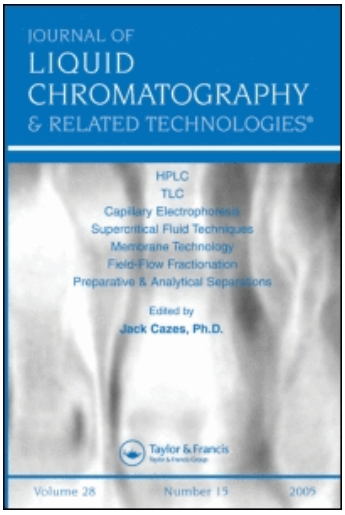

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Optimization of Conditions of Isotachophoretic Separation and Determination of New Class of Pentacoordinated Silanes

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Abstract: Optimal conditions of separation and determination of two newly obtained higher coordinated silanates of the group λ^5 have been performed. The optimization concerned 1-(N-perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxacyclopentane-3-on)]ate and 1-(N-perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxa-4,4-dimethyl-cyclopentane-3-on)]ate. Due to low mobility of analyzed ions, elaboration of a new termination electrolyte was necessary. As a main component of this electrolyte, a newly synthesized compound – 4,4′-bis{1-(N-perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxacyclopentane-3-on)]ate} was used. Separation of analyzed compounds was carried out in a time under 11 minutes. Optimal separation of the analyzed mixture was obtained in five steps under 12000 V voltage with preseparation and analytical columns applications. Voltage decreasing below 10000 V caused a decrease in the difference of analyzed ions mobility.

Keywords: Isotachophoresis, Pentacoordinated organosilicon derivatives

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INTRODUCTION

Theoretical bases of isotachophoresis (ITP) were elaborated during investigation of ion migration at the beginning of the XX century. Capillary isotachophoresis is an analytical method based on a separation process of ions (cations or anions), which are formed in zones in an electric field by the use of adequate leading and terminating electrolyte, according to their decreasing mobility and which are moving with the same velocity.[1]

Two different electrolytes are applied in ITP. A mixture of separated ionic substances is inserted between these systems. The first electrolyte (leading, Ld) consists of more mobile ions (cations or anions, depending of conditions) than the ions in the analyzed sample. The terminating electrolyte ions (Tm) are less mobile than the least mobile component of the separated mixture.[2]

Acidity (pH) of the separated mixture must be adjusted according to the type of determined ions; they must possess the same sign as ions of the leading and terminating electrolyte. Using the ITP method, it is possible to determine only cations or anions. Electrodes are connected to direct current with adequate voltage. During the separation, ions migrate one by one to the right electrode, according to the decreasing mobility, starting from leading electrolyte ions up to terminating electrolyte ions.[3]

Applications of capillary isotachophoresis in various areas (in pharmacy, biochemistry, and food chemistry) were extensively described.[4–9] Isotachophoretic methods are used for analytical, as well as industrial separation purposes.[9] The main application of capillary isotachophoresis is not only direct analysis but also sample preparation for analysis by other electrophoretic techniques. In such cases, isotachophoresis can serve as a method of a concentration of compounds, which are contained in trace amounts in the samples.[10]

The ITP method was applied, first of all, to such ionic compounds as: organic acids and bases, inorganic cations and anions, amino acids, peptides, nucleotides and nucleosides, biopolymers, and synthetic ionic polymers.[2] Also proteins: lisosome a, lisosome b, cytochrome c, and rybonucleases were separated.[1] At present, determination of water insoluble compounds by the isotachophoresis method is also possible, due to the use of organic solvents.

It is expected that isotachophoretic methods will be applied to analyses of mixtures with numerous constituents due to the development of adequate detectors. Medicinal applications are observed mainly in the analysis of human hemoglobin's, isolated from red blood cells and in analysis of serum components of uremia suffering patients.[1] ITP analysators equipped in conductometric detectors are widespread tools in pharmaceutical determination.[1]

An effort to search for optimum conditions of analysis for these compounds was caused by the lack of information in the chemical literature concerning hypercoordinated compounds. Pentacoordinated silicon derivatives (described here) are biologically active compounds containing systems similar to the natural systems.[10–14] Some of the hypercoordinated silicon compounds are engaged in chirality transfer from silicon atom to carbon atom. These compounds could be applied as model ones. It is supposed, that the silicon transport in biological systems can be based on hypercoordinated silicon compounds. Therefore, analysed pentacoordinated organosilicon compounds should be applied as model systems for this type of processes. Taking under consideration their wide biological activity, solubility in water, the lack the mutagenity, as well as the possibility for applying as plants growth regulators, are studies aimed at more recognition of this group of compounds seems to be well founded.

