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PHASE-HETEROGENEOUS ZONES IN CAPILLARY ISOTACHOPHORESIS OF LOW-SOLUBILITY BASES

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SUMMARY

The effective mobility of a low-mobility base is regulated by a coupled protolytic and precipitation equilibrium. Assuming that the latter is rapid enough, a procedure for calculating parameters of the isotachophoretic phase-heterogeneous zone of a base in the steady state is suggested. The effect of the leading ion concentration on the effective mobilities as well as the correctness of migration, separability and stability of zones in slightly alkaline electrolyte systems are considered theoretically and experimentally verified for a group of basic drugs. The relationships ascertained serve as a basis for satisfactory separation of compounds of very similar basicities and ionic mobilities but having different solubilities. This is demonstrated by the analyses of two model binary mixtures involving amitriptyline and nortriptyline or moxastine and embramine. Practical analyses, though partly limited by the fact that the heterogeneous zones are analytically not quite stable (a minute zone "bleeding" occurs) and therefore the detection limit is adversely affected, are possible.

INTRODUCTION

In the course of experiments concerning the isotachophoretic determination of ionization constants and ionic mobilities of weak monoacidic bases, we have observed that some bases behave anomalously. This applied namely to bases involving an aliphatic amino group in a side chain attached to a cyclic structure ($pK_{a,B} \approx 9$) under the conditions of isotachophoresis (ITP) with slightly alkaline leading electrolytes ($pH_L \approx 8$). The visual evaluation of the recorded steps showed that they are regular, thus indicating correct ITP migration, but the steps were unexpectedly high which implied that the effective mobilities are lower than would follow from the basicities of these compounds and the acidity conditions in the zones. Moreover, the effective mobility values were apparently affected by the concentration of the leading ion to an extent which distinctly exceeded the effect of ionic strength. Some pairs of bases having very similar ionization constants and ionic mobilities exhibited remarkable differences in effective mobilities. This effect was clearly not caused by a specific interaction with the counter ion (the results were practically the same when using borate, diethylbarbiturate or phosphate buffers as leading electrolytes), but it seemed to correlate to some extent with the solubility of the free base in water.

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Generally, the condition of solubility of all forms of a substance is considered necessary for ITP analysis. Assuming that there exists a sufficiently rapid heterogeneous precipitation equilibrium between the dissolved and undissolved fraction of the free base in its suspension, it may be possible to accept an hypothesis of regular ITP migration of such a heterogeneous zone. (The existence of "micellar" zones was described earlier[1].) According to the acidity conditions in the zone, the adapted "concentration"[a] is distributed as the free undissolved and dissolved base and its protonated form:

$$\bar{c}_B = [B]_s + [B]_l + [HB^+]_l$$

In this paper we have attempted to verify the above hypothesis. If the correct ITP migration of the heterogeneous zones and their stability were confirmed, it would be possible to use for the separation of bases, beside the commonly employed factors (differences in ionic mobilities, ionization constants, interactions with the counter ion and solvation), also the differences in the solubilities of the free bases.

THEORETICAL

When neglecting the effect of the ionic strength (in ITP it usually does not exceed 10 mmol l^{-1}) on the protolytic equilibrium, the molar fraction of the protonated form of a monoacidic fairly soluble base, x_{HB}, is affected only by the pH and does not depend on the concentration

$$x_{HB} = [H^+]/([H^+] + K_{a,B}) \qquad (1)$$

see curve 1 in Fig. 1.

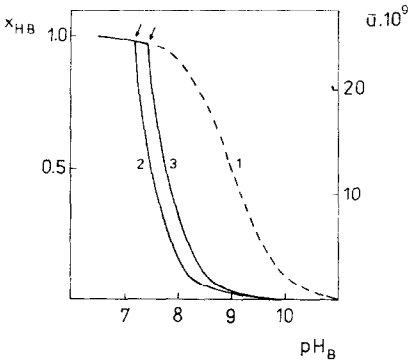

Fig. 1. Molar fraction of the protonated form, x_{HB}, and effective mobility, $\bar{u}$, of bases ($pK_{a,B}$ = 9.0; u_{HB} = $25 \cdot 10^{-9}$ m^2 V^{-1} s^{-1}) as a function of the base zone acidity, pH_B. 1, Readily soluble base; 2,3, for explanation see the text.

