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Application of Isotachophoresis for Determination of Anionic Forms of Phosphonic Acids*

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Using a capillary electrophoresis analyser, the concentrations of anionic forms of 2-phosphonobutane-1,2,4-tricarboxylic acid (PBTC) and N-trismethylenephosphonic acid (NTMP) were determined by the isotachophoresis method. The measurements were performed at pH = 4.2, 6.0 and 8.0. In each case the proper leading/terminating electrolyte system was selected on the basis of literature and authors' own research. The results of isotachophoretic analyses were found to be in good agreement with the results derived from dissociation constants determined by potentiometric method. Considerable difficulties were encountered in interpretation of the results obtained for pH = 8.0 since individual steps in the isotachopherograms were fuzzy and distorted probably due to incomplete separation of the analysed sample into zones. Knowledge of the kind and concentration of ligand forms of chelating compounds, to which PBTC and NTMP belong, can be useful for reclamation of soils polluted with heavy metals.

Key words: capillary electrophoresis, isotachopherogram, phosphonic acid, stability constant, species distribution

Isotachophoresis [1,2] belongs to electrophoretic techniques. It is used to separate ions and as an analytical method. In isotachophoresis, a sample of the analysed solution is introduced into a capillary between two electrolytes: the leading one and the terminating one. If anions are to be analysed, the leading electrolyte must contain an anion whose mobility is greater than that of the anions to be determined, whereas the terminating electrolyte contains the least mobile anion. These same principle applies to an analysis of cations. In the electric field individual ions gather into contiguous zones and move with a constant speed towards the electrodes. The length of the zones and the determined ions' concentrations in the zones stabilize to satisfy the following equation

$$v = v_i = u_i E_i$$

where: v – the velocity of an ion in the electric field; u_i – the mobility of the ion; E_i – the electric field intensity in zone.

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Separation in electrophoresis is based on differences in the electrophoretic mobility of determined ions. The electrophoretic mobility of a particular ion in solution depends on the ion's charge and radius and the solution properties (pH, ionic strength and composition). If the composition of solution is proper one, it can increase separation selectivity by changing an analyte's mobility. As the concentration of solution and ionic strength increase, the mobility of ions decreases. Also complexing agents are added. The buffer's pH affects form of the analytes, especially that of weak acids and bases. Water and nonwater electrolyte systems are used in capillary electrophoresis. If the ions to be separated have similar mobility and pK, better results can be obtained if water is replaced by other solvents, *e.g.* methanol. Nonbuffer electrolytes can be also used, but then the electrolytes in an electrode vessels must be frequently changed [3].

An efficiency of the electrophoretic separation of ions is limited by diffusion and convection because of the heat generated by passage of current. This adverse effects can be significantly reduced by the use of stabilizing fillers such as agar gels or polyacrylamide. Despite its long analysis time, the gel electrophoresis is still used today.

Due to application of a small-diameter capillaries, considerable advances in the efficiency of electrophoretic methods have been made. Currently the capillaries with an inside diameter of 0.075–0.5 mm are standard. As a result, the amount of generated heat can be substantially reduced even if a voltage of 30 kV is used. Sometimes the capillary is revolved during measurement to eliminate convection. Migration in the electric field in a capillary medium is accompanied by an electroosmosis. In a solution the capillary's wall is negatively charged due to ionisation of silanol groups (Si-OH). An electrical double layer with electrokinetic potential ζ is formed at the interface. As a result, a liquid in the capillary flows towards cathode. Since the ionization of the silanol groups and the negative charge on the capillary's wall increase, the electroosmotic flow increases with a buffer pH. A characteristic feature of the electroosmotic flow is its flat profile which has a beneficial effect on the efficiency of electrophoretic separation. The electroosmotic flow can be reduced by increasing a viscosity of the solution or lowering its dielectric constant by adding organic solvents to the electrolyte. Most electrolyte systems include soluble long-chain polymers such as methyl-2-hydroxylethyl cellulose (MHEC). To restrict electroosmosis the capillary walls are modified by coating quartz tubes with methyl cellulose or polyamide. Surfactants interact with the walls of the capillary. As a buffer ionic force increases, the electrokinetic potential and the electroosmotic flow decrease.

