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ZONE ELECTROPHORESIS OF ORGANIC ACIDS AND BASES IN WATER-ALCOHOL SOLVENTS

V. JOKL, J. DOLEJŠOVÁ and M. MATUŠOVÁ

Department of Analytical Chemistry, Pharmaceutical Faculty of the Charles University, Hradec Králové (Czechoslovakia)

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SUMMARY

The electrophoretic behaviour of ten organic acids and four bases as model samples in water–methanol and water–ethanol has been studied with respect to the organic solvent concentration in the supporting electrolyte and its pH. The changes of the permittivity of the solvent influence the absolute velocity of migration as well as the relative effective mobility (via changes in pK_a). Using the electrophoretic data, pK_a values of the observed substances in mixed solvents have been approximated. Some separations of barbiturates in aqueous ethanol are presented.

INTRODUCTION

Zone electrophoresis in other than aqueous solvents can be useful for the separation of organic acids and bases that are poorly soluble in water when not ionized. Only a few studies of such systems have been published, and the research has been mostly unsystematic. One of the reasons for this lack of interest may be the more difficult specification of the experimental conditions in such media, especially the actual acidity which is fundamentally important for electrophoretic separation of weak electrolytes. For example, some pigments[1-3], quinoline bases and phenols[4], and nucleic acids[5] have been separated in non-aqueous or mixed media. In an effort to improve the results of the separations, non-aqueous solvents have been applied to mixtures of inorganic and complex ions[6-8]. Recently some general criteria of the application of non-aqueous and mixed solvents to electromigration were outlined[9]. In this study we have attempted a systematic examination of the electrophoretic behaviour of some organic acids and bases in mixed water–alcohol solvents, with special reference to the changes in their protolytic properties caused by the influence of the permittivity of the mixed solvent.

EXPERIMENTAL

Apparatus

The electrophoresis was performed on the HVE Camag apparatus (125 V/cm,

paper Whatman No. 2, cooling with tap water to about 18°). The pH of the solutions was measured on the pH meter OP-201/2 (Radelkis) with glass and saturated calomel electrode; a salt bridge protected the calomel electrode from diffusion of the organic solvent. The electrode system was calibrated for use in mixed solvents in 0.1 m lithium succinate buffer according to Bates[10] after the graphical interpolation of the values given.

Chemicals

The model samples studied in our experiments were organic acids and bases (eventually their salts). Internal standards for electroosmosis and relative electrophoretic mobility were antipyrine (pK_a 1.45) and tetraethylammonium ion (as iodide)[11]. The samples were applied as 0.05 M solutions in 50% ethanol; the concentration of $(C_2H_5)_4NI$ was 0.02 M.

As supporting electrolyte we used a universal buffer prepared as follows: aqueous solutions of 0.67 M citric acid (A) and 1 M ethylenediamine plus 0.5 M Tris (B) were combined in various ratios to a final volume of 20 ml. Then water and organic solvent were weighed up to a final weight of 200 g. In this way a series of buffers was prepared containing 0, 20 and 40% (w/w) of the organic solvent. The solutions with 60% of the organic solvent contained only 10 ml of the buffer mixture owing to the decreased solubility of the buffer substances. The pH values of the solutions were measured. The pH dependence of the universal buffer on the ratio of A to B in aqueous solutions is shown in Fig. 1. The minimum buffer capacity is in the pH range 6–7.

Methods

The glass electrode and one end of the salt bridge were immersed in the mixed solvent for 3 h before the pH measurement. In the 60% mixed medium the potential was steady after 10 min; in other media it stabilized earlier.

The water–alcohol buffer solutions described above also served as mobile phases in descending paper chromatography. The R_F values determined were used to correct the mobilities with respect to sorption by the carrier.

The ethanol content in the water–ethanol solutions was determined by the Widmark method. The liquid phase from the impregnated chromatography paper was obtained by pressing out before or after a 60-min electrophoretic run.