[a] The term "concentration" here means the overall analytical concentration of all forms of a base including the undissolved fraction.

The overall solubility, S (mol l^{-1}), of a monoacidic sparingly soluble base depends on the pH of the solution according to the relationship[2]

$$S = S_0(1 + [H^+]/K_{a,B}) \tag{2}$$

where S_0 is the solubility of the free (non-protonated) base; the physical value of S is limited only by the solubility of the salt of the base. In eqn. 2 the term $S_0[H^+]/K_{a,B}$ represents the concentration of the protonated form HB^+ and hence its molar fraction depends on the pH and S_0 as well as on its "concentration", $\bar{c}_B$ (see curves 2 and 3 in Fig. 1)

$$x_{HB} = [HB^+]/\bar{c}_B = S_0[H^+]/K_{a,B}\bar{c}_B \tag{3}$$

where $\bar{c}_B$ is the adapted "concentration" in the ITP zone.

The effective mobilities, $\bar{u}_B$, of a monoacidic base are directly proportional to the molar fraction of the protonated form HB^+

$$\bar{u}_B = u_{HB}x_{HB} \tag{4}$$

(u_{HB} is the ionic mobility of the base) and their values can be obtained by combining eqns. 1 and 4 or 3 and 4 for fairly or sparingly soluble bases respectively. Therefore the corresponding plots of $\bar{u}_B$ *vs.* pH are shaped in an analogous manner (Fig. 1).

In plots 2 and 3 characteristic breaks appear (indicated by arrows). At pH values lower than correspond to these breaks the overall solubility of the base, S, is higher than the adapted "concentration", $\bar{c}_B$, and the base behaves as a well soluble one; its effective mobility is regulated only by the zone acidity. On the contrary, at higher pH the adapted "concentration" is higher than the solubility, S, and the effective mobility of the base is determined by the pH as well as by the solubility of the free base, S_0, and its adapted "concentration", $\bar{c}_B$. Thanks to selected model parameters, the curves 2 and 3 in Fig. 1 have a double meaning:

(a) For a pair of equally strong and mobile bases in borate operational systems with the concentration of the leading ion K^+, c_L = 10 mmol l^{-1}, curve 2 is valid for a base having a solubility of S_0 = 0.1 mmol l^{-1} and curve 3 for a base with S_0 = 0.2 mmol l^{-1}. The course of the curves indicates possible separability based on the differences in S_0.

(b) For a base having solubility S_0 = 0.1 mmol l^{-1}, curve 2 is valid in borate operational systems with c_L = 10 mmol l^{-1} (according to the regulatory function $\bar{c}_B$ = 6.2 mmol l^{-1}); in the same diluted systems with c_L = 5 mmol l^{-1} ($\bar{c}_B$ = 3.1 mmol l^{-1}), curve 3 is valid. In this instance the course of the mobility curves shows the differences in effective mobility at the same pH_L caused by the change of $\bar{c}_B$ adapted to different c_L.

However, under real conditions of ITP with a particular counter ion system, the practically important pH interval in the zone of base is substantially narrower than that depicted in Fig. 1; it is hardly wider than one pH unit (*cf.*, Fig. 6).

On the basis of the above assumptions, the algorithm for the calculation of the zone parameters of a low-solubility monoacidic base in the steady state by the RFQ method[3] was modified. The necessary input data are: the ionic mobilities of H^+, u_H,

leading ion, u_L, counter ion, u_R, and the base, u_{HB}; ionization constants of the counter ion, $K_{a,R}$, and the base, $K_{a,B}$; concentration of the leading ion, c_L, and counter ion in the leading zone, $c_{R,L}$; solubility of the free base, S_0. The algorithm neglects the concentrations of H^+ and OH^- in the summing expressions and it involves the following steps of the iteration procedure:

calculation of

pH_L: $$[H^+]_L = K_{a,R}(c_{R,L} - c_L)/c_L$$

adapted concentration according to the regulatory function:

$$\bar{c}_B = c_L u_{HB}(u_{HB} + u_R)^{-1}(u_L + u_R)u_L^{-1}$$

solubility of the base:

$$S = S_0(1 + [H^+]_B/K_{a,B})$$

decision whether $S \geqslant \bar{c}_B$:

(a) If the answer is "yes" then the effective mobility of the base is:

$$\bar{u}_B = u_{HB}[H^+]_B([H^+]_B + K_{a,B})^{-1} \tag{5}$$

(b) If $S < \bar{c}_B$ then:

$$\bar{u}_B = u_{HB}S_0[H^+]_B/K_{a,B}\bar{c}_B \tag{6}$$

The next steps, *i.e.*, the calculation of the effective mobility of the counter ion in the zones of the leading ion and the base, concentration of the counter ion in the zone of base and finally the evaluation of the RFQ function are the same as in the original procedure[3]. In the first iteration cycle the pH_L is substituted for pH_B and thereafter the pH_B is successively approximated to minimize the RFQ function until the correct value of pH_B and other parameters of the zone of base in the steady state are attained. An appropriate program was written in BASIC for a microcomputer. The program was used for computing simulated effective mobilities presented in the following parts of this paper.

A space diagram (Fig. 2) illustrates the dependence of the effective mobilities of bases having the ionic mobility, $u_{HB} = 30^a$ on the base strength and solubility for ITP performed in the operational system E (see Table I). Bases with parameters located in the plane ABC behave as readily soluble ones. Otherwise the mobilities decrease with decreasing strength and solubility of the base. The existence of the zone of base is theoretically limited by the mobility of the terminating ion (indicated by the arrow). Diagrams for bases having ionic mobility values higher or lower than 30 are correspondingly shifted up or down along the vertical axis but their shape is analogous to that shown in Fig. 2.

[a] All mobilities are expressed in $10^9 \cdot m^2\ V^{-1}\ s^{-1}$ in this paper.

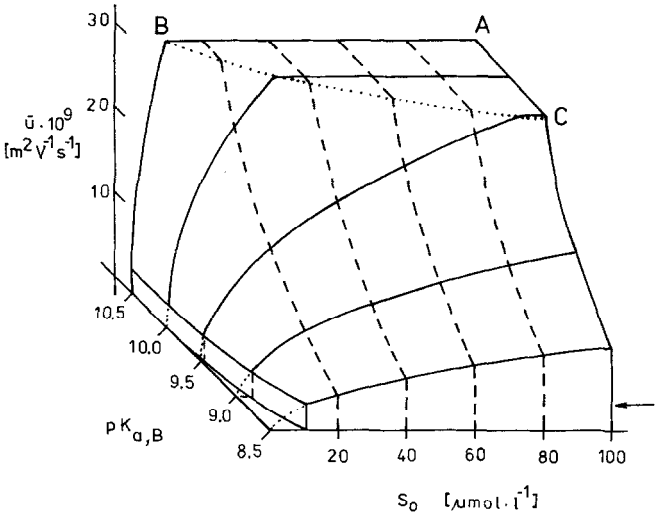

Fig. 2. Space diagram of the dependence of the effective mobility $\bar{u}$, of low-solubility bases with ionic mobility $u_{HB} = 30 \cdot 10^{-9}$ m^2 V^{-1} s^{-1} on their strength, p$K_{a,B}$, and solubility, S_0, in operational system E.

TABLE I

OPERATIONAL ELECTROLYTE SYSTEMS

MES = 2-Morpholinoethane sulphonic acid; EACA = 6-aminohexanoic acid.

System	*Leading electrolyte, L*			*Terminating electrolyte, c (mmol l^{-1})*
	Cation, c_L (mmol l^{-1})	*Counter ion, c_R (mmol l^{-1})*	*pH (exptl.)*	
A, ref. 8	K^+, 10	Acetate, 20	4.74	Acetic acid, 20
B	K^+, 10	Borate, 110	8.17	L-Histidine, 10
C	K^+, 10	Borate, 60	8.43	L-Histidine, 10
D	K^+, 10	MES, 15	6.43	EACA, 10
E	As in B but diluted 1:1 (c_L = 5)		8.13	L-Histidine, 10
F, ref. 5	K^+, 5	2-Pyridine carboxylate, 10	5.35	Formic acid, 10
G	As system E; L = Na^+		8.12	L-Histidine, 10

EXPERIMENTAL

Apparatus

The ITP experiments were carried out with the use of a CS isotachophoretic analyser (URVJT, Spišská Nová Ves, Czechoslovakia) equipped with a non-thermostatted PTFE capillary column (200 mm × 0.3 mm), a conductivity detector and a 30-μl sampling valve.

