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Note

Isotachophoretic determination of the ionic mobilities and ionization constants of weak monoacidic bases by a simple computer-aided slope–intercept method

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In recent years isotachophoresis (ITP) has become an attractive analytical tool for the analysis of drugs, especially basic ones. Much attention has been paid to the optimization of the migration process by selecting appropriate electrolyte systems and operating conditions. The ionic mobilities, u, and ionization constants, pK_a, are the fundamental physical-chemical parameters of protolytes that determine (together with the parameters of the leading electrolyte) the effective electrophoretic mobility, $\bar{u}$, of analytes under given conditions and, consequently, the whole course of ITP (the formation and stability of zones, their order and separability). The pK_a values of the analytes are usually known, but this may not be the case if the compounds of interest are, *e.g.*, newly synthesized drugs. The data on ionic mobilities, especially for organic protolytes, are often lacking.

ITP data obtained under strictly defined conditions allow the estimation of the u and pK_a values of protolytes. In contrast to other instrumental methods, ITP has certain advantages: minimum consumption of the sample and no special demands on its purity. The u and pK_a obtained by ITP should be fully sufficient for the above optimization purposes, even though such values do not possess thermodynamic or absolute character.

For fully ionized compounds with known pK_a, u can easily be evaluated by ITP, *e.g.*, by measuring the potential gradient in the sample zone relative to a reference compound[1]. Several computer-aided ITP methods for simultaneous determination of u and pK_a have been devised, involving measurement of a series of effective mobilities (or relative step heights) for a sample in a number of leading electrolytes of various pH_L values. Some methods minimized the difference between experimental and simulated ITP data by refining the input u and pK_a values[2,3], or a set of experimental ITP data is processed by non-linear regression involving iterative calculations of the steady state[4]. Another original approach lies in the measurement of the zone conductivity of the compound in two different leading electrolytes and construction of the isolines of these conductivities (the so-called isoconductors) in an u–pK_a system of coordinates; the intersection of such lines yields the required values[5]. All these methods[2–5] involve relatively complicated calculations and they have been applied only in connection with anionic ITP.

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Direct determination of the ionic mobilities of weak bases including drugs ($pK_a < 6$) by cationic ITP under the conditions in which they are completely protonated is beset by obstacles[6] caused by the relatively high effective mobility of the hydrogen ion which cannot serve as a terminator for low-mobility base cations (it penetrates through the zones and disturbs ITP migration). In this paper a simple slope–intercept method is presented for determining u and pK_a for such bases by using a linear transformation of the $\bar{u}_{X,X} = f(pH_X)$ function (which is computed from only two experimental points). The applicability of this method is exemplified by evaluation of the parameters of weak organic bases (including drugs) in acetate leading electrolytes.

EXPERIMENTAL

Apparatus

A commercial ITP analyzer (URJVT, Spišská Nová Ves, Czechoslovakia) equipped with a single PTFE capillary column (200 mm × 0.3 mm), a conductivity detector and a 30-μl sampling valve was employed in all experiments.

Materials

All chemicals and drugs were of analytical or pharmacopoeial purity and were used without additional purification. Formic acid and acetic acid were purified by isothermal diffusion; twice-distilled water was used as a solvent.

Operating electrolyte systems

The leading electrolytes were prepared from standardized stock solutions of potassium hydroxide and acetic acid without any additives (Table I). The terminating electrolyte was always 0.01 *M* in formic acid; hence H^+ was the terminating ion[6].

Procedure

Aqueous solutions containing tetraethylammonium iodide and the base studied (or its hydrochloride for medazepam), each 0.2 m*M*, were injected. Chlordiazepoxide was initially dissolved in 0.1 *M* hydrochloric acid; its injected solution was 10 m*M* in hydrochloric acid. The analysis was performed at a driving current of 50 μA which was switched to 20 μA after 800 s; under such conditions, the time elapsed

TABLE I

ELECTROLYTE SYSTEMS

	Leading electrolyte		*Terminating electrolyte*
	System 1	*System 2*	
Cation	K^+	K^+	H^+
c_L (m*M*)	10	10	
Counter ion	Acetate	Acetate	Formate
$c_{R,L}$ (m*M*)	40	13	10
pH_L (calculated)	4.28	5.28	

between the start of analysis and the passage of the first zone boundary through the detector was approximately 15–17 min. All analyses were carried out in duplicate or triplicate.

Calculations

All mobilities are expressed in $10^9 \cdot m^2\ V^{-1}\ s^{-1}$. The effective mobilities, $\bar{u}_{X,X}$, of the bases were determined with the use of tetraethylammonium ($u = 33.8$) as the reference ion, according to

$$\bar{u}_{X,X} = 60.9/(h_{X,\,rel} + 0.80) \quad (1)$$

where $h_{X,\,rel}$ is the relative step height of a base X[7].

