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Microionization constants: novel approach for the determination of the zwitterionic equilibrium of hydroxyphenylalkylamines by photometric titration

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Abstract

The record of the formation of the phenolate and the zwitterionic form in the course of titration by photometry makes it possible to estimate the tautomeric equilibrium, K_Z, between the zwitterionic and the uncharged form of an ampholyte, provided that (1) the absorptivity of the phenolate and the zwitterionic form are identical and (2) the absorptivities of both forms are distinct from the absorptivities of the protonated and the uncharged form. The relation between the absorbance and K_Z, the degree of titration and the degree of overlapping of the basic and the acid ioinization constant is given. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Ampholytes of the hydroxyphenylalkylamine and hydroxyphenylaminoalkanol type offer branched protolysis equilibria, e.g. the entire ionization scheme of pholedrine can be given as shown in Scheme 1.

This particular equilibrium may be generalized so that it is applicable to any substance of the type under discussion (see Scheme 2).

The macroscopic ionization constants for this two-step system are defined by

$$K_1 = \frac{[Z][H^+]}{[Z^+]} \quad (1)$$

and

$$K_2 = \frac{[Z^-][H^+]}{[Z]} \quad (2)$$

in which [Z] is the concentration of the electrically neutral forms, that means the sum of $[Z^\pm]$ and $[Z^0]$.

With regard to the branched protolysis, this results in

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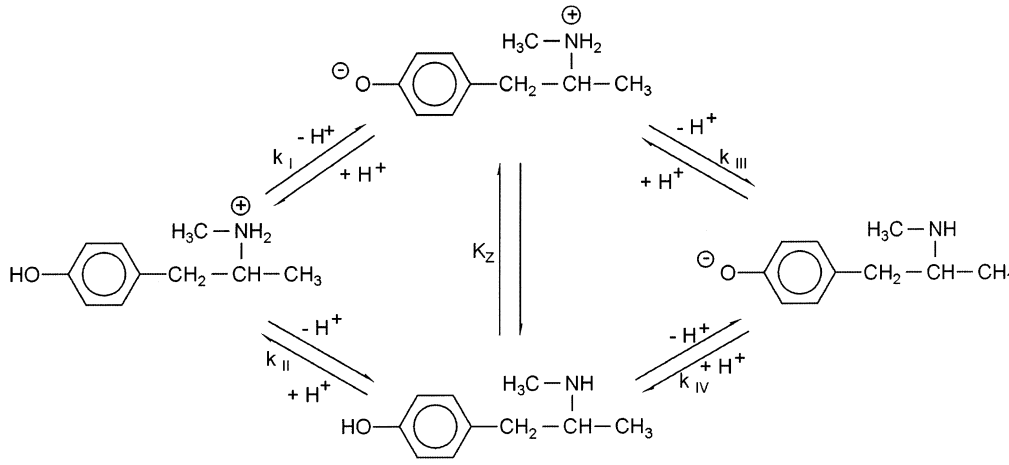


Scheme 1. Ionization of pholedrine.

$$K_1 = \frac{[Z^{\pm}][H^+] + [Z^0][H^+]}{[Z^+]} \quad (3)$$

and

$$K_2 = \frac{[Z^-][H^+]}{[Z^{\pm}] + [Z^0]} \quad (4)$$

and the micro-ionization constants

$$k_I = \frac{[Z^{\pm}][H^+]}{[Z^+]};\ k_{III} = \frac{[Z^-][H^+]}{[Z^{\pm}]} \quad (5)$$

$$k_{II} = \frac{[Z^0][H^+]}{[Z^+]};\quad k_{IV} = \frac{[Z^-][H^+]}{[Z^0]}. \quad (6)$$

Therefore, the microscopic constants are related to the macroscopic constants by the following equations (Adams, 1916)

$$K_1 = k_I + k_{II} \quad (7)$$

$$\frac{1}{K_2} = \frac{1}{k_{III}} + \frac{1}{k_{IV}}. \quad (8)$$

The remaining constant in Scheme 2, the zwitterionic constant, K_Z, characterizes the tautomeric equilibrium.