Materials

Tricyclic antidepressants: amitriptyline hydrochloride (AT); nortriptyline hydrochloride (NT) (partially N-demethylated AT). Antihistamines: moxastine hydrochloride (MX); embramine hydrochloride (EM) (brominated MX). Local anaesthetics: tetracaine hydrochloride (TC) (a basic ester); heptacaine hydrochloride (HC) (a basic carbamate). The above basic drugs, obtained from the State Institute for Drug Control, Prague, were of pharmacopoeial purity. Aqueous stock solutions containing 1 mmol l^{-1} of a drug were appropriately diluted before the ITP separation. All other chemicals were of the highest attainable purity.

The effective mobilities of bases were determined with the use of tetraethylammonium (u = 33.8, ref. 4) as the reference ion, according to

$$\bar{u} \cdot 10^9 = 60.9/(h_{rel} + 0.8) \quad m^2\ V^{-1}\ s^{-1}$$

where h_{rel} is the relative step height of a base[5].

The simulated steady state parameters were computed by using an IQ 151 microcomputer (ZPA, Nový Bor, Czechoslovakia). The earlier published mobility and ionization constants (except for the bases studied) used in the simulations were: u_H = 362.5; u_{Na} = 51.9; u_K = 76.1 (all ref. 5); borate, u = 33.0 (ref. 6), pK_a = 9.14; MES, u = 28.0, pK_a = 6.10; L-histidine, u = 29.6, pK_a = 6.04; EACA, u = 28.8, pK_a = 4.37 (all ref. 7).

RESULTS AND DISCUSSION

Some parameters of the basic drugs studied are presented in Table II.

The pK_a values in Table II indicate that in slightly acidic operational systems A or F (Table I) all the bases are fully ionized and hence the effective mobilities determined are their ionic mobilities; $\bar{u}_B = u_{HB}$.

TABLE II

PHYSICAL-CHEMICAL CONSTANTS OF THE COMPOUNDS STUDIED

Unless stated otherwise our results obtained by turbidimetry according to ref. 2.

Compound	*Molar mass of base (g/mol)*	*$pK_{a,B}$*	*$S_0 \cdot 10^4$ (mol l^{-1})*
Amitriptyline, AT	277.3	9.4[a]	0.46
Nortriptyline, NT	263.4	9.7[b]	1.7
Moxastine, MX	269.3	9.5	4.6
Embramine, EM	348.2	9.3	0.57
Tetracaine, TC	264.3	8.2[c]	12
Heptacaine, HC	362.5	7.6[d]	0.55[e]

[a] Ref. 2.
[b] Ref. 9.
[c] Ref. 10.
[d] Ref. 11.
[e] Ref. 12.

Effect of the leading ion concentration on the effective mobilities

In this series of experiments the operational systems A to D (Table I) were used and the leading electrolytes were diluted to adjust the leading ion concentration to c_L = 4, 6, 8, 10 m*M*. The systems B–D were selected according to the strength and the solubility of the bases: system B for NT, AT, MX and EM; C for TC; D for HC. The working conditions of ITP were adjusted to suit the individual electrolyte dilutions (see Table III). These conditions were found empirically for system B with regard to the increase in voltage and then were kept constant also for systems A, C and D. At very low mobilities (in concentrated leading electrolytes) a mixed zone of the base and the terminator was sometimes formed thus making the evaluation of the base step height impossible; in such cases the base was used directly as the terminating electrolyte (10 m*M*).

The results are presented in Fig. 3. All the compounds exhibit an increase in effective mobility in system A at the lowest dilution, c_L = 4 m*M*, which is probably connected with the decreased ionic strength. Since one can assume an analogous effect when working with systems B–D, the observed individual mobility values were substituted for ionic mobilities, u_{HB}, in the simulation calculations.

The effective mobilities of compounds NT and MX in system B differ negligibly from the results obtained in system A. NT and MX are fairly strong and relatively soluble bases (Table II). Their solubility, *S*, at pH_B (*ca.* 7.9) is much higher than is the adapted concentration in the zone, $\bar{c}_B$ (*cf.*, NT in Fig. 4), and therefore they behave as readily soluble bases.

