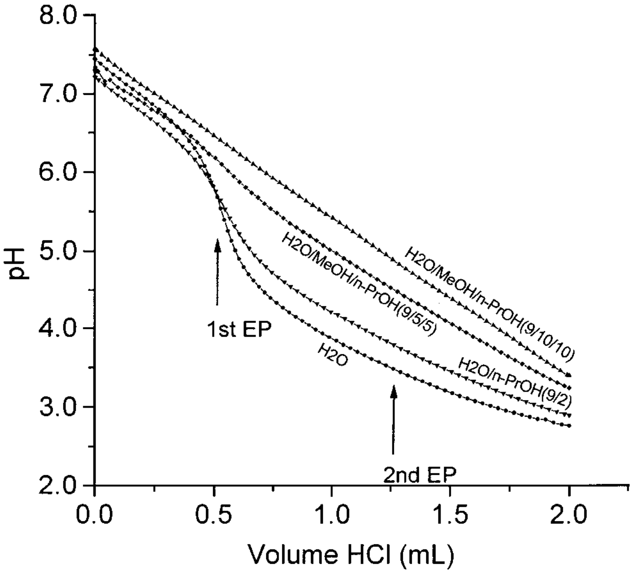

Figure 2. **Titration results for antibiotic 1 obtained in different solvent mixtures using 0.1 M HCl as titrant.**

The values of pK_a (2) change most dramatically with change in solvent composition. This phenomenon can be explained in terms of the differing degrees of charge separation involved:

$$K_a(1):\quad NH_2^+{\cdot}CO_2H{\cdot}CO_2H = NH_2^+{\cdot}CO_2H{\cdot}CO_2^- + H^+$$

$$K_a(2):\quad NH_2^+{\cdot}CO_2H{\cdot}CO_2^- = NH_2^+{\cdot}CO_2^-{\cdot}CO_2^- + H^+$$

$$K_a(3):\quad NH_2^+{\cdot}CO_2^-{\cdot}CO_2^- = NH{\cdot}CO_2^-{\cdot}CO_2^- + H^+$$

The equilibrium expressed by K_a(2) involves ionization of a species with no net charge to two oppositely charged ions and would be expected to be strongly influenced by a change in dielectric constant. This argument is similar to that used to rationalize the effects of organic solvent content on the dissociation constants of organic indicators,[2,3] and is also demonstrated by the dramatic change in pK_a generally observed for carboxylic acids in contrast to amines. (For an example of this compare the pK_a values of acetic acid and tris(hydroxymethyl)amino methane in water/DMSO mixtures as reported by Mukerjee et al).[5] The presence of multiply charged species complicates the explanation of the fairly large observed change in pK_a(1) observed in this work, since K_a(1) generates no separation of charge, leading to the expectation of a small change with change in solvent. Perhaps

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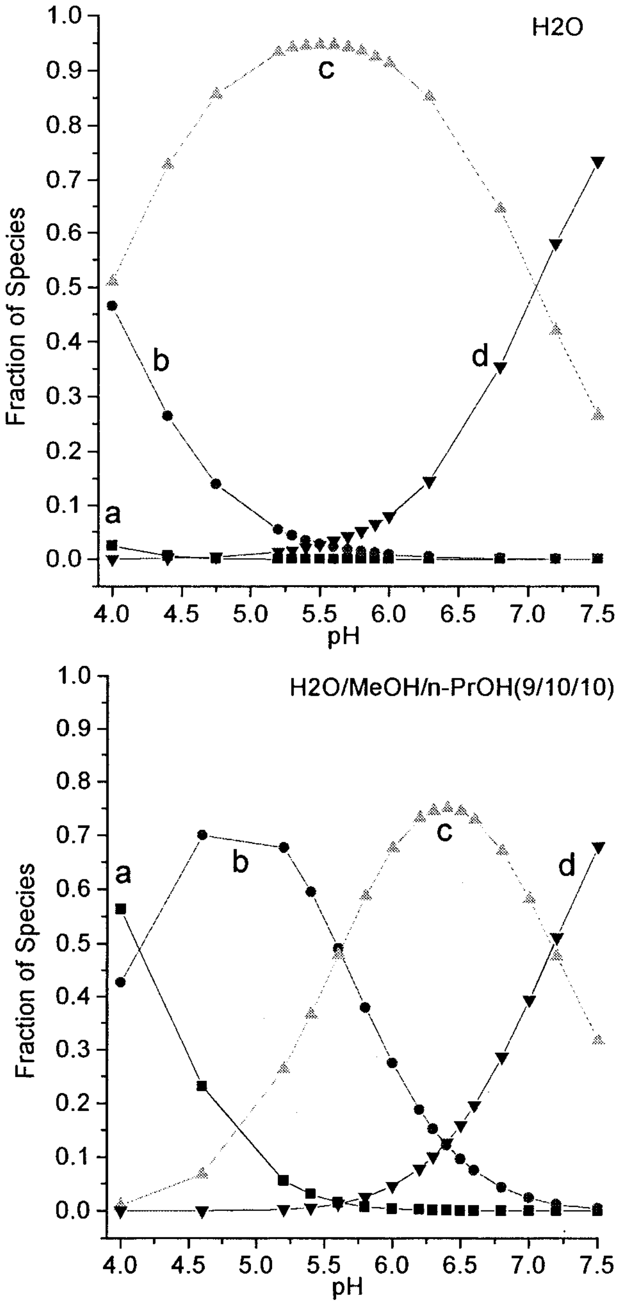