For the electrophoresis, 0.5 μl of the solutions of samples and mobility standards was applied to paper impregnated with the supporting electrolyte. The duration of the electrophoresis was from 10 (aqueous buffers) to 90 min (buffers in 60% ethanol); the absolute migration distance of $(C_2H_5)_4N^+$ was *ca.* 15 cm. Two parallel independent experiments were carried out.

Compounds 1–12 (see Table II) were detected under UV; pilocarpine, procaine and the internal standards were detected chemically by use of the Dragendorff reagent. Mobility was determined in the most intensive region of the zone. In a few cases tailing was observed.

Calculations

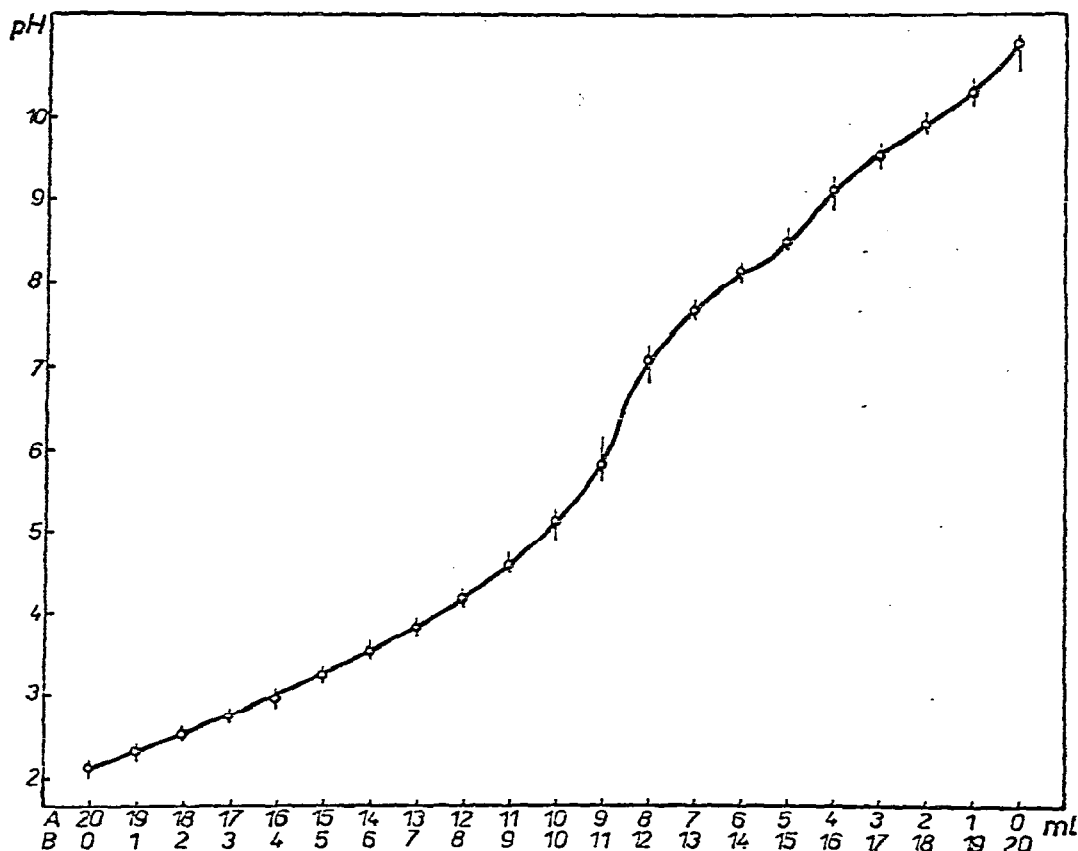The mobilities are given as values relative to the tetraethylammonium ion

Fig. 1. Variation of the pH of the universal buffer with the ratio of components: A, millilitres of 0.67 M citric acid; B, millilitres of the mixture 1 M ethylenediamine + 0.5 M Tris, made up to 200 g.