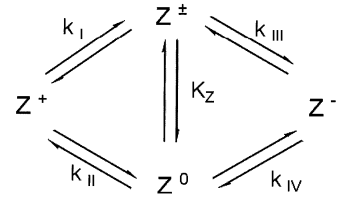
The relation to the microconstants is given by

$$K_Z = \frac{k_I}{k_{II}} = \frac{k_{IV}}{k_{III}}. \quad (9)$$

The molecular interpretation of ionization-dependent phenomena requires the knowledge of microconstants. Their accurate determination is important for a complete understanding of the chemical behaviour and biological activity of ampholytes. Especially in a biological context, the fraction of the different active species at a particular pH is important and has to be considered in QSAR studies.

Macroscopic pK_a values are typically known to an accuracy of about 0.01 units, while the zwitterionic constants K_Z are often not known. As they can differ by several orders of magnitude, their knowledge is significant for estimating the fraction of the efficient species, for example in QSAR studies. The methods and problems of the determination were discussed by Albert and Serjeant (1984) and Polster and Lachmann (1989).

The method of Edsall et al. (1958) appears to be the method exclusively used for the determination of microconstants for the ampholytes under discussion. It is based on the different absorption spectra of the two phenolate forms Z^- and $Z^{\pm}$ and the cation, Z^+, and the uncharged molecule,



Scheme 2. Ionization of an ampholyte.

Z^0, in relation to the pH. Edsall defined a function, α, which represents the fractions, F, of all the phenolic hydroxyl groups in solution that are ionized

$$\alpha = \frac{\text{concentration of all phenolates}}{\text{concentration of all molecular species}} \quad (10)$$

$$= \frac{[Z^{\pm}] + [Z^-]}{[Z^+] + [Z^{\pm}] + [Z^0] + [Z^-]} = F_{Z^{\pm}} + F_{Z^-}$$

$$1 = F_{Z^+} + F_{Z^-} + F_{Z^{\pm}} + F_{Z^0} = F_{Z^+} + F_{Z^-} + F_Z. \quad (11)$$

Furthermore, Edsall defined a variable, M, the relation between ionized and non-ionized phenolic groups and the pH, respectively:

$$M = \frac{[H^+]\cdot\alpha}{1-\alpha}.$$

Combined with Eqs. (5), (6) and (10), this results in

$$M = \frac{k_I[H^+] + k_{II}k_{IV}}{[H^+] + k_{II}}$$

and in logarithmic form,

$$pM = pH - \log\frac{\alpha}{1-\alpha}.$$

A plot of pM vs. α will extrapolate to pk_I when $\alpha = 0$ or $[H^+] \to \infty$ and, when $\alpha = 1$ or $[H^+] \to 0$, to pk_{IV} and, finally, when $\alpha = 0.5$, to pk_{II}

$$k_{II} = \frac{[H^+](k_I - [H^+])}{[H^+] - k_{IV}}.$$

These calculations are valid if the absorptivities of the two phenolate forms, $Z^{\pm}$ and Z^-, are identical at the wavelengths chosen for the analysis. Hydroxyphenylaminoalkanols fulfil this criterion with a close approximation.

The Edsall method was criticised by Niebergall et al. (1972) since the method depends upon a smooth line being drawn through the data points, which results in investigator bias. Edsall already pointed out that the function pM is very sensitive to small errors in α when α is very close to zero or unity. Hence, the points near the ends of the curve show more scatter than those near the middle. To overcome the problem, Niebergall firstly rearranged the above equation to a linear form and then carried out a non-linear regression analysis, respectively. Neither estimation solves the principal problem already mentioned by Edsall: the experimental data are rearranged to give derived variables that are plotted. These derived variables are inherently less accurate then the raw experimental values used to obtain them. Additionally, Edsall defined the limits of his method. The zwitterionic constant should lie between 5 and 0.2.

It was the objective of this investigation to find out an alternative method for the determination of the zwitterionic constant, K_Z, even beyond the limits of the Edsall method.

2. Materials and methods

2.1. Materials

The materials were as follows: tyramine hydrochloride (analytical grade, melting point 209°C) from Sigma (Deisenhofen, Germany) and pholedrine sulphate (AB-DDR) from Isis Pharma (Zwickau, Germany).

All the other samples (pharmacopoeial grade) were purchased from Synopharm (Barsbüttel, Germany).

All other reagents were of analytical grade, from commercial sources, and were used without purification.

