

HYDROXYETHYLIMINODIACETIC ACID IN THE ISOTACHOPHORETIC SEPARATION OF ALKALI CATIONS

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N-hydroxyethyliminodiacetic acid (HIDA) has been used for the optimization of the isotachophoretic separation of alkali and alkaline earth metal cations. The required separation effect appears at the leading-electrolyte pH = 6–7. The separation of K⁺, Na⁺, Li⁺, Ba²⁺, Sr²⁺, Ca²⁺, and Mg²⁺ has been achieved using HIDA as the complexing co-counterion. In the dependence on the leading-electrolyte pH and on the HIDA concentration in it various migration sequences can be achieved in the isotachophoretic steady-state. Alkali metal cations present in the model mixtures were determined in the concentration range $(1-125) \cdot 10^{-5} \text{ mol l}^{-1}$ with the relative standard deviation that was in most cases less than 1%. Detection limits for individual ions (for the injected sample volume of 25 μl) were within the limits of $(6.4-18) \cdot 10^{-6} \text{ mol l}^{-1}$. The separation of the alkali metals in mineral and drinking water and in the lyophilized blood demonstrate the practical use of the agent under study in the ITP analysis

Optimization of the isotachophoretic (ITP) separation conditions of the alkali and alkaline earth metal cations was subjected to a number of previous studies¹⁻¹⁰. The solution of this problem is closely connected with the ITP application in the analysis of these cations in waters^{5,8}, in pharmaceuticals^{9,10}, and in the biological materials⁴.

In spite of the fact that methanol solutions offer better conditions, e.g., for the complete separation, in most of the previous studies water was used as the solvent. Due to the small relative differences in the absolute ion mobilities of certain alkali metal ions in water, the complex formation equilibria play a decisive role in the optimization of the separation conditions in this solvent.

The separating effect due to the complex-formation equilibria can be achieved in ITP analysis of alkali metals by the addition of an anionically migrating or neutral ligand into the leading electrolyte solution^{2-4,6-10} when the separation proceeds in the cationic regime. However, the alkaline earth metal cations can be separated also in the anionic regime, if the chelating agent is added that forms with these cations the anionically migrating chelates⁵. In such a case the separation should be performed at a higher value of pH so that the decomposition of the chelate during

its migration would be negligible¹¹. With respect to these practical limitations, the alkaline earth metal cations separation in the anionic regime is used rather seldom.

The so far proposed electrolyte systems for the ITP separation of alkali metals in the cationic regime that make use of the separating effect of the anionically migrating ligands have practical limits due to the substantially different concentrations of individual cations in the sample⁴, they can be used only in a rather narrow range of pH values (cf.³), the electrolyte solutions cannot be in a simple way prepared in the required purity⁴, or agents that are not commercially available have to be used⁸. Due to these facts new electrolyte systems suitable for the ITP separation of alkali and alkaline earth metal ions are still to be searched.

The aim of our study was to investigate the possibility of the use of N-hydroxyethyliminodiacetic acid (HIDA) for the ITP separation of alkali metals. Our choice of the complexing agent arose from its excellent separating properties in the zone-electrophoretic separation of metal cations¹²⁻¹⁴. We have concentrated our efforts to the investigation of the effect of its concentration in the leading-electrolyte solution on the effective mobilities of the alkali metal cations at various pH values. A lower attention has been paid to the K⁺, Rb⁺, and Cs⁺ ions as for them the complexing effect of the agent does not apply and their separation in the aqueous solutions can be achieved using common crown ethers^{6,7}.

The practical use of the electrolyte systems containing HIDA in the function of the anionically migrating ligand was tested by the analyses of water and lyophilized blood.

EXPERIMENTAL

Apparatus

The CS Isotachophoretic Analyzer (Vývojovo-výrobný závod prístrojov jadrovej techniky, Spišská Nová Ves, Czechoslovakia) with the separation unit with column-coupling was used for this study, the analyzed samples were sampled by a sampling valve (25 µl). The zone lengths from the conductivity detectors of the analyzer were measured using an electronic circuitry¹⁵.