Compounds AT and EM are in fact as strong bases as NT and MX but they are by one order less soluble. In system B they form zones of pH_B 7.6 to 7.8 and under such conditions their solubility is lower than the adapted "concentration", $\bar{c}_B$, in the whole concentration range studied (Fig. 4). The effective mobilities strongly depend on the concentration of the leading ion. The experimental values exhibit a distinctly parallel course with respect to the simulated function. The positive shift might be caused by an inaccurate value of S_0 or by its temperature dependence in the non-thermostatted apparatus; as follows from eqn. 6, the calculation of the effective mobility is influenced by the value of S_0. The compound HC is a poorly soluble and relatively weak base and therefore it was analysed in system D. Also in this instance the solubility at pH_B (5.8 to 6.0) is lower than the adapted "concentration" and the ITP behaviour of HC is similar to that of AT and EM.

TABLE III

WORKING CONDITIONS OF ITP IN DILUTED SYSTEMS

t' = Time of current switching; t_L = approximate time elapsed before the passage of the first zone boundary through the detector.

c_L (m*M*)	*Separation current* (μ*A*)	t' (min)	*Detection current* (μ*A*)	t_L (min)
4	10	30	7.5	33
6	15	30	10	35
8	25	20	15	27
10	30	20	20	26

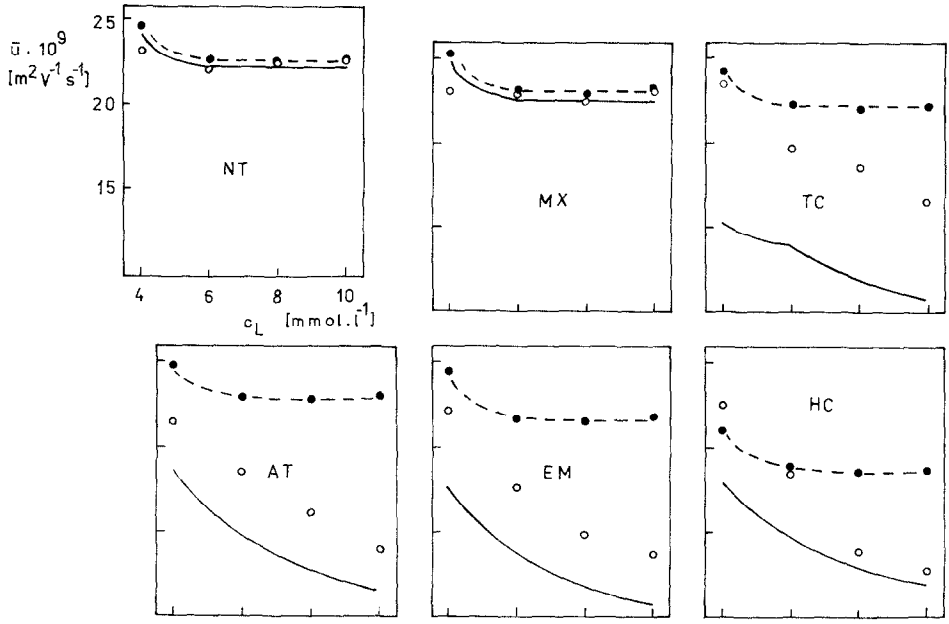

Fig. 3. Dependence of effective mobilities, $\bar{u}$, of bases (see Table II) on the concentration of the leading ion, c_L. Experimental data: ⅛, in system A; ⓐ, in systems B (for NT, AT, MX, EM), C (for TC) and D (for HC); solid line, simulated $\bar{u}$ *vs.* c_L function.

Compound TC, thanks to its basicity and solubility, exhibits a characteristic break in the simulated function $\bar{u}_B$ *vs.* c_L in system C; this is caused by the relationship between the solubility and adapted "concentration" (*cf.*, Fig. 4): at $c_L = 4$ mM, $S > \bar{c}_B$; at $c_L = 8$ or 10 mM, $S < \bar{c}_B$. The experimental effective mobility values roughly follow this trend.

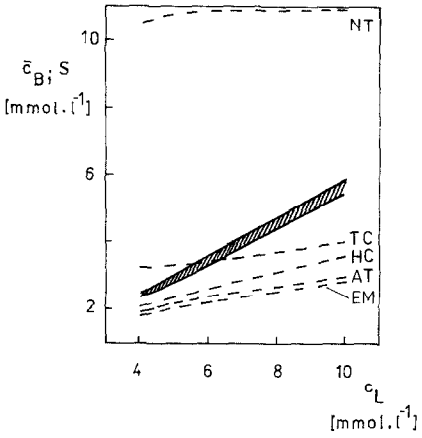

Fig. 4. Dependence of calculated adapted "concentrations", $\bar{c}_B$, of bases and their solubilities, S, in their own zones (in systems B–D) on the concentration of the leading ion, c_L. ———, c_B (very similar values); – – –, S.