The aim of this work was to elaborate the optimum conditions of separation and determination of two newly obtained pentacoordinated silanates: 1-(N-perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxacyclopentane-3-on)]ate and 1-(N-perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxa-4,4-dimethylcyclopentane-3-on)]ate by the capillary isotachophoresis technique. However, in order to do this, it was necessary to apply adequate terminating electrolytes with mobility lower than the mobility of analyzed ions. Therefore, a new pentacoordinated compound: 4,4′-bis{1-(N-perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxacyclopentane-3-on)]ate} was used.

EXPERIMENTAL

The ES-silanate compounds were prepared by the method described in the literature.[15] The compounds synthesized in Department of Organic and Applied Chemistry of University of Podlasie, i.e., 1-(N-perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxacyclopentane-3-on)]ate (ES-1) and 1-(N-perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxa-4,4-dimethylcyclopentane-3-on)]ate (ES-3) (Figure 1), served as the materials for investigation. In the optimization process the following reagents were applied: deionized water from Merck (Darmstadt, Germany) and methylene chloride from Merck (Darmstadt, Germany), prepared from equal volumes of following solutions: HCl, CH_3COONa, CH_3COOH solution from POCH (Gliwice, Poland).

In order to prepare the standard solutions (ES-1 and ES-3) adequate amounts of these compounds were weighed to obtain concentrations $1.0 \cdot 10^{-3}\,\text{mole} \cdot \text{L}^{-1}$, $5.0 \cdot 10^{-4}\,\text{mole} \cdot \text{L}^{-1}$, and $7.5 \cdot 10^{-4}\,\text{mole} \cdot \text{L}^{-1}$. The weighed portions were dissolved in deionized water and subjected to

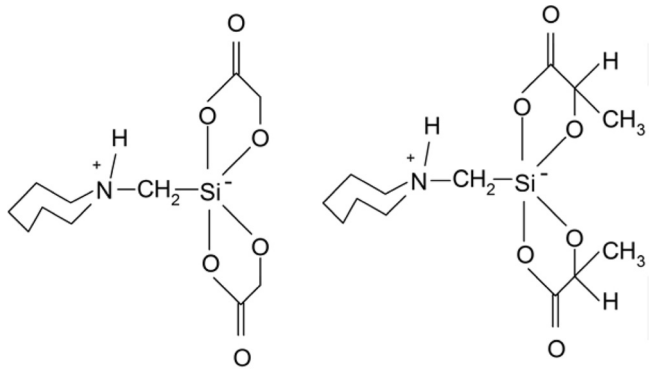

Figure 1. Structures of analyzed compounds: a. 1-(N-perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxacyclopentane-3-on)]ate (ES-1); b. 1-(N-perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxa-4,4-dimethylcyclopentane-3-on)]ate (ES-3).

analysis. The terminating electrolyte contained an aqueous solution of 4,4′-bis{1-(N-perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxacyclopentane-3-on)]ate} (ES-4, Figure 2). Leading electrolytes Ld-1 and Ld-2 for an analytic column, exhibiting pH = 3.5, were prepared from equal volumes of following solutions: HCl solution ($2 \cdot 10^{-3}$ mole $\cdot$ L^{-1}), CH_3COONa solution ($1.5 \cdot 10^{-3}$ mole $\cdot$ L^{-1}), CH_3COOH solution ($1.5 \cdot 10^{-3}$ mole $\cdot$ L^{-1}). The terminating electrolyte contained an aqueous solution of 4,4′-bis{1-(N-perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxacyclopentane-3-on)]ate} (10^{-3} mole $\cdot$ L^{-1}).