Figure 3. **Fraction of species existing in solution as (a) protonated, (b) neutral or zwitterion, (c) monoanion, and (d) dianion. Solvents are indicated.**

stabilization of the two charges of the neutral zwitterionic species increases the sensitivity of pK_a(1) to changes in dielectric constant.

The pK_a's calculated in this way are uncorrected for the changes in the standard potential of the electrode used in the pH measurements due to changes in the solvent. Such changes can be substantial in certain solvent mixtures.[5-7] To investigate this possibility, pH values were measured in HCl solutions of known concentration in water and 1:1:1 water:methanol:*n*-propanol, solvents in which any change in response of the electrode would be maximized. At HCl concentrations of 0.001M, a solution in water exhibited a pH of 3.04, and in the 1:1:1 mixture, 3.20; at 0.00001M, pH's of 5.25 and 5.45 were observed, respectively. These changes are small compared to the large shifts in the dissociation constants observed in the titrations and were not applied to the pHs used in the calculations of the pK_a's. A practical reason for not correcting the measured pH values for solvent effects is that during the actual processing, pH of the crystallization solution is monitored using a glass electrode with similar response to the one used in the titration experiments. Therefore, the target pH values calculated from the uncorrected data will more closely match those observed in the manufacturing setting.

The populations of the species present in solution at a given condition may be calculated from the dissociation constants and the pH. This can be done by simultaneous solution of the linear equations derived from the equilibrium expressions and the conservation of mass. The equilibrium mole fraction of each species can be plotted as a function of pH as shown in Figure 3. In water, where the dissociation constants are relatively widely spaced, the proportion of the desired monoanion is highest at a pH of 5.5–5.6, and reaches a maximum of 95% of the total in solution. In 9:10:10 water: methanol:*n*-propanol the pK_a's are closer leading to a lower maximum proportion of monoanion with respect to the aqueous solution. The target pH, at which the maximum is observed, is shifted to 6.4, a considerable change with respect to the value obtained in water.

Figure 3 shows that use of the target pH derived from the water titration data in the pH adjustment of the aqueous/organic solution, or any other error in the adjustment of pH during the crystallization, would significantly reduce the proportion of the desired species at the conclusion of the crystallization, which is carried out at high alcohol concentrations to reduce the solubility of the product. Therefore the pK_a results obtained in the actual crystallization solvent can be used to maximize the concentration of the crystallizing species during the isolation.

In summary, the potentiometric titration of the antibiotic compound **1** in water and water/alcohol mixtures shows changes in the pK_a's of the compound, a knowledge of which can be exploited in the design of efficient isolation procedures. The optimum pHs were calculated for different solvent mixtures and were shown to have shifted significantly with changes in solvent, due to the differential changes in the dissociation constants.

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Supporting Information Available

Derivation of the simultaneous solution for K_a(2) and K_a(3), and a description of the method used to calculate ionic populations from the dissociation constants. This material is available free of charge via the Internet at http://pubs.acs.org.

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