Reagents

The solutions were prepared using water demineralized by reverse osmosis and a mixed-bed-ion-exchanger column (Rodem-1, Okresní průmyslový podnik, Tišnov, Czechoslovakia) and purified by a continuous circulation through the column with the mixed-bed ion exchanger in a polytetrafluoroethylene column.

Reagents used for the preparation of the leading and terminating solutions were supplied by the following manufacturers: histidine (Reanal, Budapest, Hungary); N-morpholinoethane sulphonic acid (MES), N-(2-acetamido)-2-aminoethane sulphonic acid (ACES), and hydroxyethyl cellulose 4 000 (Serva, Heidelberg, F.R.G.); N-ethylmorpholine (Fluka, Buchs, Switzerland); ammonia and acetic acid (Lachema, Brno, Czechoslovakia). N-hydroxyethyliminodiacetic acid (HIDA) was prepared using the method described by Vieles et al.¹⁶. Histidine, MES, ACES,

and HIDA were purified by the repeated precipitation of the aqueous solutions by distilled ethanol or acetone. Precipitation and all subsequent operations were made in polyethylene or fused silica vessels. The same rules were applied also to the isothermal distillation of ammonia, acetic acid, and N-ethylmorpholine as well as to the preparation of the leading- and terminating electrolyte solutions.

RESULTS AND DISCUSSION

The composition of the operational (electrolyte) solutions used (Table I) has been chosen so that the buffer counterions (acetate, MES, ACES) contributed in a minimum degree to the formation of complexes with the cations under study. The numerical values of stability constants for the counterions mentioned above^{17,18} and for HIDA¹⁹ show that HIDA forms complexes with the alkaline earth metal ions the stability of which can be by 3–4 orders of magnitude higher in comparison with the respective complex with the buffer counterion as a ligand. These data indicated that HIDA would affect the mobilities in the sequence $Ba^{2+} \leq Mg^{2+} < Sr^{2+} \ll Ca^{2+}$.

In the ITP separation of cations the effective stability constants play a decisive role^{20–22} and therefore we have concentrated our attention to the investigation of the leading-electrolyte pH effect (in the range 5.0–8.0) and of the HIDA concentra-

TABLE I

Systems used. Abbreviation used: HIDA — N-hydroxyethyliminodiacetic acid, MES — N-morpholinoethane sulphonic acid, ACES — N-(2-acetamido)-2-aminoethane sulphonic acid, HIS — histidine, HEC — hydroxyethylcellulose, HAc — acetic acid, diethylb.a. — diethylbarbituric acid, N-ethylmorph. — N-ethylmorpholine, β -Ala — β -alanine

Parameter	System No.			
	1	2	3	4
Solvent	water	water	water	water
Leading ion	NH_4^+	NH_4^+	NH_4^+	NH_4^+
Concentration, $mmol\ l^{-1}$	10	10	10	10
Counterion	HAc	MES	ACES	diethylb.a.
Co-counterion	HIDA	HIDA	HIDA	HIDA
Concentration, $mmol\ l^{-1}$	5	3	3	2.5
pH of the leading electrolyte	5.0	6.2	6.8	8.3
Additive to the leading electrolyte	HEC	HEC	HEC	HEC
	0.1%	0.1%	0.1%	0.1%
Terminating ion	β -Ala	HIS	HIS	N-ethylmorph.
pH of the terminating electrolyte	4.8	6.0	6.3	7.9

tion effect ($0-5 \text{ mmol l}^{-1}$) on the effective mobilities of the alkalis. Using the leading electrolyte of pH 5 (system No. 1, Table I) the retardation of the alkaline earth metal ions was small due to the lower values of the effective stability constants. On the other hand, the use of the leading electrolyte of pH 8 (system No. 4, Table I) resulted in negative systematic errors in Ca^{2+} determination. The expected separating effect was observed in the leading electrolyte of pH 6.2 and, particularly, of pH 6.8 (systems Nos 2 and 3, Table I). The more detailed investigation of the HIDA concentration effect was carried out only in these two leading electrolytes. The experimental results obtained were derived from the response of the conductivity detector in the analytical column and they are given in Figs 1 and 2 in the form of the dependence of the step height of the given cation (RSH_M) on the HIDA concentration in the leading electrolyte solution. The step heights of Na^+ zone in the respective leading electrolytes were used as the reference values.

