

CENTRIFUGALLY ACCELERATED PAPER CHROMATOGRAPHY
OF INORGANIC IONS

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Since the original work of CONSDEN, GORDON AND MARTIN¹ paper chromatography has been applied to the problems of separation of both organic and inorganic mixtures that were difficult or time-consuming to separate by more conventional methods of analysis. The period of the order of hours usually necessary for the development of chromatograms in order to obtain satisfactory separation has been, however, the major drawback to applying this technique to the analysis of labile compounds, unstable valency states, and radioelements of short half-life. Attempts have recently been made to speed up the flow of solvent through the paper by the use of volatile solvents², or by developing the chromatograms at higher temperatures³⁻¹⁰. Although these modifications when applied to some particular problems gave more satisfactory results than conventional paper chromatography, they suffer, however, from the drawback that it is difficult to obtain equilibrium conditions with volatile solvents, and that changes in composition of the species or valency states may occur at higher temperatures.

An interesting innovation recently introduced by CARONNA¹¹ and developed in particular by McDONALD and coworkers¹²⁻¹⁷ has permitted the development of chromatograms in very short times by combining chromatography with centrifugal acceleration. This procedure has been successfully applied to the separation of dyes¹⁵, amino acids^{15, 16}, chloroplast pigments¹⁸, ⁸²Br- and ¹³¹I-labelled organic substances¹⁹, human serum lipoproteins¹⁷, 2,4-dinitrophenylhydrazones of carbonyl compounds²⁰, and some substances of the vitamin B₆ group²¹. The only separation of inorganic ions by this technique reported in the literature is that of Cu, Cd, Bi, and Hg, by INDOVINA AND RICOTTA²². The success achieved in the rapid separation of organic compounds and the improved results over those given by ordinary chromatographic techniques prompted us to extend the application of centrifugally accelerated paper chromatography to inorganic and radiochemical problems. The present communication reports the initial results of a study of fundamental factors that govern good and reproducible separation of inorganic ions by this technique. The study was made with Fe(III), Co(II), Ni(II), Mn(II), Cu(II), and Zn(II) ions with R_F values that extend over a wide range.

APPARATUS

In the literature descriptions have been given of several apparatus for centrifugal

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paper chromatography^{11-17, 19, 23-28} as well as various modifications in the procedure. When we undertook the present work two models of the apparatus (Chromatofuge Modèle T. 65, and Centrifugeur pour chromatographie par Ets. Jouan) were commercially available. Because of the lower price and its simplicity, the apparatus made by Etablissements Jouan was chosen for these studies. The apparatus is shown in Fig. 1. Its chief characteristics are: rate of rotation: variable between 500 to 1200 rev./min; size of paper: up to 45 cm diameter. Dimensions of the apparatus: diameter 50 cm; height 26 cm; weight 4 kg.

The apparatus consists essentially of a detachable developing tank (of Perspex or stainless steel, that of Perspex being used in the present work) provided with a removable cover fastened by three pins. A Petri dish with the solvent placed in the tank permits an easy saturation of the atmosphere within the tank with the vapour of the solvent. The circular sheet of paper is held in position at the centre of the tank on a Teflon disk coupled magnetically to the motor. During rotation the paper disk assumes a taut horizontal position. Each tank is equipped with a magnetic disk covered with Teflon to which the rotation is imparted by a magnetic drum attached to the motor. Thus every tank is air-tight.

The circular paper is spotted, by means of a micro pipette, with the solution to be analysed on previously marked points on a circle of 4.5 cm radius. To prevent inward movement of the spots, the developing solvent was fed at 3 cm from the centre of the paper with the aid of a glass atomiser (Fig. 2). The desired delivery of the solvent in the form of a fine continuous stream could be adjusted by regulating the pressure of air and the diameter of the capillary connecting the atomiser with the solvent vessel.