(conventional mobility $+100$), and corrected for electroosmosis and sorption by the carrier. They were calculated as follows:

$$U = \frac{l_x \cdot R_{F,s}}{l_s \cdot R_{F,x}} \cdot 100 \tag{1}$$

where l_x and l_s are the migration distances of the sample (x) and the standard ion (s) from the zone of antipyrine or (below pH 3) from the start.

The calculation of the theoretical course of the mobility curve (expressing the dependence of the relative effective mobility U on pH of the migration medium) was performed by means of the relationship[12]

$$U = (u_{HB} \cdot [H^+] + u_B \cdot K_a)/([H^+] + K_a) \tag{2}$$

If the acid, HB, or its conjugated base, B, are electrically neutral species, eqn. 2 is simplified. The relative ionic mobilities, u, were calculated (when not experimentally

ascertainable) from the empirical relationship[11] valid under given experimental conditions:

$$u/z = 1470 \cdot M^{-1/2} - 29 \tag{3}$$

where M is the molecular weight and z the charge.

The pK_a values of the substances studied were determined from the electrophoretic data by means of logarithmic analysis using eqn. 2 transformed to the form:

$$(u_{HB} - U)/(U - u_B) = K_a/[H^+] \tag{4}$$

In the linear plot $\log(u_{HB} - U)/(U - u_B)$ against pH, the intersection point of the straight line with the ordinate gives the value of pH $= pK_a$. Another way to approximately determine pK_a was based on finding the pH value for which $U = u/2$; under this condition[12] again pH $= pK_a$. The value of u was estimated by extrapolation from the experimental mobility curve. This method was useful when the waves on the mobility curves were not fully developed.

RESULTS AND DISCUSSION

With regard to a consistent qualitative composition within the whole pH range as well as to the solubility in the mixed media a universal buffer was prepared consisting of organic components only. Despite this, its concentration had to be halved for the 60% solutions of alcohols.

Sorption by the carrier under the experimental conditions used was controlled by means of descending chromatography (Fig. 2). R_F values varied slightly with pH as well as with the alcohol content. The sorption was generally higher in systems with lower alcohol content. The sorption of most electrolytes was lower when they were ionized. However, the sorption of phthalic and diethylbarbituric acids and of the stronger bases pilocarpine and procaine was almost independent of both pH and alcohol content, R_F being higher than 0.8. The tetraethylammonium ion migrated uniformly at various pH values; with increasing alcohol content the R_F values decreased from 1 to 0.85.
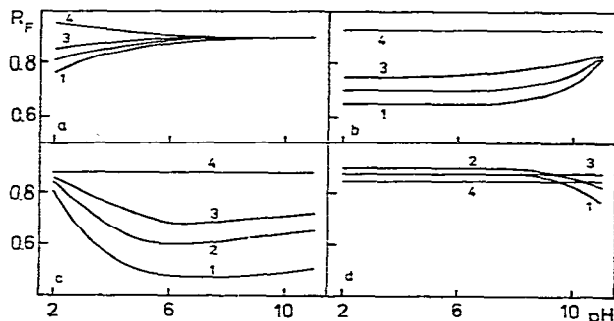
Fig. 2. Descending paper chromatography using water–ethanol buffers as the mobile phases: (a) benzoic acid; (b) 4-nitrophenol; (c) 1-aminonaphthalene; (d) procaine. 1–4 = 0, 20, 40, and 60% ethanol.

Changes in composition of the mixed solvent

These were examined for the water–ethanol system. The composition of the liquid pressed out from the paper (without electrophoresis) differed from the original one used for the impregnation (Table I). The overall trend in the deviations can probably be explained by an irreversible sorption of a constant portion of water and ethanol in the form of a cellulose–water–ethanol complex. Of greater importance for the evaluation of electrophoretic experiments was a variation in the composition of the interstitial liquid during the electrophoresis, because it is the proper migration medium. Although the apparatus is adapted to reduce evaporation and is kept cool, some loss of the more volatile component occurs, proportional to its content. For this reason the values in more concentrated alcohol solutions are less reliable.

