

Electrophoretic Separation and Determination of Small Amounts of Copper(II), Iron(III), and Iron(II) Ions in Some Inorganic Chemicals by Indirect Densitometry

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The feasibility to separate small amounts of copper(II) salts in lead(II) acetate, aluminium sulfate, and iron(III) chloride, of iron(III) salts in lead(II) acetate and aluminium sulfate, and of iron(II) salts in copper(II) sulfate from the main component by means of paper electrophoresis has been studied. Besides, the applicability of the indirect densitometry of photographic negatives for the determination of the separated contaminants has been investigated. It has been found that 0.2–1% of copper(II), iron(III), and iron(II) ions, respectively, may be determined in above chemicals. The results have been evaluated statistically. The relative deviations of the determination have been within the range of 3–10%.

In spite of its separation efficiency and high sensitivity, the paper electrophoresis has not been widely used in the field of inorganic chemistry till now. In this study, we tried to use the paper electrophoresis for the separation of admixtures of ionic character from the main component and to determine them without elution. This combination does not require any special equipment and is not tedious.

As a matter of fact, we pursued the determination of copper(II) ions in lead(II) acetate, aluminium sulfate, and iron(III) chloride, of iron(III) salts in lead(II) acetate and aluminium sulfate, and of iron(II) salts in copper(II) sulfate. The major problems in the use of this procedure involved the fixation of experimental conditions for a full separation of mixture as well as the selection of a method suitable for the quantitative evaluation of electrophoretic patterns.

On the basis of the papers hitherto published we went in search of the conditions suitable for separation. But these papers were mostly concerned only with the qualitative separation of approximately equimolar mixtures of ions. These separations involved the use of complex-forming substances as efficient components of the supporting electrolyte, namely mineral acids [1, 2] or their salts [3] and more frequently chelate-forming organic compounds mainly from the group of carboxylic hydroxy acids (lactic acid [4, 5], hydroxy-isobutyric acid [6, 7], tartaric acid [4, 5], and citric acid [5, 8]) or polycarboxylic amino acids [9]. It was found that the *N*-(2-hydroxyethyl)iminodiacetic acid (HIDA) [10, 11] possessed the properties exceptionally suited for the electrophoretic separation of cations. The regular and sharply marked zones of its chelates were a very good prerequisite of its utilization to quantitative purposes.

The densitometric estimation usual in paper chromatography and electrophoresis is very simple in contrast to elution methods. However, it has some drawbacks due

to time instability of the coloured products formed in detection reactions. This method was criticized by Keil [12] who also proposed the „indirect“ densitometry of the negatives obtained by photographing the detected chromatograms in reflected light and employed this procedure for the quantitative evaluation of protein hydrolysates.

In this study, the principle proposed by Keil was applied for the electrophoresis of above inorganic systems and the procedure was improved by using better equipment. The reproducibility of different determinations was evaluated statistically.

Experimental

Chemicals and solutions

N-(2-Hydroxyethyl)iminodiacetic acid was prepared by condensation of ethanolamine with iminodiacetic acid [13]. The preparation of model mixtures was carried out by using:

a) 20% aqueous solutions of the salts $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$, $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$;

b) standard 1% solutions of admixtures, prepared as follows: Cu(II) solution (from $\text{Cu}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), determined complexometrically with murexide; Fe(III) solution (from $\text{Fe}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$, mildly acidified with HNO_3), determined complexometrically with cinnamohydroxamic acid [14]; Fe(II) solution (from $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ — the solution was bubbled through under addition of Pt — black by hydrogen till the reaction of Fe(III) was negative), determined after oxidation by H_2O_2 complexometrically with cinnamohydroxamic acid.

The mixtures for analyses were prepared so as to contain 5% of main salt and 0.01—0.05% of admixtures. When the samples of lead(II) acetate with an admixture of Fe(III) as well as the samples of copper(II) sulfate with an admixture of Fe(II) were prepared, HIDA was added into solution in such an amount that its final concentration should be 10^{-1} M.

Electrolytes

a) 5×10^{-2} M solution of HIDA was prepared from 2×10^{-1} M solution of HIDA sodium salt by dilution and adjusting to convenient pH by means of 1 M- HNO_3 under potentiometrical control;

b) 0.33 M solution of citric acid.

Detection reagent

1% aqueous solution of sodium diethyl dithiocarbamate (Cupral).

Equipment and working procedure

The horizontal electrophoresis with cooling and the working procedure described in previous papers [10, 11] were used. The samples were spotted with a $10 \mu\text{l}$ micropipette. The detection was performed by drawing a dried electrophoretic pattern through the detection solution and drying it in air.