2.2. Apparatus

The apparatus comprised a piston burette E 457 Metrohm (Herisau, Switzerland).

2.3. Procedure

Solutions containing 1×10^{-2} M of the substances were made in distilled water degassed by boiling. The titration was carried out at 25°C in a beaker sealed into a water jacket for thermostatic control. The beaker was fitted with a pH-electrode assembly. A 100 ml portion of a solution was titrated with carbonate-free potassium hydroxide solution (1 M) under stirring and in a nitrogen atmosphere. For each step of titration (50 μl and then 100 μl steps), the pH was registered, and the

absorbance of the solution was measured in a 1-mm cell at the wavelength selected. The absorbance thus measured was corrected for the absorbance of the completely protonated substance by subtracting the absorbance of a solution in hydrochloric acid (0.1 M). If the pH range of the titration exceeded pH 10, the stochiometric degree of titration, τ_{st}, was corrected for the concentration of hydroxyl ions to give the corrected degree, τ_{corr}

$$\tau_{corr} = (\tau_{st} 0.01 - [OH^-])\, 100.$$

S_0 was calculated by non-linear regression using Origin V.5.

3. Results and discussion

3.1. Principle

The procedure is based on two assumptions of the Edsall method:

1. On ionization of the phenolic hydroxyl group, the maximum in the absorption spectrum of the compounds studied shifts from about 275 nm to about 295 nm. The absorption at a wavelength longer than 295 nm is practically zero for the unionized phenolic hydroxyl group Z^+ and Z^0, whereas it is large for the phenolate forms $Z^\pm$ and Z^-.
2. The absorptivities of the two phenolate forms $Z^\pm$ and Z^- are identical.

The principle is to be explained with the (theoretical) example of an ampholyte with no overlapping ionization characteristics ($pK_2 - pK_1 > 3$). Then, in the course of titration of the cation Z^+ with a base, first, Z^+ is totally deprotonized to the zwitterion, $Z^\pm$, and the uncharged form Z^0, respectively, and thereafter, the loss of the second proton takes place resulting in the anion Z^-. A plot of the phenolate forms, expressed in terms of α, against the degree of titration, expressed in terms of τ, is illustrated in Fig. 1. From $\tau = 0$ to $\tau = 1$ only $Z^\pm$ and Z^0 are formed. The slope, S_0, of the curve represents the degree of zwitterion formation:

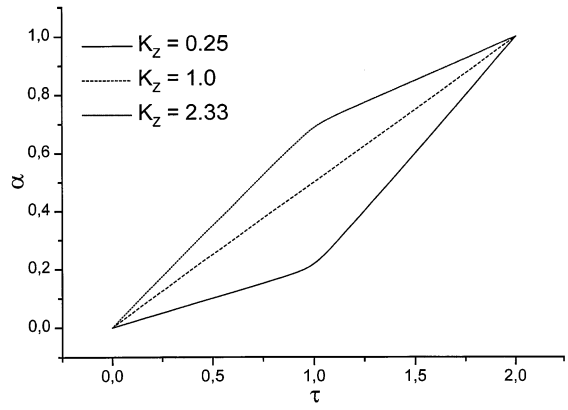


Fig. 1. Photometric titration of ampholytes with $\Delta pK_a = 3$ and different zwitterionic constants, K_Z.

$$S_0 = \frac{[Z^\pm]}{[Z^\pm] + [Z^0]} = \frac{[Z^\pm]}{[Z]} = \frac{F_{Z^\pm}}{F_Z}. \tag{12}$$

In reality, for the substances under discussion, the pK_a values are separated by 0.8–1.6 units. Therefore, the ionization processes are overlapping, which means that in the course of titration, the electrically neutral form, Z, coexists with the anion, Z^-. The shape of the curve depends on the difference in pK_a values of the ionization process expressed as $K_{2/1}$.