The evaluation of u_X and $pK_{a,X}$ for bases was performed by means of the IQ-151 32-kbyte microcomputer (ZPA Nový Bor, Czechoslovakia) and our own program written in BASIC 6. The program involves an iteration procedure for minimizing the RFQ function[8] and occupies 1.6 kbytes of the computer random access memory (RAM); a single computation takes about 90 s. Alternatively, the calculation can be accomplished by using a programmable pocket calculator, *e.g.*, Texas Instruments TI-59; a single calculation takes about 15 min.

RESULTS AND DISCUSSION

The effective mobility, $\bar{u}_{X,X}$, of a base X in its own zone is defined by

$$\bar{u}_{X,X} = [H]_X u_X/([H]_X + K_{a,X}) \quad (2)$$

which can be transformed to the equation of a straight line

$$\bar{u}_{X,X} = -K_{a,X}\bar{u}_{X,X}/[H]_X + u_X \quad (3)$$

with a slope of $-K_{a,X}$ and an intercept u_X. To evaluate the parameters of this straight line it is sufficient to determine the effective mobilities, $\bar{u}_{X,X}$, in two leading electrolytes having the same fixed concentration of the leading ion, c_L, but two different concentrations of the same counter ion, $c_{R,L}$ (and, consequently, differing in pH_L). The starting input data are: the ionic mobilities of the leading ion K^+ (76.1), of acetate as the counter ion (42.4) and of H^+ (362.4); $pK_{a,R}$ (4.76) and c_L as constants, $c_{R,L1}$, $c_{R,L2}$ and corresponding experimental values of $\bar{u}_{X,X1}$ and $\bar{u}_{X,X2}$ as variables. Initially the starting values of u_X and $pK_{a,X}$, *i.e.*, $(u_X)_0$ and $(pK_{a,X})_0$ are calculated by using the original $[H]_L$ values. Thereafter, $(u_X)_0$ and $(pK_{a,X})_0$ are used to calculate $[H]_X$ by the RFQ method (a subroutine) and the whole cycle is repeated with the refined $(u_X)_i$ and $(pK_{a,X})_i$ values until the difference between $(pK_{a,X})_{i+1}$ and $(pK_{a,X})_i$ is less than an arbitrary constant, here 0.01 (*cf.*, Fig. 1). As shown in Fig. 2, the simulated function $\bar{u}_{X,X} = f(\bar{u}_{X,X}/[H]_X)$ is not rectilinear after the first approximation, when $[H]_L$ is substituted for $[H]_X$, since the real $[H]_X$ in the zones of the bases is different (lower in this instance). Therefore a straight line connecting the two experimental points would give erroneous values of u_X and $pK_{a,X}$. Iterative approximations of $[H]_X$ make

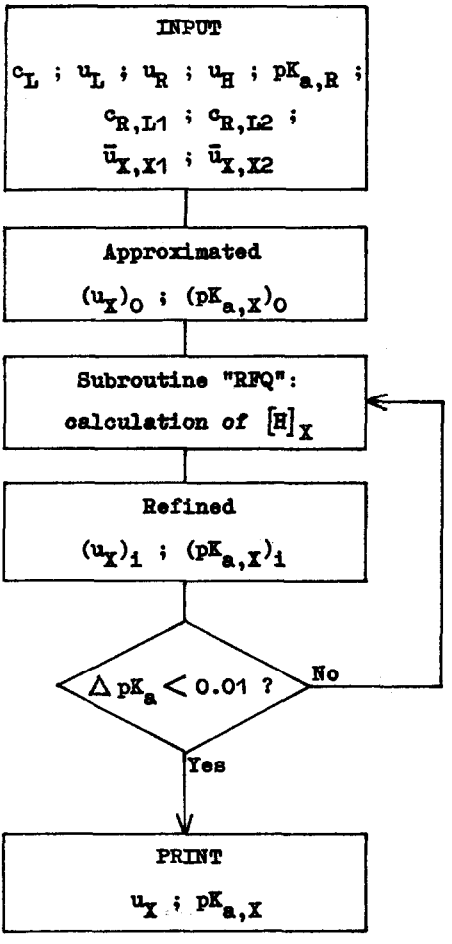

Fig. 1. Schematic flow chart of the program for computing u and pK_a for weak bases from the pairs of experimental values of the effective mobilities, $\bar{u}_{X,X1}$ and $\bar{u}_{X,X2}$.

the function 3 straighter and the calculated u_X and $pK_{a,X}$ converge to the true end values.