The conditions for the separation of different mixtures were determined on the basis of preliminary qualitative experiments as given in Table 1. Other ions which give intensively coloured diethyl dithiocarbamate complexes, namely Ni(II), Co(II), Cr(III),

Table 1

Experimental conditions for electrophoretic separation: 20°C, voltage gradient 15 V cm⁻¹

Main component	Admixture	Electrolyte	Necessary time
aluminium sulfate aluminium sulfate lead(II) acetate	Cu(II) Fe(III) Fe(III)	HIDA, pH 2.7	1 h
lead(II) acetate copper(II) sulfate iron(III) chloride	Cu(II) Fe(II) Cu(II)	HIDA, pH 2.1 HIDA, pH 1.8 0.33 M citric acid	1 h 1/2 h (15°C) 1 1/2 h

and UO_2^{2+} have mobilities dissimilar to those of Fe(III), Fe(II), and Cu(II) and they thus do not interfere with determination [8, 11]. But in the solution of HIDA having pH 2.7, Ni(II) and Cu(II) do not separate perfectly so that the determination of Cu(II) in the presence of Ni(II) may not be possible.

It was found that the above mixtures might be spotted in the form of solutions of the simple salts in most cases and it was not necessary to transform them in HIDA complexes. Only the separation of Fe(III) from lead(II) acetate or from aluminium sulfate required to spot the samples in the form of complexes. In opposite case, the zones of Fe(III) had irregular shape which was not convenient for quantitative evaluation.

Indirect densitometry of electrophoretic patterns

The quantitative evaluation of electrophoretic patterns was based on the method of indirect densitometry [12]. The original method of Keil was modified as follows: The electrophoretic patterns were photographed on the document 4° DIN orthochromatic standard film. An exposure speed of 8 sec and stop number of 11 were used while the electrophoretic patterns were illuminated by two 60 W electric bulbs from the distance of 40 cm. The densitometric evaluation of negatives was carried out by means of high-speed photometer „Zeiss“ which is usually used for scanning emission spectra. The

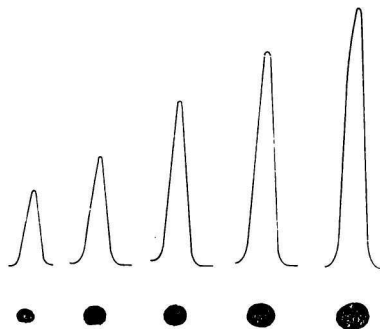


Fig. 1. Electrophoretic pattern and the corresponding densitometric curve for the determination of Fe(III) present in the amounts of 0.2–0.4–0.6–0.8–1.0% in aluminium sulfate.

slit width of photometer always amounted to 1 mm while its height was adjusted for every negative separately according to the size of the biggest spot. Instead of manual shift of negative in photometer and plotting the numerical values of optical density which were to be worked up graphically, the instrument was adapted for automatic drift by means of a synchronous motor and joined to a line compensation recorder (KBT 1/EN, VEB Messgerätewerk, Magdeburg) which was adjusted to the recording of current. The densitometric curves showed several maxima corresponding to individual zones on negative. The determination of Fe(III) in aluminium sulfate is given in this paper as an example (Fig. 1). The areas of maxima were evaluated by the use of planimeter (polar planimeter K-800 MOM Budapest).

The background correction was given up and the base line was determined in the same way as in gas chromatography. The reproducibility of the method thus modified was tested by statistical evaluation of the results.

Results

Verification of the linearity of calibration curves

The direct densitometry of chromatograms and electrophoretic patterns is based on the linear relationship between the area demarcated by the maximum of densitometric curve

Table 2

Fluctuation of values about regression lines for the determination of copper(II), expressed by standard deviations (s) and variation coefficients (v)

Main component	Admixture	Added [%]	Found* [%]	s [%]	v [%]
$\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$	Cu(II)	0.2	0.19	0.011	5.58
		0.4	0.42	0.025	6.49
		0.6	0.61	0.020	3.35
		0.8	0.78	0.037	4.66
		1.0	1.00	0.032	3.25
$\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$	Cu(II)	0.2	0.20	0.008	4.00
		0.4	0.41	0.013	3.25
		0.6	0.60	0.019	3.22
		0.8	0.79	0.024	3.07
		1.0	1.00	0.011	1.10
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	Cu(II)	0.2	0.20	0.016	8.05
		0.4	0.39	0.032	7.99
		0.6	0.59	0.037	6.24
		0.8	0.77	0.043	5.47
		1.0	1.01	0.033	3.35