EXPERIMENTAL AND RESULTS

Fe(III), Co(II), Ni(II), Mn(II), Cu(II), and Zn(II) chloride solutions in *N* HCl were spotted on the paper (diameter 38 cm), either separately or as a mixture. Butanol-HCl (1:1, v/v) mixtures, in which the concentration of the acid was varied, were used as solvents. Acetone-HCl-H₂O mixtures, well known for giving good resolution of many of the above ions in conventional chromatography²⁰, were also tried. The spots on the chromatograms were detected by means of an ammoniacal solution of 8-hydroxyquinoline in ethanol.

The following types of paper were used in the present studies: Whatman No. 1, Whatman No. 3 MM, Arches No. 302, Arches No. 304 and Ederol No. 202.

Effect of the type of paper on the nature of the chromatogram

As already observed by McDONALD *et al.*¹⁵ in the case of Whatman No. 1 paper, our solvents gave with Whatman No. 1, Whatman No. 3 MM, Arches No. 302 and Arches No. 304, elliptical chromatograms, the long axis coinciding with the machine direction of the paper. Almost circular patterns were obtained with Ederol No. 202. Measurement of the R_F values of Fe(III) in different directions in the case of elliptical chromatograms showed variations of about 12%, while in the case of Ederol No. 202 the R_F was found to be constant, *viz.* 0.17 for butanol-*N* HCl, in all directions of the paper. The detailed study reported in the present article was therefore carried out with Ederol No. 202. In order to compare the R_F values on centrifugal chromatograms with those obtained with conventional chromatographic procedures, separa-

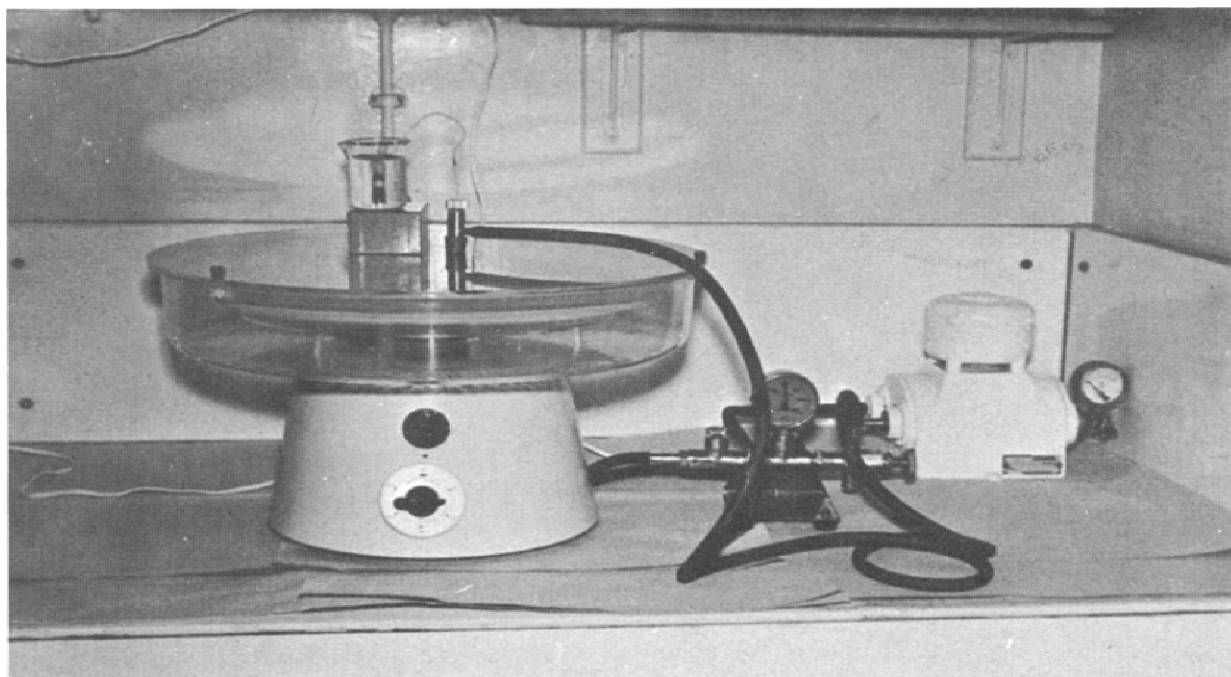


Fig. 1. Apparatus used for centrifugal chromatography.