It can be shown in a simple way that for the given leading and reference ion the RSH values are inversely proportional to the effective mobilities of the ionogenic

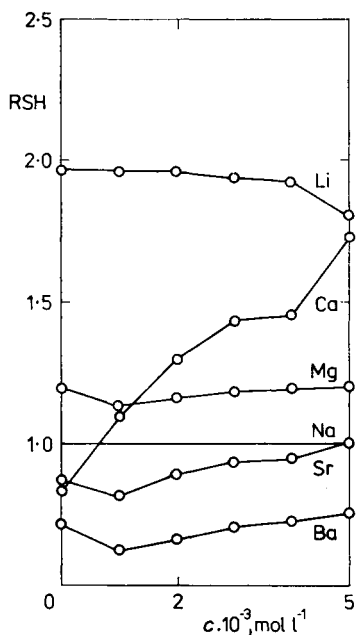


FIG. 1

Relative step heights of cations for different concentrations of HIDA (in mmol l^{-1}) in the leading electrolyte of pH 6.2

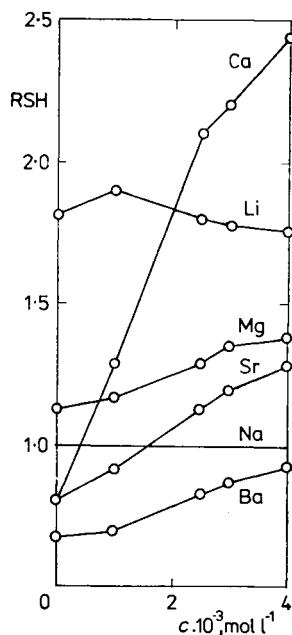


FIG. 2

Relative step heights of cations for different concentrations of HIDA (in mmol l^{-1}) in the leading electrolyte of pH 6.8

substances^{4,21}. Assuming that in our case the changes of HIDA concentration have no effect on the effective mobilities of NH_4^+ (leading ion) and Na^+ (reference ion) and that the complex species have a zero elementary charge (and therefore also zero actual mobility) the following relation can be derived^{4,21}

$$\text{RSH}_M = A + B\beta_{ML}[\text{L}]_M, \quad (1)$$

where A is a constant, comprising the actual mobilities of the leading-, reference-, and complex-forming cation M . B is a constant containing the actual mobilities of the leading- and reference ions. β_{ML} is the respective stability constant and $[\text{L}]_M$ is the free ligand concentration in the metal M zone.

In the simplest approximation we can assume a linear relation between the value of $[\text{L}]_M$ and the complexing agent concentration in the leading electrolyte (errors made due to this assumption — cf.²²). Because of the relation between RSH_M and $[\text{L}]_M$ given above we can expect also a linear relation between RSH_M and the HIDA concentration in the leading electrolyte solution, $[\text{L}]_{LE}$.

Parameters of the linear regressions corresponding to the experimental data given in Figs 1 and 2 are summarized in Table II. For the leading electrolyte of pH 6.2 the correlations are better expressed only for Sr^{2+} and, particularly, for Ca^{2+} . For Ba^{2+} , Mg^{2+} , and Li^+ the effect of HIDA is minimal in spite of the distinct separation of Mg^{2+} and Na^+ as well as of Sr^{2+} and Ba^{2+} that can be achieved with this agent (Fig. 3); the separation of these ions is difficult without the use of the complexing agent⁸.