TABLE I

CHANGES IN ETHANOL CONTENT IN THE MIXED SOLVENT

Original liquid (%, w/w)	20	40	60
Pressed out before electrophoresis (%, w/w)	17.50	37.28	60.40
Pressed out after 1 h electrophoresis (%, w/w)	16.70	34.98	54.64
Relative loss of ethanol during electrophoresis (%)	4.57	6.17	9.54

Absolute migration velocity

As the content of alcohol in the mixed solvent increases so the velocity of ionic migration rapidly decreases. In 60% ethanol it is almost ten times less than in the aqueous solution. The viscosities of water, methanol, and ethanol are similar, and so the reason for the diminished mobility must be sought elsewhere. The permittivity of the mixed solvent is being much less than that of water, and this leads to increased formation of ion-pairs and hence to a decrease in the effective mobility. Following the Debye–Hückel theory, the relationship between the speed of the migration of tetraethylammonium ion and the permittivity ($\varepsilon^{-3/2}$) of the water–ethanol solvent at various pH values was examined. The dependence is not linear; the influence of the mixed medium is complex, and beside the permittivity other factors are influential, mainly changes in the solvation of the ions.

Mobility curves

The model substances were organic acids and bases, covering a wide range of pK_a values and different chemical types (aromatic carboxylic acids, nitrophenols, an enolic compound, an aromatic amine, heterocyclic bases, and an aliphatic amine). The solubility of non-ionized substances in water varied from considerable (e.g. 2-aminobenzoic acid) to practically nil (e.g. 8-quinolinol). Table II lists the molecular weights and pK_a values of the model substances.

The electrophoresis was performed with all the samples in aqueous solution and in three water–ethanol and three water–methanol mixed solvents. The acidity of the supporting electrolyte was varied in the pH range 2–11 at ca. 0.5 intervals. The experimental values of corrected relative mobilities were used to draw the mobility curves as the function $U = f(pH)$. Some typical examples of a monobasic and dibasic acid and a base are shown in Figs. 3–5. The theoretical mobility curves were calculated by means of eqns. 2 and 3, and they enabled us to classify the experimental curves.

TABLE II

MOLECULAR WEIGHTS (M) AND pK_a VALUES (WATER, 25°)[13,14] OF THE SUBSTANCES UNDER EXAMINATION

| No.	Substance	M	pK_{a1}	pK_{a2}
1	5-Sulphosalicylic acid	218.2	2.9	
2	Salicylic acid	138.1	3.0	
3	Phthalic acid	166.1	2.95	5.40
4	2-Aminobenzoic acid	137.1	4.95	2.05 (pK_{a0})
5	Benzoic acid	122.1	4.20	
6	4-Hydroxybenzoic acid	138.1	4.6	9.3
7	2-Pyridinecarboxylic (picolinic) acid	123.1	5.5*	
8	4-Nitrophenol	139.1	7.15	
9	5,5-Diethylbarbituric acid	183.3	7.98	
10	3-Nitrophenol	139.1	8.40	
11	1-Aminonaphthalene	144.2	3.92	
12	8-Quinolinol (oxine)	145.2	5.02	9.8 (−OH)
13	Pilocarpine	208.3	7.0	
14	4-(2-Diethylamino)ethoxycarbonylaniline (procaine)	236.3	9.09**	