Chelating compounds, to which PBTC and NTMP belong, can be used for the electrokinetic reclamation of soils polluted with heavy metals and radioactive metals [4–8]. They form charged soluble complexes with different forms of ligands and alter the electrokinetic potential of the solid phase, facilitating the electrokinetic remediation of contaminated soils. Thus for practical purposes it is essential to know what kind of ligand forms and in which concentrations occur in considered systems.

Using as an example of PBTC and NTMP, a potential application of the isotachophoresis to quantitative and qualitative analysis of the anionic forms of these acids is presented.

EXPERIMENTAL

The investigations were carried out for three solution pH values: 4.2, 6.0 and 8.0 for which proper leading/terminating (LE/TE) electrolyte systems had been prepared. The LE/TE systems for the isotachophoretic tests were selected on the basis of literature and authors' own research [6–8]. The anionic ligand forms were analysed for above pH values.

The system of electrolytes at pH = 4.2 was:

- LE: HCl – 1.0×10^{-2} mole/dm^3, β-alanine – 5.0×10^{-2} mole/dm^3, methyl-2-hydroxylethyl cellulose (MHEC) – 0.05%, pH = 4.10;
- TE: CH_3COOH – 5.0×10^{-3} mole/dm^3, L-histidine (HIS) up to pH = 4.37 (these compositions are based on the data from *Critical Reviews in Analytical Chemistry*).

The system of electrolytes at pH = 6.0 was:

- LE: L-histidine monohydrochloride (HISCl) – 1.0×10^{-2} mole/dm^3, HIS – 1.5×10^{-2} mole/dm^3, MHEC – 0.05%, pH = 5.90;
- TE: monosodium glutamate – 5.0×10^{-3} mole/dm^3, pH = 6.45.

The system of electrolytes at pH = 8.0 was:

- LE: HCl – 1.0×10^{-2} mole/dm^3, tris(hydroxymethyl)amionomethane (TRIS) – 2.0×10^{-2} mole/dm^3, MHEC – 0.05%, pH = 8.15;
- TE: 2-(N-morpholino)ethane sulphonic acid (MES) – 1.0×10^{-2} mole/dm^3, TRIS – 1.5×10^{-2} mole/dm^3, pH = 7.88.

All the reagents from which leading electrolyte and terminating electrolyte were prepared were of purity class: pure for analysis (POCh: NaOH, HCl; Aldrich: β-alanine, MHEC, HIS, HISCI, TRIS, sodium glutamate; Sigma: MES). The analysed acids: PBTC and NTMF, with lower purity, were purified through crystallization.

2-Phosphonobutane-1,2,4-tricarboxylic acid (Fig. 1a) dissociates itself in five stages. Using the potentiometric method and the BEST computing software [9], the dissociation equilibrium constants (as pK_a) in 0.1 M KCl at 25°C were determined to be: $pK_{a1} = 1.74$, $pK_{a2} = 3.98$, $pK_{a3} = 4.96$, $pK_{a4} = 6.82$, $pK_{a5} = 10.65$. The values and the SPEPLOT program [9] were used to plot distribution curves for the particular ionic forms of the acid depending on the solution pH (Fig. 2). The obtained results are in good agreement with the dissociation constants of PBTC in a 0.1 M solution of ammonium tetramethylnitrate at 25°C found in the literature: $pK_{a1} = 1.8$, $pK_{a2} = 4.0$, $pK_{a3} = 4.9$, $pK_{a4} = 6.8$, $pK_{a5} = 10.8$ [10].

N-Trismethylene phosphonic acid (Fig. 1b) dissociates itself in six stages. The dissociation equilibrium constants (as pK_a) for this acid in 0.1 M KCl at 25° C are: $pK_{a1} = 0.30$, $pK_{a2} = 1.50$, $pK_{a3} = 4.64$, $pK_{a4} = 5.86$, $pK_{a5} = 7.30$, $pK_{a6} = 12.10$ [11].

The concentration distribution curves for ionic forms of NTMP are shown in Fig. 3.

Isotachophoretic measurements were performed using an EA100 capillary electrophoresis analyser manufactured by Villa Labeco (Slovakia). The separation of ions and their migration towards an electrodes and the detectors take place in 2 capillaries with a diameter of 0.3 and 0.8 mm and a length of 90 and 160 mm, respectively, arranged in series. By using capillaries with different inside diameters one can analyse solutions containing ions considerably differing in their concentrations, increase the selectivity of the analysis and reduce its duration. Ions whose concentration is very high, in comparison with other ions, after passing through first capillary can be lead out of measuring system. The analyser is equipped with a conductivity detector and a UV detector.