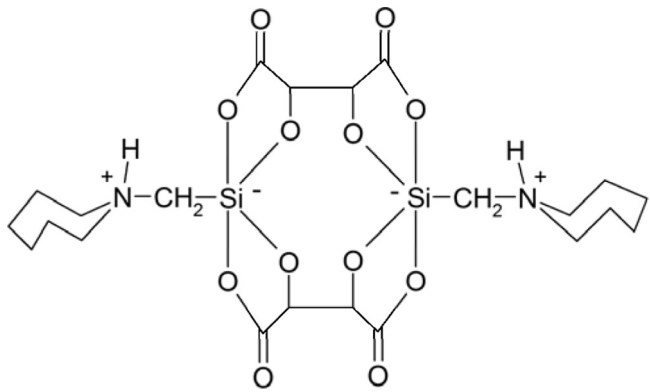

Figure 2. Structure of a compound used in terminating electrolyte 4,4′-bis{1-(N-perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxacyclopentane-3-on)]ate}.

Apparatus

Analyses were carried out by means of a capillary electrophoresis analysator EA 202 M produced by Villa Labeco s.r.o. in Spisska Nova Ves (Slovakia) equipped with an: injection block with a container for terminating electrolyte, preseparation column (capillary diameter 0.8 mm, length 90 mm), bifurcation block with a electrode block of the preseparation column, analytic column (capillary diameter 0.3 mm, length 160 mm), and electrode block of the analytic column, UV detector, two conductometric detectors with a measurement range between 30 kΩ and 20 MΩ, steering unit – personal computer PC containing converter AD/DA. After drawing standard curves for individual compounds, optimization conditions of the isotachophoretic separation of the mixture was carried out.

The ^{1}H, ^{13}C spectra were performed on Varian Mercury 400 (Varian, Inc., Palo Alto, USA) spectrometer (400 MHz) at 25°C, using deuterated methyl sulfoxide as solvent and TMS as internal standard. IR spectra were obtained on a Magna-IR 760, Nicolet (KBr), (Thermo Fisher Scientific, Waltham, GB). The infrared spectra of ES-silanates have been examined in the region 3500–450 cm^{-1} to assign the characteristic group frequencies in the compounds synthesized. The electronic absorption spectra in the visible range were obtained on a Beckman DU68 (Beckman Coulter, Inc., Fullerton, USA) spectrophotometer using 1 mL quartz cells at room temperature. The UV/Vis spectra were recorded digitally (0.5 nm step) over the range 400–900 nm. The samples concentration was $4 \cdot 10^{-3}$ mole $\cdot L^{-1}$ in CH_2Cl_2.

1-(N-Perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxacyclopentane-3-on)]ate: ^{1}H NMR (DMSO), δ (ppm): 1.5–1.8 (4H, CCH_2C), 1.6–1.9 (4H, CCH_2C), 2.59 (2H, $SiCH_2$), 3.03–3.18 (4H, NCH_2Si), 3.32 (4H, NCH_2), 3.96 (4H, CH_2COO), 8.06 (1H, NH). ^{13}C NMR (DMSO), δ (ppm): 23.55, 25.41, 25.43, 49.05, 62.73, 173.82. UV/Vis (CH_2Cl_2): λ_{max} ($lg\varepsilon$) = 303 (2.48), 325 (2.51). IR (KBr) ν_{max}: 3431, 3118, 2939, 2862, 1726, 1472, 1460, 1240, 1100, 579, 533, 489, 457 cm^{-1}.

1-(N-Perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxa-4,4-dimethylcyclopentane-3-on)]ate: ^{1}H NMR (DMSO), δ (ppm): 1.28 (12H, CCH_3), 1.53–1.63 (4H, CCH_2C), 1.68–1.81 (4H, CCH_2C), 2.4 (2H, NCH_2Si), 2.89–3.26 (4H, NCH_2), 8.13 (1H, NH). ^{13}C NMR (DMSO), δ (ppm): 20.30, 23.59, 25.60, 49.23, 64.33, 68.74, 175.59. UV/Vis (CH_2Cl_2): λ_{max} ($lg\varepsilon$) = 283 (2.45), 325 (2.51), 450 (2.65), 459 (2.66). IR (KBr) ν_{max}: 3430, 2980, 2938, 1728, 1463, 1392, 1343, 1240, 1100, 538, 481 cm^{-1}.