TABLE IV

CALCULATED PARAMETERS: pH, SOLUBILITY AND MOBILITY IN THE BASE AND TERMINATOR ZONES

Compound	*System*	pH_T	$S_{B,T}$ *(mM)*	$\bar{u}_{T,T}$	$\bar{u}_{B,T}$	pH_B[a]	$\bar{u}_{T,B}$[a]	$\bar{u}_{B,B}$[a]
AT	B	6.97	12.4	3.1	22.8	7.60	0.8	11.5
EM	B	6.97	12.2	3.1	21.6	7.58	0.8	10.7
TC	C	7.11	17.0	2.3	20.7	7.86	0.4	10.8
HC	D	5.06	19.1	4.8	18.6	5.79	1.1	12.0

[a] Values calculated for c_L = 10 m*M*.

Correctness of the ITP migration

The efficiency of the self-sharpening effect at the leading ion, K^+–base zone boundary lies outside this discussion. For evaluating the situation at the base–terminator boundary it is first necessary to consider the solubility of bases in the terminator zone; the pH_T is practically independent of c_L. (The bases NT and MX which are soluble in their own zones were not considered.)

The solubilities of the bases in the terminator, $S_{B,T}$, are more than twice the values of their adapted "concentrations", $\bar{c}_B$ (*cf.*, Fig. 4); hence the relation 5 holds for the calculation of $\bar{u}_{B,T}$. By comparing the calculated mobilities, in all cases considered the following inequalities are valid[13]:

$$\bar{u}_{B,T} > \bar{u}_{T,T} \quad \text{and} \quad \bar{u}_{T,B} < \bar{u}_{B,B}$$

Therefore the base–terminator boundary exhibits a self-sharpening effect in both directions and the migration of the individual bases is correct.

In the set of compounds studied we have concentrated on the separation of the pair AT–NT and MX–EM. These are chemically closely related substances; their separation would be of practical importance for pharmacokinetic studies, purity control, etc. Considering the sensitivity requirements, time consumption and the determined effective mobilities, the operational system E (Table I) was chosen: the separation current of 30 μA was decreased to 10 μA after 500 s; the terminating histidine can be replaced by β-picoline which has similar parameters.

To substantiate theoretically the separability of the above pairs of bases in the system E, the effective mobilities of bases in their own and neighbouring zones were compared[13]. The mobilities of NT and MX were calculated according to eqn. 5 and those of AT and EM according to eqn. 6. For the zones of bases the following inequalities hold:

zone NT (pH_B 7.91), $\bar{u}_{AT,NT}$ (11.1) < $\bar{u}_{NT,NT}$ (22.7)
zone AT (pH_B 7.75), $\bar{u}_{NT,AT}$ (22.8) > $\bar{u}_{AT,AT}$ (16.4)
zone MX (pH_B 7.91), $\bar{u}_{EM,MX}$ (10.6) < $\bar{u}_{MX,MX}$ (23.0)
zone EM (pH_B 7.73), $\bar{u}_{MX,EM}$ (23.1) > $\bar{u}_{EM,EM}$ (15.5)

It is clear that the self-sharpening effect of the boundaries is established in both directions for these pairs of bases and that the migration is again correct. (The

differences in the effective mobilities are distinct and the above trend is valid in the whole range of c_L investigated, *i.e.*, also for other dilutions of the system B.)

Stability of zones and the zone existence diagram

Calibration graphs for some of the bases were examined as the criterion of the zone stability. Fig. 5 depicts the calibration graphs for drugs in the concentration range 20–100 μM for ITP in both slightly acidic (A or F) and alkaline (E) operational systems. (The leading electrolyte of system A was diluted 1:1, c_L = 5 mM, for this purpose.)