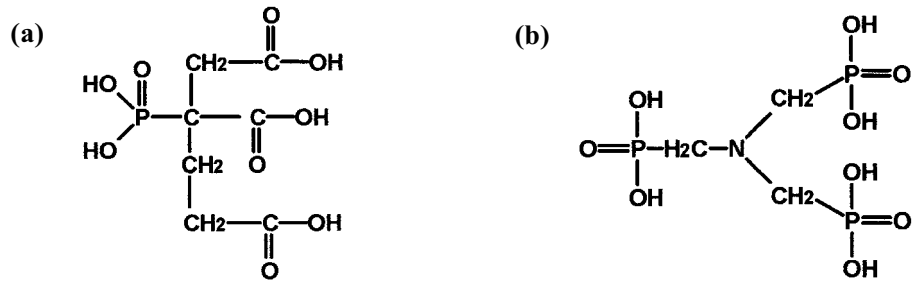

Figure 1. Structural formulas of PBTC (a) and NTMP (b).

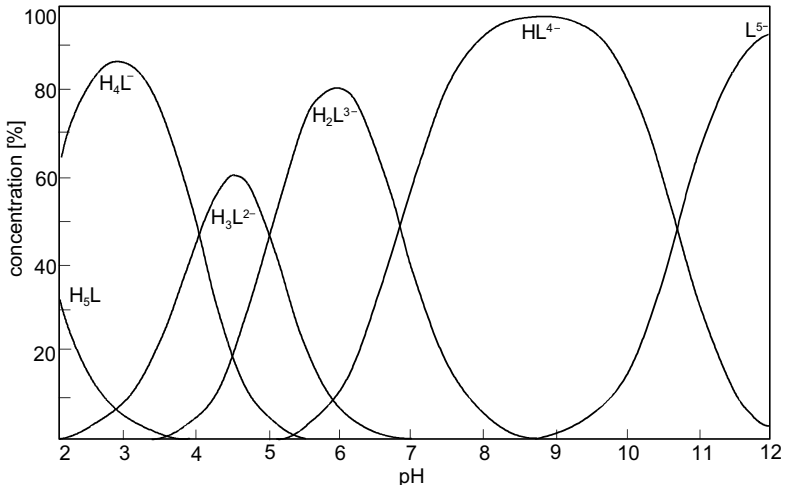

Figure 2. Species concentration distribution curves for 0.0025 M PBTC (molar % to total PBTC concentration), as a function of pH at 0.1 M ionic strength and 25°C.

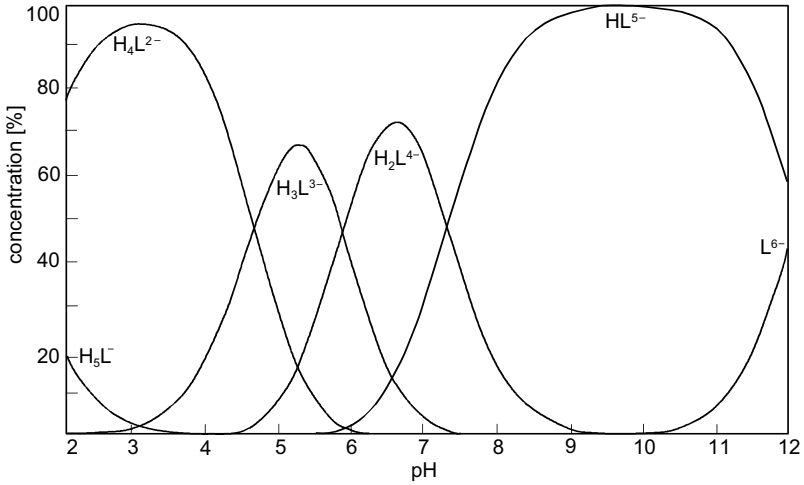

Figure 3. Species concentration distribution curves for 0.0024 M NTMP (molar % to total NTMP concentration), as a function of pH at 0.1 M ionic strength and 25°C.