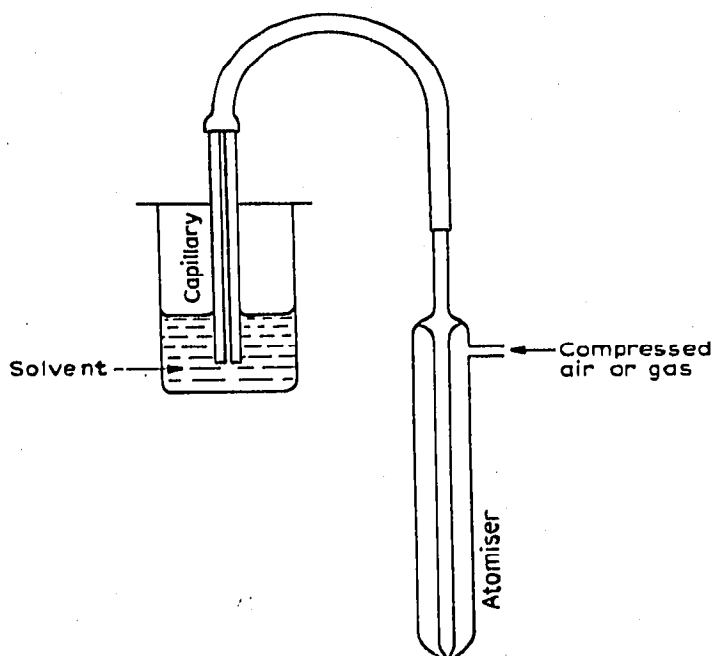


Fig. 2. Device for feeding the developing solvent to the paper.

tions were also realised side by side by the ascending technique on 3.5 cm wide Ederol No. 202 paper strips, since the R_F values of these ions on this paper have not been reported in the literature.

Effect of the solvent and its flow rate on the chromatograms

The pattern of the chromatograms and the quality of separation were greatly influenced both by the nature of the solvent and by its rate of flow. Volatile solvents, e.g. acetone-acid mixtures, are not favourable for centrifugal chromatography owing to evaporation of the solvent from the paper. A higher delivery rate of the solvent in order to compensate for the evaporation often leads to flooding of the paper, which gives rise to elongated and diffused bands on the chromatogram. Thus volatile solvents demand a careful adjustment of the flow rate for a given speed of rotation of the paper. Less volatile solvents, e.g. butanol-HCl mixtures, are most suitable for this technique and give a wider operation range. In the present work the flow rate of the solvent for "minimal wetting" of the paper was always determined in each case by preliminary experiments guided by the conditions described by McDONALD and coworkers¹⁵, viz., "the ideal flow rate ought to be such that on stopping both the flow of solvent and the rotor, the perimeter of the wetted area does not increase appreciably on standing". For Ederol No. 202 paper this flow rate varied within the range 1.5-2.25 ml/min, depending on the rate of rotation of the paper and composition of the solvent.

Effect of the rate of rotation of the paper on the chromatograms

Thick papers, namely Whatman No. 3 MM, Arches No. 304, and Ederol No. 202, could be utilised for the whole range of the rotation speeds of the apparatus; Arches No. 302 was less suitable, while the disks of Whatman No. 1 could not stand a speed higher than 500 rev./min with the solvents used.