TABLE II

Parameters of the regression equations $\text{RSH}_M = A + B[\text{L}]_{LE}$ characterizing the step heights of the metal M on the HIDA concentration in the leading electrolyte solution at the chosen values of the leading electrolyte pH

Metal	pH 6.20				pH 6.80			
	A	B	r^a	n^b	A	B	r	n
Ba	0.68	0	0.2298	16	0.62	88.26	0.9060	20
Sr	0.84	24.88	0.7972	20	0.76	147.18	0.9367	22
Mg	1.18	0	0.2260	20	1.06	96.99	0.8831	21
Ca	0.92	156.66	0.9652	19	0.88	417.14	0.9848	22
Li	1.92	0	-0.3391	20	1.82	0	0.3306	18

^a Correlation coefficient; ^b number of experimental data.

Using the leading electrolyte of pH 6.8, the influence of HIDA on the effective mobilities is more distinct. The experimental results (Fig. 2) as well as the slopes of the corresponding regression lines (Table II) indicate that the retardation effect of this complexing agent corresponds to the assumption (see above) that was made using the tabulated stability constants. An isotachophoreogram of the model mixture of cations (Fig. 4) shows that the migration sequence is in agreement with the RSH values. Because we did not observe any migration anomalies at the concentrations used²⁰, the tabulated parameters of the regression equations (Table II) can be used for the evaluation of HIDA concentration that must be present in the leading electrolyte solution to achieve the required migration sequence of the alkali and alkaline earth metal ions.

The mostly very low values of correlation coefficients are connected with the simplifications that are introduced by the use of linear regression (cf. above) and also with the experimental errors themselves. The effect of the measuring electrode surface coating on the response of the conductivity detector for polyvalent ions^{23,24} was the main source of errors in our experiments. The regular cleaning of the electrodes by 100 V pulses²⁵ guaranteed a very good long-term reproducibility of the RSH value measurements (during 12 months). The measured data were characterized by the

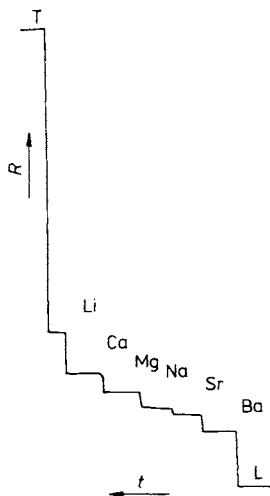


FIG. 3

Isotachophoreogram of the separation of the model mixture of cations in the system No. 2 (Table I). Driving currents $I_1 = 250 \mu\text{A}$, $I_2 = 45 \mu\text{A}$. Injected volume $25 \mu\text{l}$

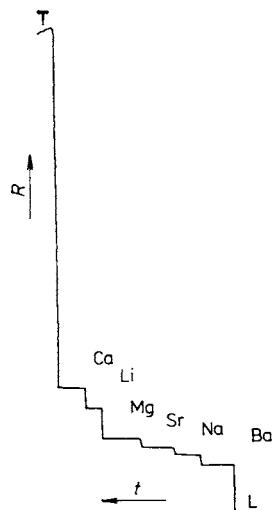


FIG. 4

Isotachophoreogram of the separation of the model mixture of cations in the system No. 3 (Table I). Experimental conditions as in Fig. 3

relative standard deviations in the range from 0.5 to 3% for 90% of the results, with the maximum value of the relative standard deviation in the whole set of measurements not exceeding 6%.

