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Rapid Communication

Evaluation of ionization constants of drugs in aqueous organic mixtures from reversed phase high-performance liquid chromatography

A. Gustavo Gonzalez, M. Angeles Herrador and Agustin G. Asuero

Department of Analytical Chemistry, University of Seville, E-41012 Seville (Spain)

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Summary

General methods for evaluating ionization constants in organic aqueous mixtures based on reversed-phase high-performance liquid chromatography (RP-HPLC) measurements are briefly revisited and presented in a form suitable for easy calculations.

Solvent effects on the dissociation of acid solutes are of great interest in chemical and biomedical analysis. It is well known that the poor solubility in water of many drugs necessitates the use of water-miscible cosolvents in order to prepare solution of these compounds (Rubino and Berryhill, 1986). Organic cosolvents are required for drugs that are to be administered in soft gelatin capsules, suppositories, typical preparations or injections (James, 1986). The stability, absorption and activity of a drug are strongly affected by the degree of ionization and lipid solubility of the drug (Asuero, 1988).

The changes in the Gibbs standard free energy accompanying the ionization of an acid HA in any mixed solvent (aqueous organic mixture) relative to that in pure water are referred to as the solvent or medium effect, and may be regarded as Gibbs standard energies of the proton transfer (Gonzalez et al., 1991c).

$$\Delta G_t^{\circ}(\mathrm{HA/A}) = 2.303RT(\mathrm{pK_a^*} - \mathrm{p}K_a^{\mathrm{w}}) \qquad (1)$$

where $\mathrm{p}K_a^*$ and $\mathrm{p}K_a^{\mathrm{w}}$ refer to the $\mathrm{p}K_a$ value of the acid HA at the standard state of the mixed solvent and water, respectively. Knowledge of the acidity constants of compounds in aqueous organic cosolvents is of vital importance for inferring solvent effects.

In order to evaluate acidity constants in mixed media the preferred technique is potentiometry, although it entails some drawbacks. Apart from the requirement for the titrand concentration (at least 0.005 M), one is often faced with the problems of instability of titrant solutions in non-aqueous and partially aqueous solvents. In such cases, spectrophotometric methods are ideal tools for evaluating acidity constants from typical ab-

Correspondence to: A.G. Gonzalez, Department of Analytical Chemistry, University of Seville, E-41012 Seville, Spain.

sorbance vs pH graphs (Gonzalez et al., 1991a). However, spectrophotometric techniques are only applicable if (Gonzalez et al., 1991b): (i) the compound shows appreciable absorption at the working wavelength; (ii) the site of protonation or deprotonation is conjugated with or is an inherent part of a chromophoric group in the compound; and (iii) the conjugate acid-base species have different absorption spectra. Unfortunately, many substances do not exhibit such suitable absorption features and it is necessary to call on other procedures.

RP-HPLC is a very appropriate technique for evaluating ionization constants of acid solutes in mixed media. This technique was successfully utilized by Palalikit and Block (1980) to determine the pK_a values of a number of acids. Mobile phases consisting of aqueous organic mixtures at fixed pH and ionic strength are readily prepared. Rheodyne-type valves equipped with replaceable sample loops permit the injection of fixed volumes between 2 and 1000 μl. Commercial HPLC detectors allow one to determine low concentrations of analyte, typical detection limits being in the range 1–0.001 ng (Harris, 1991). All these features overcome the possible drawbacks associated with the concentration level of analytes in addition to its physicochemical detection. The volume of acid HA solution to be assayed is thus minimized (a few μl) and the concentration of HA solute may be of the order of μg/ml.

However, when using types of silica bonded material as stationary phases, another acid-base equilibrium is likely to occur inside the HPLC column over the range of pH values studied: the active silanol sites of silica material are deprotonated upon increasing the pH (although probably more slowly than the solute). On the other hand, these columns entail the disadvantage of deterioration of the silica support at pH above 8. Both of these problems may lead to significant deviations from the model equation (Eqn 5). Moreover, the column used can have a strong effect on the observed pK_a value. Therefore, it is advisable to operate with non-silica packing materials. Non-ionic copolymer stationary phases are interesting materials, since they are not subject to shrinkage and swelling due to changes in the ionic strength of the mobile phase and being organic and non-ionic, they should lack the active sites (as in the case of silica materials). Accordingly, the retention time measured by using these columns would be considered to be proportional to the degree of ionization of the solute, and the adsorption features of the packing material would be regarded as unchanged irrespective of the pH of the mobile phase. In this case, as indicated by Palalikit and Block (1980), the pK_a values for solutes obtained from RP-HPLC measurements agree very well with those evaluated from spectrophotometric methods.