4,4′-bis{1-(N-Perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxacyclopentane-3-on)]ate}: ^{1}H NMR (DMSO), δ (ppm): 1.48–1.59 (8H, CCH_2C), 1.72–1.89 (8H, CCH_2C), 2.1–2.2 (4H, NCH_2Si), 2.98–3.24 (8H, NCH_2), 4.16 (4H, CCHO), 7.85 (2H, NH). ^{13}C NMR (DMSO), δ

(ppm): 29.59, 25.43, 25.52, 49.99, 58.57, 74.99, 173. UV/Vis (CH_2Cl_2): λ_{max} (lgε) = 283 (2.45), 302 (2.48), 325 (2.51). IR (KBr) ν_{max}: 3300–3500, 2935, 2862, 1708, 1716, 1473, 1240, 1092, 534, 510, 447 cm^{-1}.

RESULTS AND DISCUSSION

The aim of the investigation was to separate and determine the analyzed compounds in the shortest time. Results obtained by the capillary isotachophoresis with conductometric detection are presented in tables 1–4, optimal conditions of separation and determination on isotachophoregrams (Figures 3–5).

Analyses were carried out in aqueous solutions: acidic, neutral and basic. During isotachophoretic analysis only ions with the same sign – cations or anions were determined. The separation was carried out due to differences in electrophoretic mobility of analyzed ions. These compositions of leading (Ld-1 and Ld-2) and terminating (Tm) electrolytes enabled determination of individual silanates as well as their mixture in acidic medium.

As proposed in literature, the most often applied terminating electrolytes are characterized with slightly larger mobility than compounds analysed in the present work. However, their introduction to the environment are not indifferent. Therefore, by the process of trial and error and by addition of the standard, a new terminating electrolyte was elaborated and used. It is essential for isotachophoretic separation for the leading electrolyte ions to possess higher mobility higher and terminating electrolyte – ions with lower mobility – than ions in the sample. Double salt: 4,4′-bis{1-(N-perhydroazepiniomethyl)[spirobi(1-sila-2,5-dioxacyclopentane-3-on)]ate}, showed to be an excellent terminating electrolyte with suitable mobility. This solution was applied on purpose as the terminating electrolyte, due to its lack of negative influence on the environment. Hypercoordinated derivatives of organosilicon compounds are easily soluble in water, and not poisonous. They are biologically active, some

Table 1. Common parameters of the method of ES-1 and ES-3 separation and determination

Parameters of the method	
UV filter [nm]	200
High Voltage Limit [V]	12000
Sample rate [smp/s]	50
Polarity	+ cations

Table 2. Optimum conditions of ES-1 determination on the preseparation column

Stage	Time [s]	Intensity [μA]	Comp [10 mV]	Conductometric detector
1	300	100	0	
2	120	200	50	
3	180	200	0	X

of them can be applied as plant growth regulators.[16–18] To recap, hypercoordinated organosilicon compounds show a very positive influence on the environment and live organisms. Therefore, the proprieties of these groups decided on proposing the new terminating electrolyte.

Separation and determination of the chosen compounds was carried out by the use of two dimensional capillary isotachophoresis with a switching column. Optimal conditions for separation and determination of chosen compounds have been elaborated. Quantitative analysis was based on a comparison of a zone height on the sample isotachophoregram with the zone height on isotachophoregram obtained for standard solution.

Analyzed compounds and their mixtures were subjected to various tests in order for maximum separation to obtain a number of zones containing one type of ions. Limits of zones are sharp when all zones are characterized by different effective ion mobility. It is connected also with the decrease of conductivity of a zone, i.e., increase of resistance and with changes of ion concentration in this zone.

Analyzed compounds caused many difficulties during isotachophoretic determination, because of small mobility differences. Until now, attempts to analyze these compounds have been not reported in the literature. During optimization of determination the various conditions of this method were tested: time of the analysis, current intensity, level of high voltage limitation, and different columns.

