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Ionization constants of sparingly soluble substances from aqueous titration data

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Summary

A method is described for deriving the ionization constants (pK_a values) of sparingly soluble organic substances from aqueous potentiometric titration data only. The method is equally applicable to mono- and bifunctional materials, and extends the ability to measure aqueous pK_a values. Computer-assisted data analysis provides the pK_a values with the standard errors.

The determination of ionization (or dissociation) constants is usually performed by potentiometric methods, preferably in aqueous media with their particular relevance to biological systems. Often, the poor solubility of the un-ionized species (of e.g. drugs) seems to restrict the use of this method, since precipitation during titration occurs. Alternatives may then be a determination of pK_a values based on (UV) spectrophotometric changes, or on the measurement of the solubility as a function of pH. The former method, of course, is not generally applicable, whereas the latter is a very tedious procedure (Levy and Rowland, 1971; Zimmermann, 1983, 1986). Another possibility is the use of mixed solvents (e.g. water/ethanol) in which the uncharged species does not precipitate, but, since it is doubtful whether results obtained with this method can be converted to the aqueous pK_a scale, Albert and Serjeant (1971) actually reject it (for reviews and methods see Cookson, 1974; Albert and Serjeant, 1971).

In an attempt to determine the pK_a values of some inhibitors of a cell membrane-bound protein that regulates the transport of nucleosides like adenosine, we encountered similar difficulties with respect to the solubilities of these compounds (for structures see Fig. 1). In the present work we present a potentiometric method with computer-assisted data analysis that enables the estimation of the pK_a values of these compounds in aqueous media, without the need to determine the solubilities of the materials.

The compounds (ca. 0.05 mEq in 0.1 M KCl) were potentiometrically titrated with 0.1 M KOH from a calibrated Radiometer ABU 11 micropipettor at $25.0 \pm 0.1°C$ under N_2. pH was measured with a Radiometer PHM 62 standard pH

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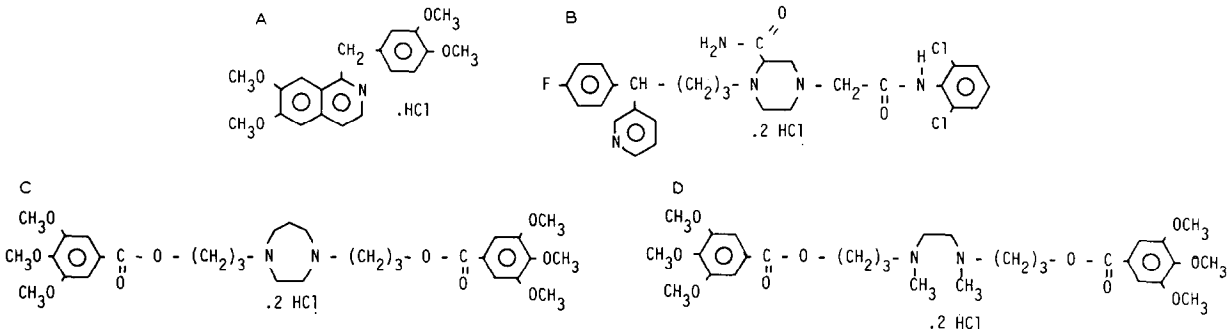

Fig. 1. Chemical structures of some inhibitors of the nucleoside transport protein. A: papaverine. B: soluflazine. C: dilazep. D: hexobendine.

meter. The volume of solution was 10–15 ml. The ionic strength was assumed to be that of 0.1 M KCl (activity coefficient 0.775). Data (values of pH and added KOH, 15–45 per titration) were analysed with the SCOGS (Stability Constants of Generalized Species) computer program (Sayce, 1968), modified as described earlier (IJzerman et al., 1984). SCOGS is used to analyse appropriate pH titration data to yield acid association constants (and hence pK_a values), stability constants of simple complexes, etc. Allowance is made for temperature, activity coefficients, volume of solution, normality of the titrant, added acid or base, following a non-linear least-squares minimization of the added volumes of alkali. All titrations were performed in triplicate.