* Value from ref. 18.
** Value from ref. 17.
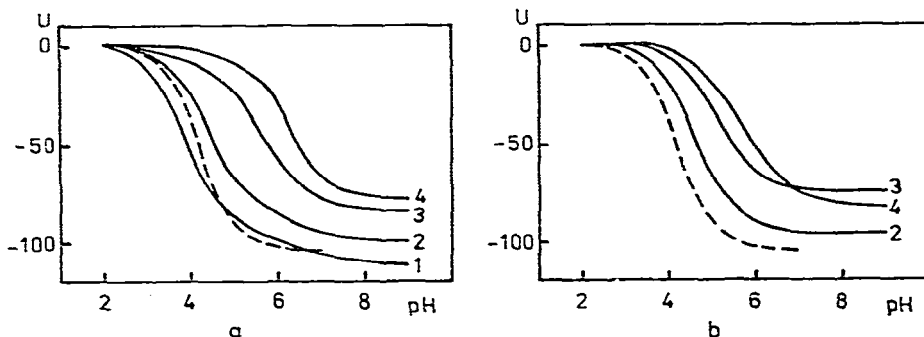
Fig. 3. The electrophoretic mobility curves of benzoic acid: (a) water–ethanol; (b) water–methanol. The dashed line is the calculated curve for aqueous solution; 1 = the experimental curve in aqueous solution; 2–4 = the experimental curves in solutions containing 20, 40, and 60% alcohol.
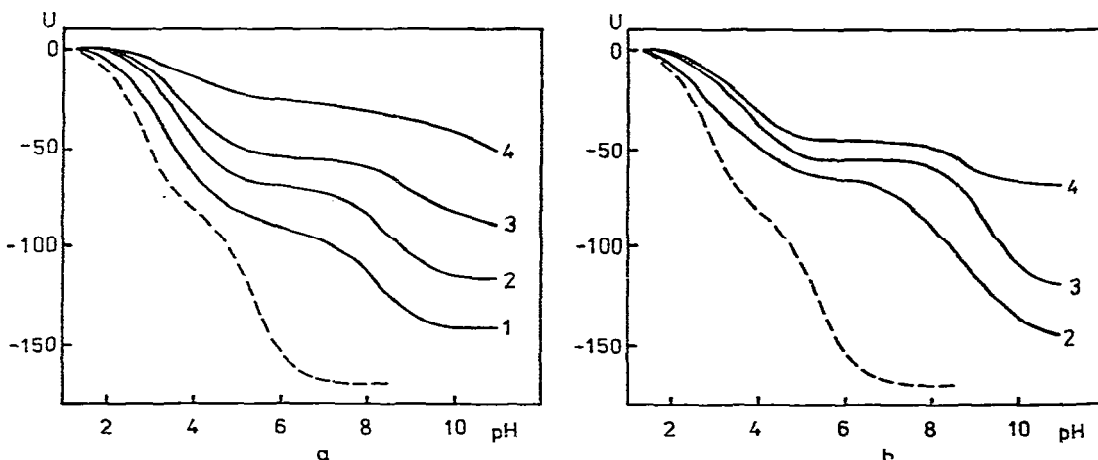

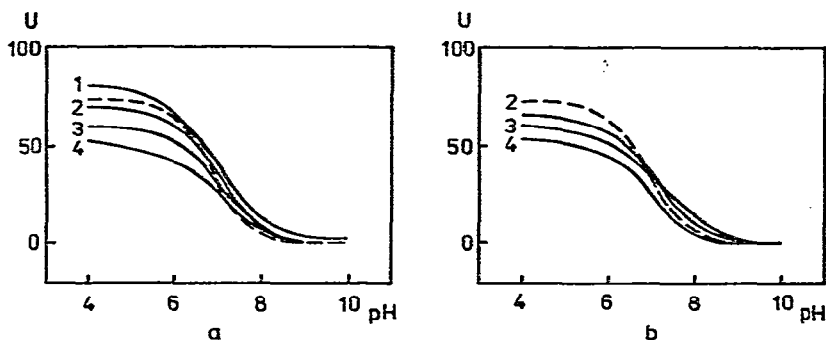Fig. 4. The mobility curves of phthalic acid.

Fig. 5. The mobility curves of pilocarpine.

The curves show that the relative mobilities in mixed media are generally lower than in aqueous solution (this does not take account of the absolute retardation of the migration discussed above). This effect increased with the content of the organic solvent, possibly owing to the solvation and the electrostatic interactions of ionic species with the buffering ions in the supporting electrolyte under conditions of decreased permittivity of the solvent. These effects were different for the substances tested and the mobility standard.