Our experiments proved that the use of HIDA as a complexing co-counterion does not lead to any analytical interference due to the simultaneous migration of other metal ions. From all the cations under study (Cu^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} , Al^{3+} , Pb^{2+} , Cr^{3+} , Mn^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+}) only Mn^{2+} migrated in the system No. 3 (Table I). These results are in agreement with the expected results as these cations form in a significant degree the negatively charged two-ligand complex species^{14,19}. In the analysis of practical samples it is necessary to consider that only the permanent organic cations and the organic bases, the $\text{p}K_a$ value of which is higher than the $\text{p}K_a$ value of the terminating cation used, can be the potential migration and analytically interfering substances. As it was already mentioned, HIDA does not render the possibility of K^+ , Rb^+ , and Cs^+ separation in aqueous electrolyte systems. As the determination of K^+ is very frequent in the analytical practice it seems reasonable to combine the retardation effect of HIDA with the effect of crown ethers on the effective mobilities of the alkali metal cations^{6,7}. The possibility of this separation combination will be discussed in our next study.

Example of the Practical Sample Analysis

In this part of our study we have verified the practical utility of HIDA as the complexing co-counterion for the analysis of mineral and drinking waters and of the lyophilized human blood.

To obtain the calibration curves (cf. Table III) we have used the mixtures prepared of reagents, in which the actual contents were vouched for by the producer or were determined by the complexometric titration ($\text{Mg}(\text{NO}_3)_2$; CaCl_2) or by gravimetry

TABLE III

Parameters of the regression equation of the calibration lines ($y = a + bx$)

Ion	<i>a</i>	<i>b</i>	<i>r</i> (<i>n</i> = 15)
Ba	0.33	71 300	0.9928
Sr	-0.03	155 700	0.9998
Li	0.21	55 600	0.9999
Na	2.37	74 200	0.9999
Mg	0.26	156 000	0.9999
Ca	0.39	139 600	0.9992

(BaCl₂; SrCl₂). The precision of these determinations is characterized by the values of relative standard deviations that are listed in Table IV.

The use of histidine as the terminating cation can unfavourably affect the precision and accuracy of the ITP determinations due to the anodic oxidation in the reser-

TABLE IV
Relative standard deviations (s_r) for the model mixture analyses

Ion	$s_r^{\min} - s_r^{\max}$ %	Concentration mmol l ⁻¹
Ba	0.89—6.99	0.01—0.05
Sr	0.38—1.11	0.01—0.05
Li	0.10—0.88	0.02—0.10
Na	0.17—0.70	0.25—1.25
Mg	0.10—0.49	0.05—0.25
Ca	0.49—1.26	0.10—0.50

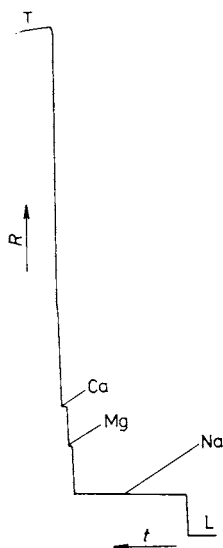


FIG. 5

Analysis of the lyophilized human blood, dilution 1 : 1 000. System No. 3 was used (Table I). Driving currents $I_1 = 250 \mu\text{A}$, $I_2 = 45 \mu\text{A}$. Injected volume 25 μl

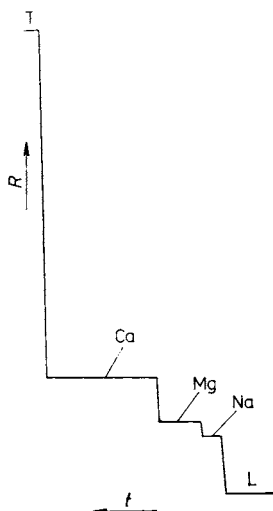


FIG. 6

Analysis of the drinking water sample, dilution 1 : 5. System No. 3 was used. Driving currents $I_1 = 250 \mu\text{A}$, $I_2 = 45 \mu\text{A}$. Injected volume 25 μl

voir of the terminating electrolyte which forms in a significant amount a cationically migrating product, the effective mobility of which falls into the interval between Ba^{2+} and Na^+ . The negative impact of the formation of this product on the quantitative analysis was eliminated by a frequent exchange of the terminating electrolyte in the reservoir.