RESULTS AND DISCUSSION

Boundary isotachopherograms (Tab. 1 and Figs 4, 6, 8) for all the three pH values were obtained only when LE and TE were in an analyser capillaries. Therefore it is possible to determine if any components or impurities which could distort a results were present in the reagents used for preparing an electrolytes. Analyses were performed for standard solutions containing phosphonic acids in concentrations: 0.5×10^{-3} and 1.0×10^{-3} mole/dm^3. The results are shown in Table 2 and Figures 1–7. Relative height and length of the isotachopherogram's steps indicate a kind of ion and concentration of the ion respectively.

Table 1. Description of isotachopherograms obtained for LE and TE with different pH values (boundary analysis without tested solutions).

pH = 4.2		pH = 6.0 zone		pH = 8.0	
$h_{rel.}$ [a]	l [b]	$h_{rel.}$ [a]	l [b]	$h_{rel.}$ [a]	l [b]
0.2259	5.92	0.6249	4.46	0.2450	1.16
0.4540	4.32			0.2743	2.74
				0.3597	23.36

[a] – relative height of isotachopherogram zone; [b] – length of isotachopherogram zone.

Table 2. Description of isotachopherograms obtained for PBTC and NTMP.

Analysis of PBTC					Analysis of NTMP				
pH = 4.2									
	c = 0.5 mol/dm^3		c = 1.0 mol/dm^3			c = 0.5 mol/dm^3		c = 1.0 mol/dm^3	
	zone					zone			
No	$h_{rel.}$ [a]	l [b]	$h_{rel.}$ [a]	l [b]	No	$h_{rel.}$ [a]	l [b]	$h_{rel.}$ [a]	l [b]
1	*0.2283*	*3.80*	*0.2306*	*2.06*	*1*	*0.2379*	*3.48*	*0.2295*	*1.76*
2	0.4114	59.74	0.4161	118.72	2	0.3245	61.50	0.3252	122.94
3	0.4523	9.32	0.4450	13.06	3	0.3510	10.72	0.3451	21.60
4	0.5286	4.56	0.5095	8.22	*4*	*0.4666*	*4.96*	*0.4639*	*5.72*
					5	0.5030	8.10	0.4998	15.46
					6	0.7054	4.80	0.7054	7.91
pH = 6.0									
1	0.2787	67.78	0.2528	132.56	1	0.3565	65.52	0.3625	130.54
2	0.3201	6.96	0.3032	11.78	2	0.4340	14.40	0.4243	29.64
3	0.3778	4.20	0.3591	6.58	*3*	*0.6284*	*3.28*	*0.6117*	*3.94*
4	*0.6263*	*3.34*	*0.6124*	*3.54*	4	0.6815	6.42	0.6829	12.94

Table 2 (continuation)

					pH = 8.0				
1	0.1677	85.74	0.1704	173.70	1	0.2220	87.52	0.3344	184.82
2	*0.2401*	*2.18*	*0.2404*	*2.52*	1[c]	0.3661	141.28		
3	*0.2615*	*3.76*	*0.2636*	*2.10*					
4	0.3294	3.26	0.3394	6.02					
5	*0.3500*	*22.20*	*0.3457*	*22.34*					

[a] – relative height of isotachopherogram zone; [b] – length of isotachopherogram zone; [c] – for NTMP concentration c = 0.75 mole/dm^3.

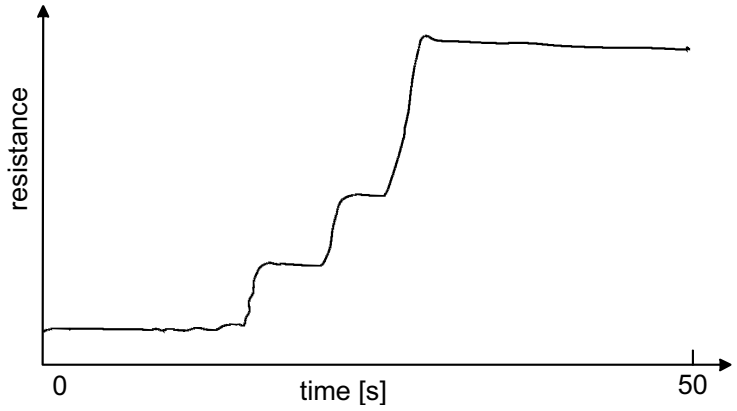

Figure 4. Boundary isotachopherogram obtained from analytical column detector for anion analysis at pH = 4.2.