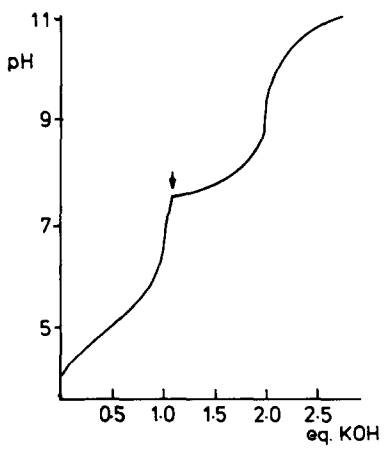

Fig. 2. Titration curve of dilazep 2HCl in 0.1 M KCl (arrow: start of precipitation).

In Fig. 2 a typical titration curve of dilazep is shown. In the first equivalence point the compound begins to precipitate (indicated by the arrow) giving rise to an aberration from 'normal' titration curves of materials remaining soluble throughout the titration. When the titration is continued, more precipitate is formed, until the second equivalence point is reached. The titration was stopped at pH 11, in order to avoid any anomaly of the glass electrode.

Since the equations in SCOGS used for the determination of the ionization constants of mono- and bifunctional acids and bases apply to soluble substances only, analysis of the titration curves with this computer program should be restricted

TABLE 1

Analysis of the titration curves of dilazep 2HCl

pK_1 ±S.E.M.	pK_2 ±S.E.M.	*n*	Comments
5.15±0.05	7.97±0.03	136	all data points
5.14±0.08	8.14±0.06	100	all data points before precipitation and after full neutralization
5.14±0.09	8.15±0.06	75	all data points before precipitation and in (second) pH jump
5.14±0.11	8.25±0.08	68	all data points before precipitation and one in (second) pH jump
n.d.	n.d.	65	all data points before precipitation

n, number of data points; n.d. = not determinable.

TABLE 2

Macroscopic ionization constants of nucleoside transport inhibitors (from 3 titrations)

	$pK_1 \pm$S.E.M.	$pK_2 \pm$S.E.M.
Papaverine HCl	6.06 ± 0.01	-
Dilazep 2HCl	5.14 ± 0.11	8.25 ± 0.08
Soluflazine 2HCl	5.09 ± 0.09	5.74 ± 0.08
Hexobendine 2HCl	4.52 ± 0.01	8.47 ± 0.01

to that part of the titration curve where precipitation has not yet occurred (see also Table 1). Using the data points from that section only, the estimated pK_a value ($\pm$S.E.M.) for the monofunctional acidic salt papaverine HCl, a nucleoside transport blocker, equals 6.06 ± 0.01 (see Table 2), which is in fair agreement with data from the literature: 5.9 (potentiometric titrations in mixed solvents, Evstratova et al., 1968), 5.95 (optical method, Kolthoff, 1925) and 6.40 (optical method, Biggs, 1954). After full neutralization the remaining papaverine base (both the soluble and precipitated fraction) is actually 'inert' to added alkali. Therefore, we considered it justified to include data points following the equivalence point in our analysis. Indeed, the pK_a value remains unchanged, again 6.06 ± 0.01.

The latter inclusion may be of relevance when dibasic acids, like dilazep 2HCl, hexobendine 2HCl and soluflazine 2HCl, all inhibitors of the nucleoside transport protein as well, are considered. In all cases realistic pK_a values were obtained from titration data covering the 'soluble' part of the curve and one datapoint in the second equivalence point as to mark full neutralization. The analysis of the titration behaviour of dilazep may serve as an illustration (Table 1).

One could argue that, due to e.g. absorption of OH^- ions to the precipitate, the pH of the suspension after full neutralization is lower than would be expected. As emerges from Table 1, this possible artifact can be circumvented by taking only one data point in the equivalence point into account (and leaving all further data points out of the analysis). Even the deliberate increase of the pH value of this single data point by 1 unit does not affect the estimated pK_a values (data not shown).

In Table 2 the pK_a values of papaverine, dilazep, soluflazine and hexobendine are gathered. From these data it appears that at physiological pH papaverine and soluflazine are predominantly present as uncharged species, whereas dilazep and hexobendine are monocations.

Finally it can be concluded that the method described in this communication enables the determination of the ionization constants of sparingly soluble mono- and bivalent substances in aqueous media, if precipitation does not occur immediately after the first addition of alkali. It avoids the use of various graphical extrapolation techniques (Streng and Zoglio, 1984) and provides estimates of the reliability of the pK_a values by the calculation of the S.E.M.

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