Attempts of optimization of separation and determination of chosen ES – silanates were carried out by changes of voltage from 10000 V to 15000 V. Experiments carried out under 10000 V failed. Obtained

Table 3. Optimum conditions of the method of ES-3 determination on preseparation column

Stage	Time [s]	Intensity [μA]	Comp [10 mV]	Conductometric detector
1	150	100	0	
2	160	200	50	
3	100	200	0	X

Table 4. Optimum conditions of determination of the mixture of ES-1 and ES-3

Stage	Time [s]	Intensity [μA]	Comp [10 mV]	Conductometric detector
1	500	100	0	
2	160	200	50	
3	100	200	0	X
4	500	50	50	–
5	100	50	0	X

isotachopherograms below 10000 V voltage showed only one compound. Voltage decreasing caused in decreasing the difference of analyzed ions mobility. 15000 V is the maximum value of voltage that is possible to obtain on the ITP analysator. The high value of voltage caused the end of analysis before the planned time. The best results of separation were obtained when high voltage was limited to 12000 V (Table 1).

A speed of ions in an electric field depends on current intensity of this field. Therefore, in various steps of the method this parameter was changed. The next stages of the optimization were separations and determinations in different values of pH of solutions of analyzed samples: acidic (pH = 4), neutral (pH = 7), and basic (pH = 11). The best results were

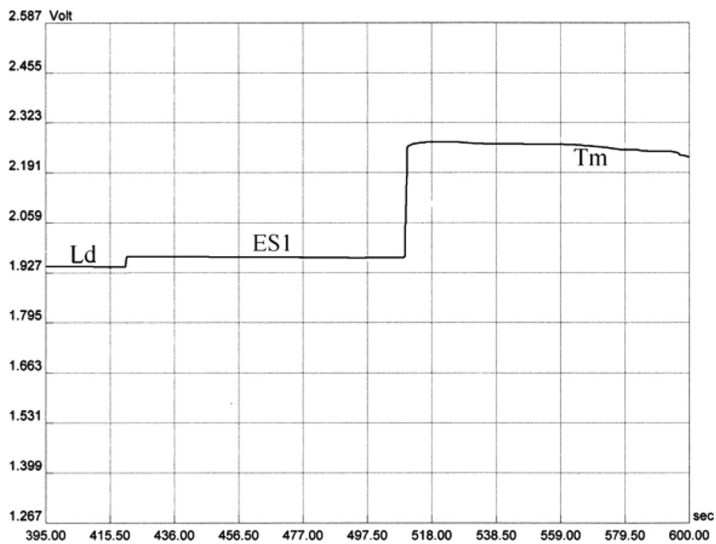

Figure 3. Isotachophoregram of ES-1 obtained on preseparation column (Conditions of ES-1 determination on the preseparation column in Table 3).

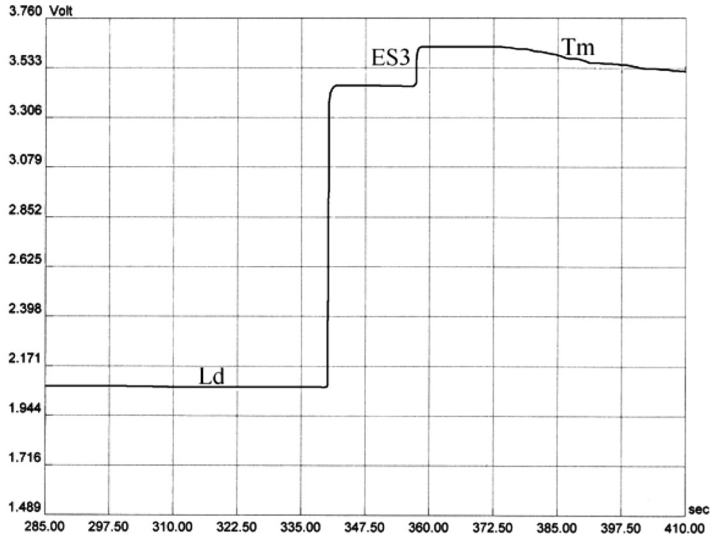

Figure 4. Isotachophoregram of ES-3 obtained on the preseparation column (Conditions of ES-3 determination on the preseparation column in Table 4).