* Average value from ten analyses.

and the amount of substance in the corresponding zone what enables us to draw the relevant calibration curve. The validity of this assumption for the indirect densitometry as well as the reproducibility of this mode of determination was tested by a mathematical, statistical evaluation of a few series of standard solutions containing concentrations of admixtures increasing within the range of 0.2–1% (referred to the main component of mixture). For each series ten parallel determinations were performed and the values obtained were worked up by the least square method. On the basis of regression values the standard deviations corresponding to various types of mixtures as well as concentrations of admixtures were calculated. The values found for the determination of copper (Table 2) may be considered as an instructive example. After statistical evaluation, it is to be found that the determination of iron(III) and iron(II) ions present as admixtures shows quite analogous parameters.

Analysis of model mixtures

The model samples were prepared so as to contain the admixture within the concentration range of 0.2–1% (of corresponding ion). For the spotted volume of 10 μ l of sample solution, it involved 1–5 μ g of admixture in 0.5 mg of sample when expressed in absolute values. Two internal standards which contained besides the main component the estimated

Table 3
Results of the analysis of model mixtures

Main component	Admixture	Added [%]	Found* [%]	<i>s</i> [%]	<i>v</i> [%]
$\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$	Cu(II)	0.24	0.24	0.024	10.0
		0.50	0.52	0.030	6.0
		0.70	0.68	0.019	2.7
		1.00	0.94	0.033	3.3
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	Cu(II)	0.24	0.23	0.016	6.7
		0.50	0.44	0.050	10.0
		0.70	0.66	0.072	10.3
		1.00	1.01	0.054	5.4
$\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$	Cu(II)	0.24	0.26	0.037	15.0
		0.50	0.54	0.035	7.0
		0.70	0.72	0.031	4.4
		1.00	0.96	0.057	5.7
$\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$	Fe(III)	0.30	0.31	0.030	10.0
		0.60	0.60	0.058	9.7
		0.90	0.92	0.059	6.6
$\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$	Fe(III)	0.30	0.31	0.020	6.7
		0.60	0.59	0.053	8.8
		0.90	0.88	0.061	6.8
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	Fe(II)	0.33	0.36	0.026	7.2
		0.66	0.70	0.030	4.3
		0.98	0.99	0.034	3.5

* Average value from ten determinations.

admixture in concentrations corresponding to lower and upper limiting values of the investigated concentration range were spotted together with samples for electrophoretic determination. The areas of maxima on densitometric curve corresponding to standards were used for drawing the calibration curve. The content of admixtures in the model samples was determined graphically by the use of calibration curve as usual. The standard deviations and variation coefficients were calculated from ten parallel determinations. The results are summarized in Table 3.

Discussion

The question was to separate the small amounts of one substance from the large excess of main component in the mixtures under investigation. In general, the shift of concentration ratio to extreme values affects the quality of separation in an unfavourable manner. At the same time, the scope of applicability of method is given by the limiting concentrations of both components at which a perfect separation still occurs and simultaneously a reliable detection of the trace component is possible. Under working conditions we used, this ratio was 1 : 500 for all systems analyzed what corresponded to 0.2% of admixture.

Regarding the deficiency of literary data [12], we considered it necessary to test the linearity of calibration curves in the investigated scope by the method of least squares. Of course, the fluctuation of values about the regression line increased with decreasing concentration, but it always remained within reasonable limits. The calculated variation coefficients were within the range of 1–5%, but in case of Cu(II) determination in iron(III) chloride the deviation reached the value of 8%.

After the linearity of calibration curves had been confirmed and the results statistically evaluated, the analysis of model mixtures containing the corresponding ion in the amount of 0.2–1% was performed. In this case, the graphical evaluation manifests a rise in standard deviations which keep within the range of 0.02–0.06%. The relative errors of determination expressed by variation coefficients are dependent on the amount determined and increase with decreasing concentration. Nevertheless, a deviation exceeded the limit of 10% in a single case. In all other cases, the variation coefficients fluctuated over the range of 3–10%. With respect to the very small amounts of substances which were determined, these results may be considered to be satisfactory since they are adequate to the efficiency of the method itself.

The accuracy of the method is lower in comparison with some other analytical methods but this deficiency is made up by high sensitivity and selectivity of the determination as well as by a minimum consumption of sample. It is especially suitable for series analyses of the small amounts of samples since it does not require any preceding chemical separation.

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