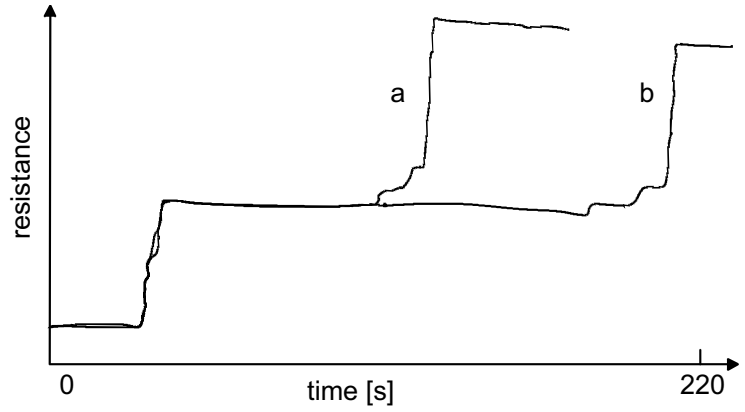

Figure 5. Isotachopherograms obtained from analytical column detector for PBTC solutions with concentrations 0.5×10^{-3} (a) and 1.0×10^{-3} mole/dm^3 (b) for anion analysis at pH = 4.2.

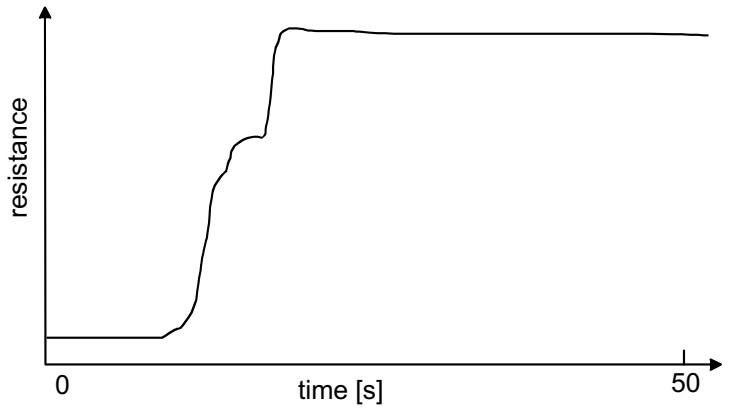

Figure 6. Boundary isotachopherogram obtained from analytical column detector for anion analysis at pH = 6.0.

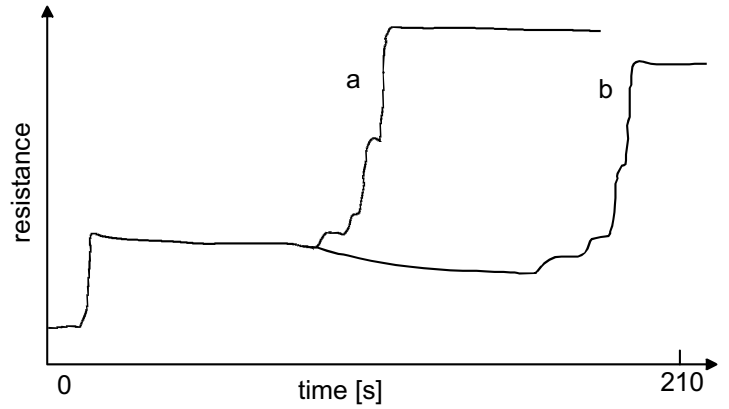

Figure 7. Isotachopherograms obtained from analytical column detector for PBTC solutions with concentrations 0.5×10^{-3} (a) and 1.0×10^{-3} mole/dm^3 (b) for anion analysis at pH = 6.0.