The corresponding linear regression equations and the correlation coefficients, k_r, are summarized in Table V. The results obtained in system E indicate that the zones of the more soluble bases (NT, MX) are quite stable; the intercept is negligibly small. The calibration graphs for sparingly soluble bases (AT, EM) are also perfectly rectilinear but the intercept has a negative value. This means that during the migration of the zone in the capillary from the injector valve to the detector a constant amount of the base is lost. This loss is independent of the amount injected and hence it represents the systematic error of the ITP determination. Under the given experimental conditions, the loss amounts to 0.2 nmol of AT or 0.4 nmol of EM. The effect of the separation current (25, 20, 10 μA) (and consequently the analysis time) was also examined but no significant dependence was observed. That is why it can be concluded[14] that the loss is a certain form of zone bleeding and not tailing. Some kind of sorption of the free base in the capillary must be assumed since in successive blank experiments (injections of the terminating electrolyte instead of the sample) the ITP records revealed successively decreasing amounts of the base residue which may interfere with further analyses. For that reason the analytical procedure was modified

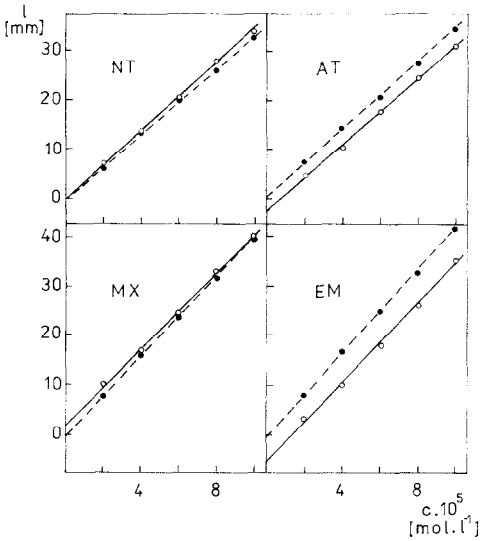

Fig. 5. Calibration graphs, l (step lengths, mm) *vs.* c (concentration of base, 10^{-5} mol l^{-1}). Full dots and dashed lines, points and regression lines in systems F (for AT, NT) and A, 1:1 (for MX, EM); empty dots and solid lines, the same in system E.

TABLE V

LINEAR REGRESSION EQUATIONS OF CALIBRATION GRAPHS

Compound	*System*	*l (mm)* =	k_r
NT	F	$3.33\ c \cdot 10^5 - 0.5$	0.9999
AT		$3.34\ c \cdot 10^5 + 0.7$	0.9999
NT	E	$3.39\ c \cdot 10^5 + 0.2$	0.9993
AT		$3.30\ c \cdot 10^5 - 2.2$	0.9991
MX	A (1:1)	$4.02\ c \cdot 10^5 - 0.5$	0.9999
EM		$4.17\ c \cdot 10^5 - 0.4$	0.9999
MX	E	$3.83\ c \cdot 10^5 + 1.8$	0.9997
EM		$3.98\ c \cdot 10^5 - 5.4$	0.9987

in such a way that after each separation the capillary was rinsed with a small volume (*ca.* 0.5 ml) of 0.1 *M* acetic acid and thereafter it was filled with fresh leading electrolyte. In this case the ITP record of a subsequent blank experiment was regular. The above procedure was employed in all analyses performed with alkaline operational systems. Finally it can be concluded that the migration of the heterogeneous zones of sparingly soluble bases is correct but the zones are not absolutely stable. In principle, quantitative analyses are possible but the detection limits are influenced negatively.

The possibilities of the ITP migration of low-solubility bases in system E are shown in the zone existence diagram[13], Fig. 6. The point T corresponds to the terminating histidine. The area of the diagram is limited on the right hand side by the line AB of strong soluble bases ($\bar{u}_B = u_{HB}$) with ionic mobility, $u_{HB} \geqslant \bar{u}_T$ (segment TA indicates possible inversion of mobilities of weak soluble bases fulfilling the limiting condition $\bar{u}_{B,T} = \bar{u}_{T,T}$). As it is necessary to consider three variable parameters of the bases (u_{HB}, $pK_{a,B}$, S_0), the vertical lines of the internal system of coordinates (that normally mark the $pK_{a,B}$) are individual for parametrically introduced values of S_0.

Separations

System E was used for a model separation of pairs of drugs under the following working regime: the separation current of 30 μA was switched to 10 μA after 500 s; the time of passage of the first boundary through the detector, t_L, was 20–21 min. The recorder chart speed was increased from 0.05 to 0.5 mm s^{-1} just after the passage of the zone of impurities.