Beside this effect, greater or lesser shifts of descending parts of the curves can be seen. The shape of this part of the curve is governed by the acidity of the compound (and the associated distribution of charged and non-charged species in an equilibrium) ; thus the shift is caused by changes of the dissociation constants in the mixed medium. The values of dissociation constants in water–alcohol media are known for only some of the substances examined. However, their approximate values can be determined from the electrophoretic data. For the majority of the substances they were found by means of logarithmic analysis of the mobility curves (see above), as shown in Fig. 6 for benzoic acid. For the dibasic acids (phthalic and 4-hydroxybenzoic acids), the shape of the experimental curve near the second dissociation step region differs greatly from the predicted one, so the pK_{a1} values were found directly from the curve (pH under the condition $U = u/2$); pK_{a2} was not determined. The experimental data, together with the available data from literature, are listed in Table III.
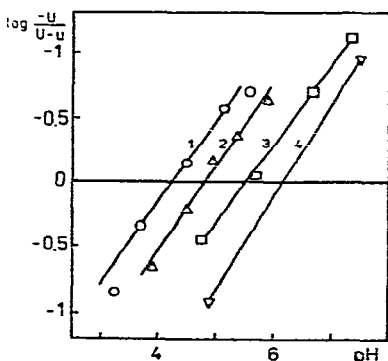

Fig. 6. Linear transformation of the mobility curves of benzoic acid in water–ethanol. Numbering the same as in Fig. 3.

TABLE III

pK_a VALUES IN WATER–ALCOHOL FROM ELECTROPHORETIC DATA

Literature values[10,15] in parentheses.

No.*	Water		Methanol (%, w/w)			Ethanol (%, w/w)		
			20	40	60	20	40	60
1	2.9	(2.9)	3.6	4.1	5.2	3.5	4.7	6.0
2	{ 3.1		3.4	3.7	4.3	3.25	3.6	4.4
	(3.0)					(3.23)	(3.75)	(4.30)
3	3.4	(2.89)	3.3	3.6	3.7	3.7	3.8	4.0
4	4.9	(4.95)	5.3	5.8	6.1	5.3	5.9	6.3
5	{ 4.25		4.6	5.1	5.6	4.8	5.5	6.2
	(4.20)		(4.60)	(5.10)	(5.65)	(4.76)	(5.40)	(6.02)
6	4.7	(4.6)	5.1	5.5	5.7	5.0	5.9	6.4
7	5.7	(5.5)	6.0	5.9	6.0	6.0	6.0	6.0
8	6.9	(7.15)	7.2	7.4	7.3	6.8	7.1	7.6
9	8.0	(7.98)	8.2	8.7	8.3	8.0	8.2	9.2
10	8.0	(8.40)	8.4	8.7	8.4	8.4	8.2	9.3
11	4.1	(3.92)	4.1	4.3	4.2	4.1	4.2	4.3
12	4.7	(5.02)	5.0	5.2	4.9	5.0	5.2	4.9
13	6.9	(7.00)	7.1	7.3	7.0	7.1	7.0	6.9
14	9.2	(9.09)	9.0	9.2	8.5	8.8	8.7	7.8

* Substances numbered as in Table II.