The parameters computed for some bases are presented in Table II. The effect of temperature and ionic strength are neglected; it may be presumed that they are compensated to a certain extent by the relative measurement of the mobilities (*cf.*, ref. 5). Hence it seems that the errors in the measurement of the effective mobilities will be the main source of uncertainty in the results. As seen in Table II, the computed $pK_{a,X}$ values are in acceptable agreement with earlier published data for most of the bases. The rather large difference between the computed and literature $pK_{a,X}$ values for 2-methylpyridine (and also the poor reproducibility of the computed result) is connected most probably with difficulties in the determination of the effective mobilities of this base since the values are very close to that of the reference ion. The cause of the large difference in the case of medazepam is not clear; the ITP behaviour

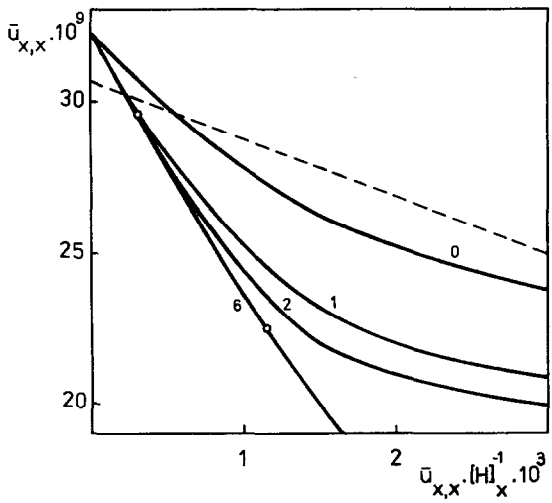

Fig. 2. Convergence pathway of the computation: simulated function $\bar{u}_{x,x} = f(\bar{u}_{x,x}/[H]_x)$ for 1,10-phenanthroline (u_x = 32.3, $pK_{a,x}$ = 5.07); the two experimental points are shown. Solid curves: 0, first approximation ($pH_x = pH_L$); 1, 2, 6, number of the iteration cycle. The dashed line connects the experimental points (not seen in the diagram) in the first approximation.

of medazepam indicates that it is a substantially stronger base than, *e.g.*, chlordiazepoxide (medazepam exhibits a considerably smaller difference in the effective mobilities determined in the two electrolyte systems employed).

The limitations of the method are first the need for sufficient buffering capacity

TABLE II

PARAMETERS FOR BASES EVALUATED FROM ISOTACHOPHORETIC DATA

$pK_{a,x}$ = ionization constant; u_x = ionic mobility; A, values computed in this study as means of two independent ITP determinations with standard deviations in parentheses; B, literature values (from ref. 11 except where stated otherwise).

Base	$pK_{a,x}$		$u_x \cdot 10^9$ ($m^2\ V^{-1}\ s^{-1}$)	
	A	*B*	*A*	*B*
Aniline	4.67 (0.01)	4.60	38.7 (0.3)	
4-Chloroaniline	4.17 (0.01)	3.98	31.7 (0.4)	
Pyridine	5.13 (0.01)	5.20	51.1 (0.2)	
2-Methylpyridine	6.42 (0.20)	5.97	41.5 (0.4)	
1,10-Phenanthroline	5.07 (0.01)	4.96	32.3 (0.3)	
Hexamethylenetetramine	4.90 (0.01)	5.18	36.8 (0.1)	
6-Aminohexanoic acid	4.44 (0.01)	4.37*	30.0 (0.4)	28.8*
Histidine	6.03 (0.02)	6.04*	29.2 (0.1)	29.6*
4-Aminoantipyrine	4.41 (0.04)	4.18	25.0 (0.5)	
Aminophenazone	5.18 (0.01)	4.94	24.2 (0.1)	
Chlordiazepoxide	4.89 (0.02)	4.6**	20.8 (0.1)	
Medazepam	5.61 (0.01)	4.4**	23.1 (0.2)	

* Ref. 9.
** Ref. 10.

of the counter-ion system in the zones of the bases, and secondly a reliably ascertainable difference between the two experimental values of the effective mobilities of the compound studied. The impossibility of termination at very low effective mobilities is another methodological limitation. Apparently the acetate system can be used to examine bases with a practical range of basicities given approximately by $pK_{a,X} = pK_{a,R} \pm^{1.3}_{0.4}$. The calculation is fairly sensitive, especially under the extreme limiting conditions, to the reliability of the experimental $\bar{u}_{X,X}$ data. For example, satisfactory convergence is not attained for β-alanine ($pK_a = 3.55$) when calculating its pK_a and u from the actual values of the effective mobilities ($\bar{u}_{X,X1} = 20.0$, $\bar{u}_{X,X2} = 11.8$); in this instance, $pH_X < 4$ and the zone exhibits distinct inversion of mobility with respect to terminating H^+. Analogous $\bar{u}_{X,X}$ values of 17.7 and 10.5 (simulated by using the RFQ function) do not differ greatly from the original ones, but here the calculation converges to the expected result.

In spite of the discussed limitations, the method proposed would be of use, because of its simplicity, to laboratories possessing only modest computation facilities. The results are sufficient for the purposes of prediction and optimization of ITP analysis. The study of compounds exhibiting basicities outside the above recommended range would be possible by using another counter-ion system provided that the effective mobilities are not affected by the base–counter ion interactions and that the free base does not precipitate under the given conditions.

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