In acidic operational systems, NT and AT have the same mobility and their separation is impossible. Fig. 7a illustrates their successful separation accomplished in system E. The detection limit of NT in the mixture is about 20 pmol and this value approaches the real limit of the ITP method; for the reasons discussed above, the detection limit of AT is an order higher and amounts to 300 pmol.

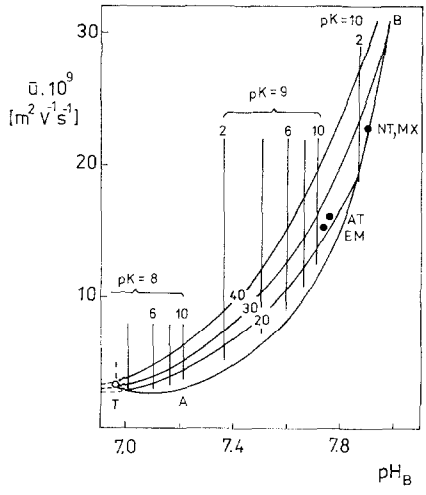

Fig. 6. The zone existence diagram in system E. The numbers 20, 30, 40 refer to the ionic mobilities of the bases, u_{HB} (10^{-9} m^2 V^{-1} s^{-1}); 2, 6, 10 are the solubilities of the bases, S_0 (10^{-5} mol l^{-1}). For a more detailed explanation see the text.

MX and EM exhibit slightly different mobilities in acidic systems but the selectivity, $(\bar{u}_{MX} - \bar{u}_{EM})/\bar{u}_{EM} = 0.05$, is poor; in system A both bases form a transient mixed zone (Fig. 7b) though the injected quantity is small (0.5 nmol of either base). On the other hand, their separation in system E (Fig. 7c) is perfect (practical selectivity *ca.*

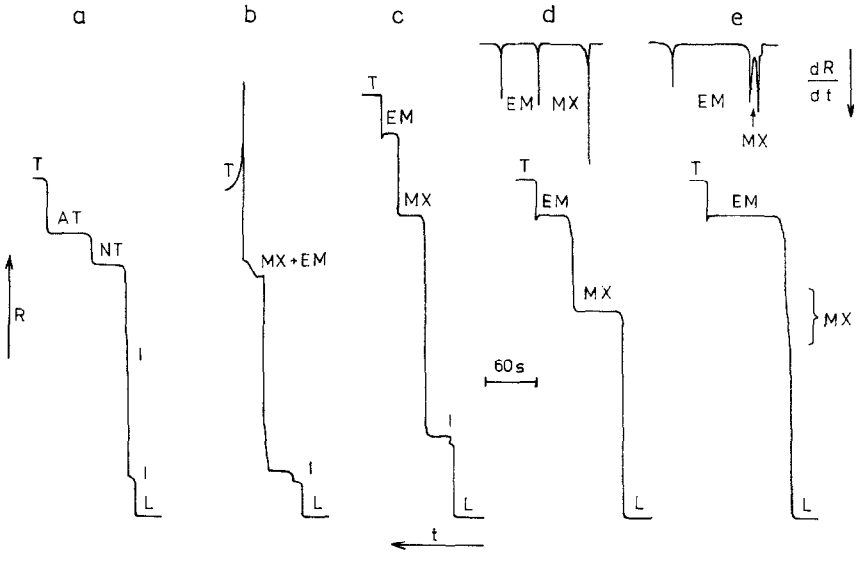

Fig. 7. Analyses of mixtures. Detection current: 10 μA. Chart speed: 0.5 mm s^{-1}. I = Impurities in the operational system. (a) 2.4 nmol AT + 1.5 nmol NT, system E; (b) 0.5 nmol MX + 0.5 nmol EM, system A; (c) 0.9 nmol MX + 0.9 nmol EM, system E; (d) 1.5 nmol MX + 1.5 nmol EM, system G; (e) 30 pmol MX + 3 nmol EM, system G.

0.2). The interfering effect of impurities (Na^+ and other metal ions which cannot easily be removed from the electrolyte system) can be overcome by modifying system E, by using Na^+ as the leading ion (system G), without any negative effect on the separation efficiency (see Fig. 7d). The detection limits are 20 pmol of MX (Fig. 7e) and 400 pmol of EM in a mixture.

All the separations described apply to pure aqueous solutions and the presence of a matrix in real samples might worsen their parameters. Practical applications to drug analysis will be published elsewhere.

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