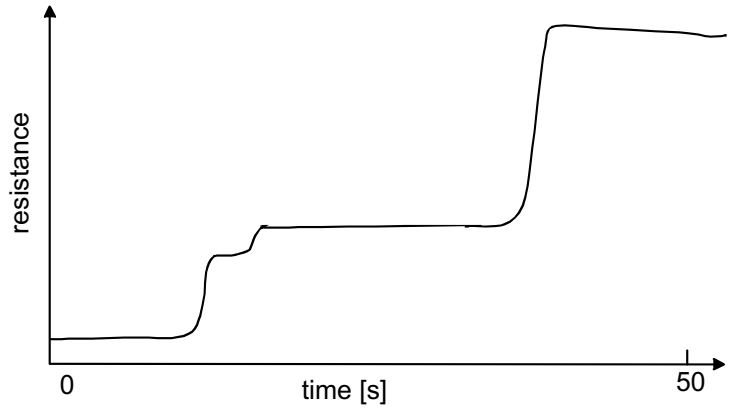

Figure 8. Boundary isotachopherogram obtained from analytical column detector for anion analysis at pH = 8.0.

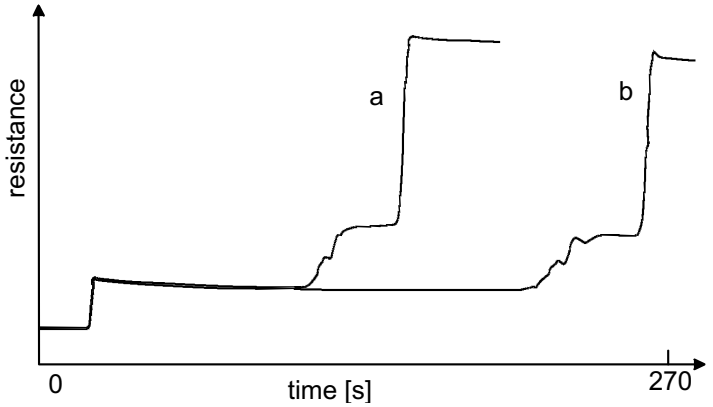

Figure 9. Isotachopherograms obtained from analytical column detector for PBTC solutions with concentrations 0.5×10^{-3} (a) and 1.0×10^{-3} mole/dm^3 (b) for anion analysis at pH = 8.0.

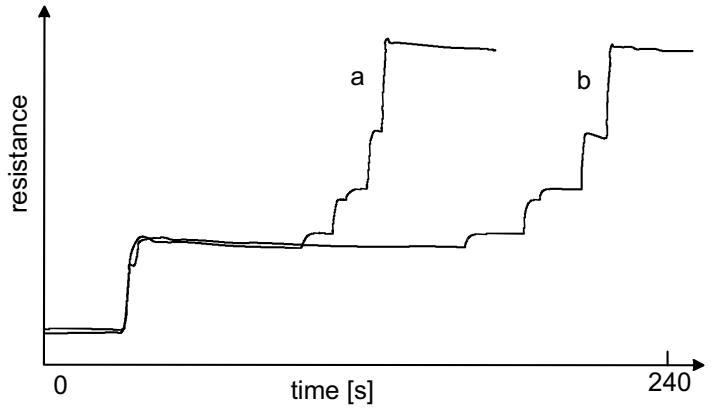

Figure 10. Isotachopherograms obtained from analytical column detector for NTMP solutions with concentration of 0.5×10^{-3} (a) and 1.0×10^{-3} mole/dm^3 (b) for anion analysis at pH = 4.2.

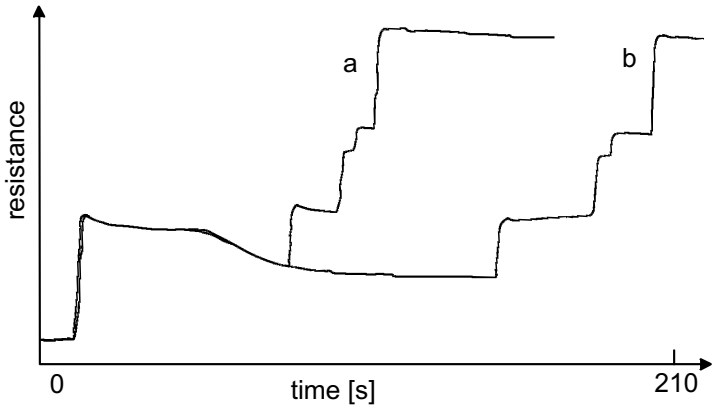

Figure 11. Isotachopherograms obtained from analytical column detector for NTMP solutions with concentration of 0.5×10^{-3} (a) and 1.0×10^{-3} mole/dm^3 (b) for anion analysis at pH = 6.0.