From Eqs. (1) and (2), it follows that

$$K_{2/1} = \frac{K_2}{K_1} = \frac{F_{Z^+} + F_{Z^-}}{F_Z^2}.$$

Combined with Eq. (11), we obtain

$$K_{2/1} = \frac{F_{Z^-} - F_Z F_{Z^-} - F_{Z^-}^2}{F_Z^2}.$$

Introducing

$$\tau = F_Z + 2F_{Z^-} \tag{13}$$

and solving for F_{Z^-} gives

$$K_{2/1} = \frac{\tau - F_Z}{F_Z^2} - \frac{F_Z(\tau - F_Z)}{F_Z^2} - \frac{(\tau - F_Z)^2}{4F_Z^2}$$

which is rearranged to the quadratic equation

$$F_Z^2 + \frac{2F_Z}{4K_{2/1} - 1} + \frac{\tau^2 - 2\tau}{4K_{2/1} - 1} = 0.$$

For F_Z it follows that

$$F_Z = -\frac{1}{4K_{2/1}-1} \pm \sqrt{\left(\frac{1}{4K_{2/1}-1}\right)^2 - \frac{\tau^2-2\tau}{4K_{2/1}-1}}. \quad (14)$$

Using the definitions of α as given by Eq. (10) and S_0 given by Eq. (12) and the relationship between τ and the fractions of phenolate given by Eq. (13), we obtain

$$\alpha = S_0 F_Z + \frac{\tau - F_Z}{2} = \left(S_0 - \frac{1}{2}\right)F_Z + \frac{\tau}{2} \quad (15)$$

$$= \left(S_0 - \frac{1}{2}\right) \times \left(-\frac{1}{4K_{2/1}-1} - \sqrt{\left(\frac{1}{4K_{2/1}-1}\right)^2 - \frac{\tau^2-2\tau}{4K_{2/1}-1}}\right) + \frac{\tau}{2}.$$

The character of the function defined by Eq. (15) is illustrated in detail by the curves shown in Fig. 2.

With decreasing difference in pK_a values, the linear section of the curve is more or less decreasing, depending on the value of the zwitterionic constant, K_Z, e.g. at $S_0 = 0.5$ ($K_Z = 1$), the curve is linear independent of the differences in pK_a (Fig. 3). In the case of very small differences in pK_a values, the slope at the origin of the curve S_0 represents the fraction of the zwitterion.

The relation to the zwitterionic constant, K_Z, is

$$K_Z = \frac{S_0}{1-S_0}.$$

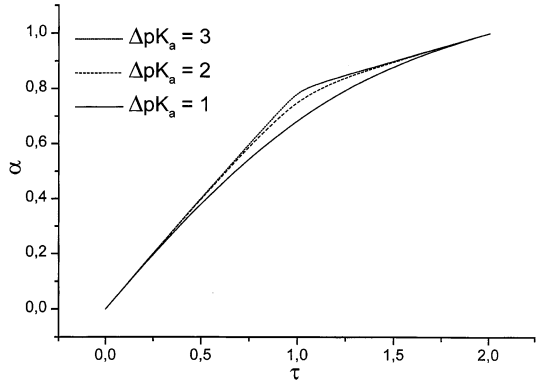


Fig. 2. Photometric titration of ampholytes with different pK_a values ($K_Z = 4$).

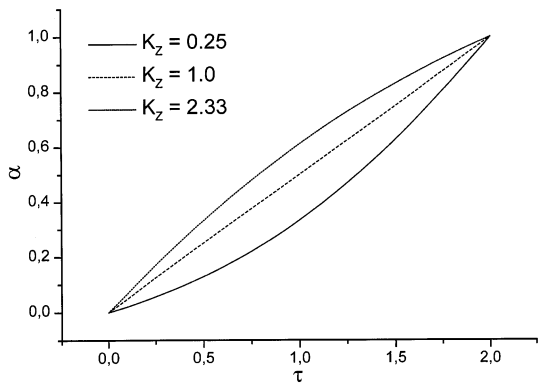


Fig. 3. Photometric titration of ampholytes with different zwitterionic constants, K_Z ($\Delta pK_a = 1$).

The combination of Eqs. (3), (4) and (12) yields

$$k_I = K_1 \cdot S_0; \quad k_{III} = \frac{K_2}{S_0}$$

$$k_{II} = K_1 \cdot (1 - S_0); \quad k_{IV} = \frac{K_2}{1-S_0}.$$

4. Results

Typical plots of α vs. τ as example are shown in Fig. 4 for oxedrine (ΔpK_a: 0.80) and isoxsuprine (ΔpK_a: 1.58).