The detection limits achieved under the conditions of the practical sample analyses for the 25 μ l injected volumes were determined for the zones passing through the detector in one second. We have obtained the following values: $7.1 \cdot 10^{-6} \text{ mol l}^{-1}$ Ca; $6.4 \cdot 10^{-6} \text{ mol l}^{-1}$ Mg; $14.0 \cdot 10^{-6} \text{ mol l}^{-1}$ Ba; $6.4 \cdot 10^{-6} \text{ mol l}^{-1}$ Sr; $13.4 \cdot 10^{-6} \text{ mol l}^{-1}$ Na; $18.0 \cdot 10^{-6} \text{ mol l}^{-1}$ Li.

TABLE V
Analysis of the mineral water "Santovka"

Ion	Tabulated mg l^{-1}	ITP mg l^{-1}	$s_r(n=5)$ %	ITP ^a mg l^{-1}	$s_r(n=5)$ ^a %
Na	372.4	326.8	0.34	322.2	1.26
Sr	—	5.8	—	5.8	—
Mg	82.7	53.5	0.82	53.6	0.46
Li	—	1.6	1.97	1.0	—
Ca	440.9	238.8	1.71	250.8	0.84

^a Analysis repeated one week later.

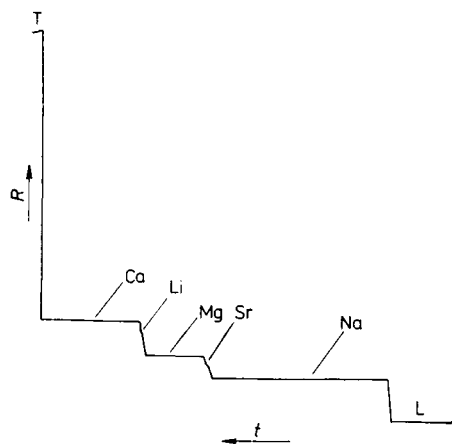


FIG. 7
Analysis of Na, Sr, Mg, Li, Ca in the mineral water "Santovka". The sample (without the precipitate in the bottle) was diluted 1 : 10. System No. 3 was used. Driving currents $I_1 = 250 \mu\text{A}$, $I_2 = 45 \mu\text{A}$. Injected volume 25 μ l

The isotachophoreogram in Fig. 5 shows that the system used makes the distinct separation of Na^+ , Ca^{2+} , Mg^{2+} , and K^+ possible. K^+ ion migrates with the effective mobility identical with that of the leading ion. Its correct determination is possible if the sample contains only negligible amounts of NH_4^+ , Rb^+ , and Cs^+ using the procedure analogous to that employed for the determination of chlorides in surface waters²⁶.

The systems investigated in this study offer the possibility of a fast ITP analysis of the alkali and alkaline earth metal ions in drinking waters (Fig. 6). Similarly as in the preceding case, also in this one the precision of the determination typical for the model samples (Table IV) has been achieved.

The isotachophoreogram shown in Fig. 7 has been obtained in the analysis of the filtrate of the "Santovka" mineral water. The results of the determination of ions (Table V) show that ITP presents reliable results. The deviations of the ITP determination from the data given on the mineral water label are probably caused by the loss of materials in the precipitate that was present in the sample.

To assess the long-term reproducibility of the ITP analyses of the alkali and alkaline earth metal ions the same sample was analyzed twice (in one-week interval). In both cases a freshly filtered sample of the same mineral water was analyzed. Freshly prepared solutions of the basic electrolytes were used for the analysis and the validity of the calibration curves was verified by the analysis of model samples. With the exception of Ca^{2+} the results of cation determination in the mineral water made in one-week interval were the same within the limits of random errors. The analysis of model samples, the composition of which was close to that of the filtrate, showed the agreement also for the determination of Ca^{2+} . It can be therefore concluded that the 5 per cent difference between the two subsequent determinations of this cation in the mineral water (Table V) is not caused by the ITP determination itself.

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