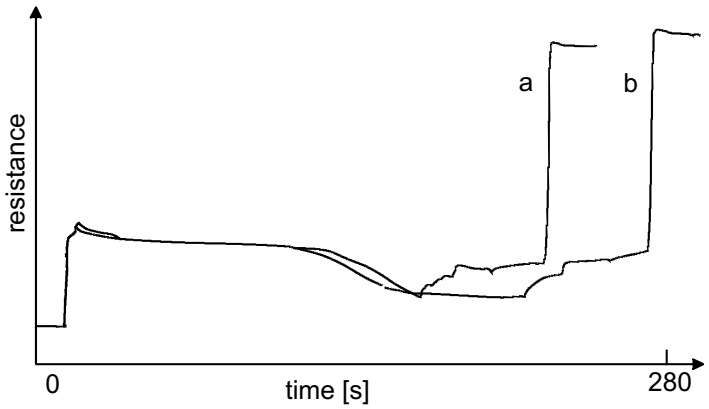

Figure 12. Isotachopherograms obtained from analytical column detector for NTMP solutions with concentration of 0.75×10^{-3} (a) and 1.0×10^{-3} mole/dm^3 (b) for anion analysis at pH = 8.0.

The isotachophoretic analysis performed for an electrolyte system at pH = 4.2, but without introducing any additional solution to the analyser (a boundary analysis), yields the isotachopherogram containing 2 short zones between lines corresponding to the leading ion (LE) and the terminating ion (TE) (Fig. 4). The zones, representing components of LE and TE or an impurities which they contain, occur at relative height of 0.23 and 0.45 and they must be taken into account in the analysis of PBTC and NTMP solutions.

From a protonation constants (if known) (Figs 2 and 3) one can calculate which of the ionic forms and in what quantities occur for specific pH values. Successive steps in the isotachophoresis correspond to the ions with ever lower mobility. Using this information one can try to assign a zones to the proper ions.

As the pH of leading electrolyte and this of terminating electrolyte change, so does a content of the particular ionic forms. Assignment of successive zone to the proper ions on basis of this correlation is difficult because of use of different electrolyte (LE and TE) systems at different pH values.

According to the PBTC concentration distribution curves, three ionic forms of this acid occur in solution at pH of 4.2: H_3L^{2-} (about 57%), H_4L^- (30%) and H_2L^{3-} (13%) (Fig. 2). Zones 2 and 4 (Tab. 2) which appear in the isotachopherogram (Fig. 5) become twice longer when the PBTC concentration is increased from 0.5×10^{-3} to 1.0×10^{-3} mole/dm^3, which indicates that they are relate to the analysed acid. At relative height h = 0.41 the longest zone probably corresponds to H_3L^{2-}. Slightly higher (h = 0.45) is zone for H_4L^- and above it a zone for H_2L^{3-} (h = 0.51).

For boundary analysis the zone which occurred at h = 0.23 is very short in the PBTC analysis. Since the zone does not become longer with increasing concentration of the PBTC, and it appears in the boundary analysis, one can conclude that it originates from LE + TE electrolyte system. The zone from the boundary analysis at h = 0.44-0.45 elongates in presence of the acid. If its length is taken into account as a background in the boundary test, the zone becomes twice longer when the PBTC con-

centration is doubled. Probably 2 ions are analysed at this height. One originates from PBTC and other one from LE/TE system.

The isotachopherograms for solution (containing PBTC) with pH = 6.0 have four zones of which a first one is clearly dominant (Fig. 7). The last zone at h = 0.62 represents LE or TE solutions. It occurs in the boundary analysis (Fig. 6) and in the analysis of the PBTC standard solutions but, it does not react to presence of this acid, regardless of its concentration. Thus the zones at relative heights 0.26, 0.32 and 0.37 correspond to 3 ionic forms of the PBTC. This is corroborated by the concentration distribution diagram (Fig. 2) which shows that at pH = 6.0 there are indeed 3 anionic forms of this acid. In state of equilibrium their quantities should be as follows: 80% H_2L^{3-}, 12% HL^{4-}, 8% H_3L^{2-}. While it can be inferred that the zone at h = 0.26 relates to H_2L^{3-}, it is difficult to relate other two zones to proper ions if one does not know latter's mobility in the medium. When the PBTC concentration is changed from 0.5×10^{-3} to 1.0×10^{-3} mole/dm^3, length of the zones increases: 1.96×, 1.69× and 1.57×, respectively. Undoubtedly, at lower concentrations (shorter zones) measuring accuracy decreases.