Table 1 summarizes the data on S_0, K_Z and the macroscopic and microscopic ionization constants for the compounds studied.

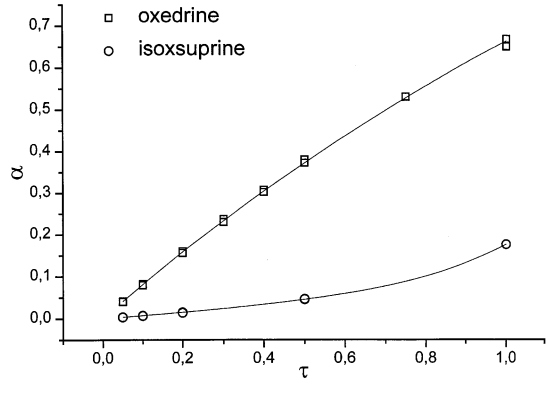


Fig. 4. Photometric titration of oxedrine ($\Delta pK_a = 0.80$; $K_Z = 4.71$) and isoxsuprine ($\Delta pK_a = 1.58$; $K_Z = 0.076$).

Table 1
Zwitterionic-, micro- and macroconstants

	S_0	K_Z	pK_1	pK_2	pk_I	pk_{II}	pk_{III}	pk_{IV}
Etilefrine	0.734	2.76	9.23	10.21	9.36	9.81	10.08	9.63
(Rodriguez et al., 1986)		1.09	8.87	9.96	9.05	9.36	9.78	9.87
(Martindale, 1993)			9.0	10.2				
Isoxsuprine	0.0705	0.076	8.19	9.77	9.34	8.22	8.62	9.74
(Martindale, 1993)			8.0	9.8				
Oxedrine	0.825	4.71	9.37	10.17	9.45	10.13	10.09	9.41
(Riegelmann et al., 1962)		2.51	9.29	10.24	9.44	9.84	10.09	9.69
(Pharmacopoea Nordica, 1963)			8.9	10.1				
Phenylephrine	0.630	1.70	8.86	10.10	9.06	9.29	9.90	9.67
(Riegelmann et al., 1962) 0.76		0.76	8.77	9.84	9.14	9.02	9.47	9.59
(Rodriguez et al., 1986)		3.39	9.01	10.23	9.13	9.68	10.11	9.58
(Martindale, 1993)			8.9	10.1				
Pholedrine	0.818	4.49	9.52	10.86	9.61	10.26	10.77	10.12
(Warren et al., 1971)			9.49	10.78				
(Müller and Holzapfel, 1983)			9.6	11.4				
(Martindale, 1993)			9.4					
Tyramine	0.738	2.82	9.48	10.66	9.61	10.06	10.53	10.08
(Riegelmann et al., 1962)		1.18	9.37	10.70	9.59	9.66	10.03	9.97
(Lewis, 1954)			9.53	10.78				
(Ogston, 1936)			9.3	10.9				

The macroscopic constants were calculated at $\tau = 0.5$ or at the isoelectrical point ($\tau = 1.0$) according to

$$pK_1 = pH - \log \frac{F_Z}{F_{Z^+}} \text{ and } pK_2 = pH - \log \frac{F_{Z^-}}{F_Z}$$

using Eqs. (10), (11) and (13) to estimate F_{Z^+}, F_Z and F_{Z^-}.

The calculated K_z values for etilefrine, oxedrine, phenylephrine and tyramine are quite different from values reported formerly (Table 1). The deviations are supposed to be caused by uncertainties using the Edsall method, as already mentioned above. For instance, the K_Z values for phenylephrine determined by Riegelmann et al. contrast markedly with those of Rodriguez et al., although the conditions of measurement (wavelength, ionic strength, concentration) were nearly identical. The plot of pM versus α for etilefrine given by Rodriguez et al. demonstrates the problem of scattering points. The method presented here provides the possibility of a first direct and immediate estimate of S_0 for the curve section from $\tau = 0.05$ to $\tau = 0.2$ independent of the exact estimation of the constants by nonlinear regression.

All the substances could be titrated at 0.01 M concentration. At this concentration, activity effects are usually small. For substances with a poor solubility, titration at 0.001 M concentration is principally possible provided that τ is carefully corrected for the concentration of hydroxyl ions.

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