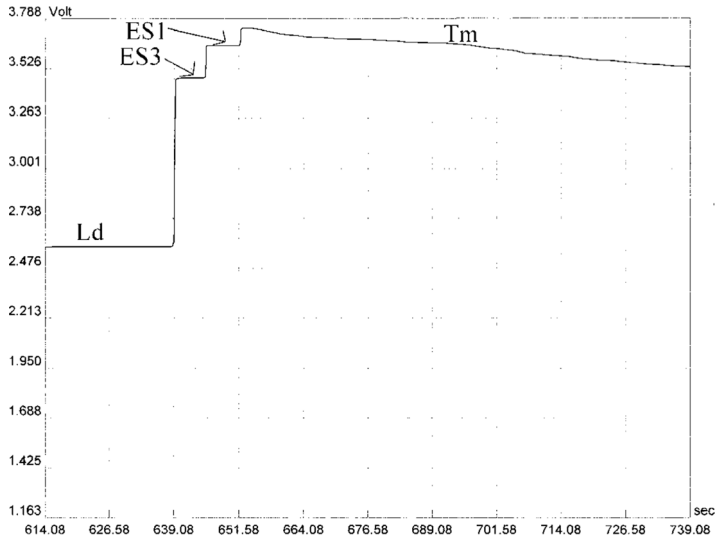

Figure 5. Isotachophoregram of the mixture of ES-1 and ES-3 obtained on the analytic column (Conditions of determination of the mixture of ES-1 and ES-3 in Table 5).

obtained in acidic medium. With the increase of pH, differences of effective mobility's of ions in the analyzed mixture, decreased.

Analyses of acidic (pH = 4) solutions allowed for complete separation of ES-1 and ES-3 as components of mixtures and for analyses of individual compounds. Standard curves were obtained in the same conditions.

Consideration of obtained isotachophoregrams (Figures 3–5) and conditions of the method (Tables 1–4) showed that the shortest time of analysis for compound ES-3 was 6 min, for ES-1–8.4 min. Good separation for a mixture of both ES-silanates was achieved in 11 min (Figure 4). The structures of analytes after analysis were examined again. They answered the initial structures of analyzed relationships.

Differences in a speed of charged particles depend mainly on their magnitude, charge, and molecular mass. It allows separating systems with various magnitude and structure of molecules.

High efficiency of separation in comparison with other electrophoretic methods and short time of analysis show that capillary isotachophoresis (ITP) can compete not only with HPLC but also with other analytic techniques. However, this method is still under investigation, as well as theoretic bases as practical applications.

Isotachophoresis allows determination of chosen ES-silanates in an aqueous solution. Sample preparation is simple (only the dissolution in water), the method is cheap, and is suitable for routine analyses. It can be considered as a "green chemistry" technique, because toxic solvents or reagents are not used.

Precision and accuracy of the obtained results by the capillary isotachophoresis method are better than these obtained by classical methods (Table 5).[2]

Table 5. Characteristic of analytical method used

Parameter	Unit	For examined ion
Precision[a]	%	2.5–3.3
Recovery[b]	%	91.5 ± 6
Linearity[c]	$Mg \cdot L^{-1}$	1–28
Limit of identification[d]	$Mg \cdot L^{-1}$	1

[a] n = 6, the samples were analyzed twice.
[b] The sample was enriched with 1.5 mL of a solution containing $1\,\mu g \cdot mL^{-1}$ of examined ion, n = 6.
[c] Correlation coefficient above 0.988.
[d] Calculated from the limit of identification and coefficients of the calibration curve.

CONCLUSIONS

A method of separation of analyzed compounds has been elaborated. It takes less than 12 min. A new terminating electrolyte has been worked out. Analyzed compounds cause numerous difficulties in electrophoretic determinations, because mobility's of their ions only slightly differs one from another. The best and the optimal separation of analysed mixture was obtained under 12000 V voltage. Below 10000 V the difference in mobility of analysed ions was decreased, and isotachpherograms showed only one type of analysed ions. Optimization of a method of determination of this class of compounds should help in research of silicon chemistry. New, elaborated method of separation and determination opens wide possibilities of investigation of biological activity of this type of substances.

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