The influence of the permittivity of a polar amphiprotic mixed solvent over the dissociation constants depends on the nature of the acid–base pair. The relationship between pK_a and $1/\varepsilon$ (owing to the dissociation of the ion pairs[16]) is for some substances almost linear; the deviations from linearity are caused by effects other than electrostatic interaction (see Fig. 7). For most non-charged acids the slope is positive. That means that their dissociation is strongly influenced by the permittivity of the medium. One exception is picolinic acid, which can, because of its neighbouring carboxylic oxygen and ring nitrogen atoms, form a chelate ring and a
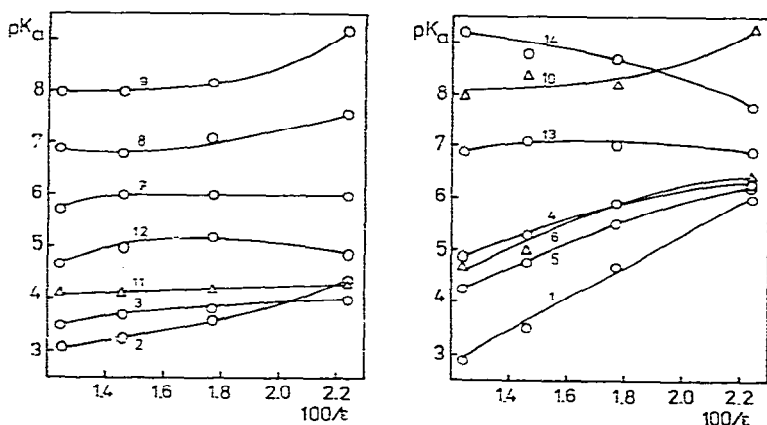

Fig. 7. The dependence of pK_{a1} on the reciprocal permittivity of the water–ethanol solvent. Numbering of the substances the same as in Table II.
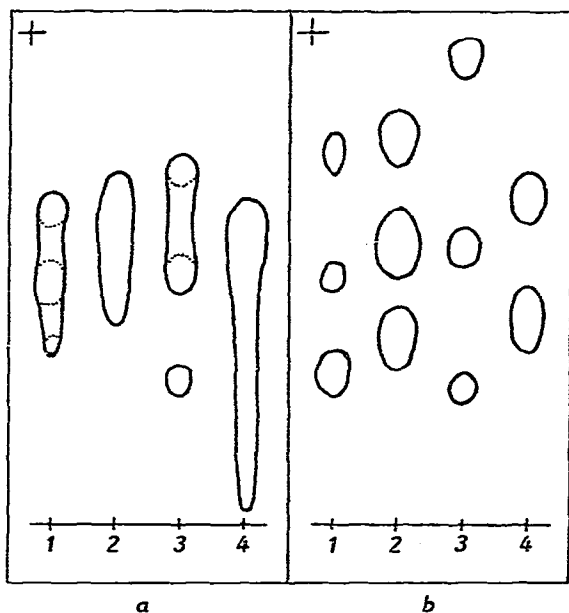
Fig. 8. Separation of some barbiturates: (a) aqueous solution, pH 8.60; (b) 40% aqueous ethanol, pH 8.66, with 1% glycerol. From top to bottom: 1 = Allobarbital + amobarbital + hexobarbital; 2 = pentobarbital + Somnyl® (barbital + aprobarbital); 3 = phenobarbital + amobarbital + hexobarbital; 4 = thiopental + pentobarbital. 5 μg of the substances were detected by fluorescence quenching at 254 nm.

dipolar ion via intramolecular hydrogen bonds; it then behaves like a cationic acid[18]. The dibasic phthalic acid, nitrophenols and the barbituric acid derivative show only a gentle slope. This class of compounds is also characterized in the literature as different from the other acids[16]. The slope for the cationic acids approaches zero or is negative. Their dissociation is influenced only a little by changes in the solvent permittivity.

It is possible to conclude that the electrophoresis of organic acids and bases in water–alcohol mixed media results in changes in the absolute velocity of migration as well as in its dependence on the pH of the supporting electrolyte. These changes can play a positive role in some separations that are incomplete performed in aqueous solution. A less favourable finding is the fact that on the carrier used only acids with low pK_a values and only pilocarpine of the bases formed well-defined zones when the content of the organic solvent was higher. The diffusion and sorption phenomena could be decreased by adding a small amount (1%) of glycerol to the supporting electrolyte, as shown by the attempts to separate some barbiturates (Fig. 8). Under the right conditions, approximate pK_a values can be determined from the electrophoretic data with the minimal supply of analysed material.

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