Theory: Consider the solute HA which is ionized in a given water-organic cosolvent mixture (charges omitted for the sake of generality)

$$HA = A + H$$

The relationship of Van Uitert and Haas (1953) is utilized to calculate the negative logarithm of the activity of the proton (pH^*) in the medium used from the reading of the pH-meter (B) as follows: $pH^* = B + \log U_H^\circ$. Values for the correction factors, $\log U_H^\circ$, are generally tabulated. Otherwise suitable methods for their determination are available (Gonzalez et al., 1992). The ionization constant for HA in the mixed solvent, K_a^* is given by

$$K_a^* = 10^{-pH^*}[A]f_A^*/[HA]f_{HA}^*$$

where f^* is the ionic activity coefficient (the 'salt effect') which is a function of the charge of species z, the permittivity of the medium D, the absolute temperature T and the ionic strength I (Glab and Hulanicki, 1981) according to:

$$-\log f^* = \frac{1.825 \times 10^6 z^2 (DT)^{-3/2} I^{1/2}}{1 + 251.45(DT)^{-1/2} I^{1/2}}$$

putting $H = 10^{-pH^*} f_A^*/f_{HA}^*$ we can write

$$K_a^* = H[A]/[HA] \qquad (2)$$

Assume now that the solute is injected into the chromatographic system using a mobile phase consisting of the solvent mixture studied at a

fixed pH* value and a constant ionic background. Taking into account that the proton transfer equilibrium is achieved much more rapidly than the distribution equilibrium between the mobile and stationary phases, the species A and HA cannot be separated chromatographically. Consequently, just one chromatographic peak is obtained for the total amount of solute (A and HA) showing a net retention time t that is the weighted average of those of the individual species

$$t = (t_{HA}[HA] + t_A[A])/([HA] + [A]) \quad (3)$$

where t_{HA} and t_A are the limiting retention times when the pH* of the mobile phase is either low enough or high enough to ensure that the solute species is the pure acid HA or the pure conjugate base A, respectively.

By rearranging Eqn 2 we obtain

$$[A]/[HA] = (t_{HA} - t)/(t - t_A) \quad (4)$$

Considering this latter relationship, Eqn 2 may be rewritten as

$$K_a^* = H(t_{HA} - t)/(t - t_A) \quad (5)$$

Thus, the pK_a^* may be graphically determined by plotting $\log(t_{HA} - t)/(t - t_A)$ vs $-\log H$. If either t_{HA} or t_A cannot be measured, the pK_a^* is determined by using the equations

$$t = t_{HA} + K_a^*(t_A - t)/H \quad (6)$$

or

$$t = t_A + (1/K_a^*)H(t_{HA} - t) \quad (7)$$

For graphical evaluation t is plotted against $(t_A - t)/H$ or $H(t_{HA} - t)$ as appropriate. In cases where both t_A and t_{HA} cannot be measured, Ramette's method (1967) applied for similar cases in spectrophotometry or in fluorimetry (Gonzalez and Pablos, 1991) may be successfully adapted considering retention times instead of absorbances or fluorescence intensities.

When the hold-up time of the column, t_0 is known (for instance, from the retention time of an unretained solute) one can use the capacity factors $k = (t - t_0)/t_0$ rather than the net retention time.

Recommended procedure: Mobile phases consisting of aqueous mixtures containing a given v/v organic cosolvent percentage must be prepared from HPLC quality organic cosolvents and aqueous potassium hydrogen phosphate (analytical grade) solution to give a final buffer concentration of about 0.015 M. The ionic strength may be kept constant by addition of potassium chloride (analytical grade) taking into account the contributions from the buffer species at different pH values (Otto and Wegscheider, 1983). Then the pH is adjusted pH-metrically and corrected for by use of the corresponding correction factors to be made to the glass electrode (Gonzalez et al., 1992). The corresponding mobile phase must be filtered through a 0.4 μm solvent titration apparatus and then degassed either by means of a flow of He or by using an ultrasonic bath. Milli-Q treated water is strongly recommended for using throughout.

Sample solutions of about 500 μg/ml or as appropriate to ensure clearly recognizable peaks are prepared in adequate mobile phase and then filtered through a 0.4 μm disposable syringe filter unit.

The use of HPLC pumps equipped with Rheodyne type injection valves is strongly recommended. The employed column should be of a non-silica packing material. Organic polymeric nonionic substances are appropriate, such as the XAD-2 copolymer (Palalikit and Block, 1980). The column may operate at 25 ± 0.1°C by means a 'low cost' water-jacket built from a suitable distillation condenser which permits the column to pass through it and a circulator thermostat.

The sample solutions (loops of 10–25 μl are available) at different pH values are injected and the chromatogram recorded. Retention times are taken from the recorder report (integrators or computerized devices). Triplicate experiments should be reformed in order to determine realistic values for the standard deviation of pK_a values.

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