According to the PBTC concentration distribution curves, there should be 2 anionic forms: HL^{4-} (amounting to 93%) and H_2L^{3-} (amounting to 7%) in the solution with pH = 8 (Fig. 2). The isotachopherograms obtained at pH = 8.0 for standard solutions containing PBTC show two well-defined zones and several fuzzy and short zones (Fig. 9). At h = 0.17 the first zone is the longest and it clearly belongs to HL^{4-}. At a similar height in the boundary analysis no zone appears (Fig. 8) and doubling of the PBTC concentration results in a zone twice as long. The zone at h = 0.35 represents the LE/TE system. Other zones in the isotachopherograms are ill-defined and coincide with zones present in the boundary analysis. It seems most sensible to relate the zone which occurs at h = 0.33 to the H_2L^{3-} ion.

Similarly as in case of the PBTC solutions, the NTMP solutions were analysed at three pH values. Obtained isotachopherograms are shown in Figs 10–12 and described in Table 2. At pH = 4.2 six zones are observed (Fig. 10). Two of them – the first one and the fourth one – represent LE or TE. Two zones also occur in the boundary analysis but in the NTMP isotachopherograms they do not increase and zone at h = 0.23 dwindles with the acid concentration. Other zones correspond to four ionic forms of NTMP. First zone is clearly the longest and if one takes into account the concentration distribution curves (Fig. 3), it specifies the concentration of H_4L^{2-} ion, which should be to about 70% of the acid concentration. At relative heights: 0.35, 0.50 and 0.71 other zones become twice longer as the NTMP concentration increases from 0.5×10^{-3} to 1.0×10^{-3} mole/dm^3 but it is difficult to relate them to a proper ions (H_3L^{3-}, H_2L^{4-}, H_5L^{-}) if their mobility is unknown in a medium in which the isotachophoretic measurement is made.

The isotachopherogram for the NTMP analysis at pH = 6.0 has four zones (Fig. 11). One of them represents the LE/TE system. The other three zones become twice longer (precisely 1.99, 2.06 and 2.01 longer) as the acid concentration increases from 0.5×10^{-3} to 1.0×10^{-3} mole/dm^3. The trace of first step, corresponding to dominant

form, is not ideal. During measurement detector registers a drop in electric resistance of the electrolyte in this zone. This indicates that either the ionic form concentration is not uniform over whole length or no complete separation has been achieved. On the other hand, the abrupt transition to a next zone seems to contradict the incomplete separation of the particular ionic forms. The concentration distribution curves do not clearly show whether this zone corresponds to H_3L^{3-} or H_2L^{4-}. At pH = 6.0 both ions should be in similar concentrations. Other three zones have a typical steplike shape. Their length and their comparison with the boundary analysis (Tab. 2) show that a step at h = 0.62 has no relationship to NTMP.

It is practically impossible to interpret the measurement results obtained for the analysis of NTMP at pH = 8.0 (Fig. 12). Although a shape of the curves indicates one long zone, the latter sinks considerably with time and it becomes impossible to use relative heights for successive steps. In addition, these steps are so ill-defined that it is difficult to separate them or even to count them. One can only suppose that the longest zone corresponds to the HL^{5-} form. At pH = 8.0 also anionic form L^{6-} (amounting to about 20%) should occur (Fig. 3).

CONCLUSIONS

Described studies illustrated that isotachophoresis can be used as an analytical method for the determination of concentration of the ionic forms of phosphonic acids. The results obtained for 2-phosphonobutane-1,2,4-tricarboxylic acid (PBTC) and N-trismethylene phosphonic acid (NTMP) are in good agreement with a relevant experimentally determined protonation constants found in the literature.

Isotachophoresis allows to determine quickly concentrations of the ionic forms of substances using very small samples. Qualitative analysis and quantitative analysis require standardization curves.

Choice of an electrolyte system with the proper pH is of primary importance since it determines correctness of the analysis.

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