Since the front portion of the spots moves slower than the rear portion, the spots in centrifugal chromatography are more compact than those observed with other chromatographic procedures. Thus a greater resolution for a relatively shorter run of the solvent is obtained. At higher speeds the spots are more compact and the separation therefore more complete.

Separation of Fe(III), Co(II), Ni(II), Mn(II), Cu(II) and Zn(II)

Mixtures of acetone, water and HCl, which gave satisfactory results with Ederol No. 202 paper strips in ascending paper chromatography, were not convenient solvents for centrifugal chromatography for the reasons already pointed out. Table I lists the variation of the R_F values of these ions when the concentration of HCl is increased in the butanol-HCl (1:1, v/v) mixture. The R_F values obtained with the ascending technique are also given for comparison. For the six ions examined and over the entire range of solvents used the R_F values on the centrifugal chromatograms are higher than those obtained by the ascending technique. INDOVINA AND RICORTA²² have also observed higher R_F values for Cu, Cd, Bi and Hg ions, in the solvent butanol-acetic acid-conc. HCl-water (45:10:1:44) in centrifugal chromatography than in the stationary technique. The R_F values have been shown to depend on the partition coefficient and the relative amounts of the two phases in contact²⁰. Since the experiments with the centrifugal and ascending techniques were carried out

with the same solvent and paper system, the partition coefficient can be assumed to be practically constant. The increase in the R_F values in the case of centrifugally accelerated chromatography can therefore be attributed to the higher rate of flow of the organic phase in relation to the aqueous phase.

TABLE I

R_F VALUES OF Fe(III), Co(II), Ni(II), Mn(II), Cu(II) AND Zn(II) OBTAINED IN CENTRIFUGAL CHROMATOGRAPHY AND BY ASCENDING DEVELOPMENT

Solvent: butanol-HCl (1:1, v/v) mixtures. Paper: Ederol No. 202. Speed of rotation: 1200 rev./min. The values in parentheses are the R_F values obtained by the ascending technique.

Normality of acid	Fe(III)	Co(II)	Ni(II)	Mn(II)	Cu(II)	Zn(II)
1	0.17 (0.092)	0.091 (0.04)	0.08 (0.043)	0.10 (0.059)	0.15 (0.086)	0.76 (0.62)
2	0.37 (0.17)	0.17 (0.088)	0.16 (0.077)	0.20 (0.092)	0.28 (0.18)	0.90 (0.73)
3	0.49 (0.28)	0.26 (0.17)	0.26 (0.15)	0.31 (0.15)	0.37 (0.23)	0.94 (0.78)
4	0.72 (0.52)	0.51 (0.34)	0.48 (0.29)	0.51 (0.25)	0.58 (0.42)	0.97 (0.96)
4.25	0.76 (0.61)	0.66 (0.46)	0.65 (0.46)	0.67 (0.46)	0.70 (0.52)	0.97 (0.96)
5	0.81 (0.57)	0.66 (0.48)	0.65 (0.46)	0.68 (0.45)	0.71 (0.56)	0.93 (0.95)
6	0.90 (0.98)	0.68 (0.48)	0.66 (0.43)	0.70 (0.49)	0.72 (0.59)	0.92 (0.95)
7	0.90 (0.97)	0.62 (0.48)	0.62 (0.44)	0.66 (0.46)	0.70 (0.57)	0.91 (0.91)
8	0.98 (0.96)	0.63 (0.45)	0.60 (0.39)	0.67 (0.43)	0.73 (0.56)	0.89 (0.82)
9	0.98 (0.98)	0.65 (0.47)	0.62 (0.34)	0.64 (0.38)	0.71 (0.56)	0.88 (0.72)
10	0.98 (0.98)	0.65 (0.57)	0.60 (0.31)	0.63 (0.38)	0.71 (0.56)	0.86 (0.67)
11	0.98 (0.97)	0.64 (0.63)	0.57 (0.29)	0.61 (0.36)	0.70 (0.54)	0.85 (0.67)
12	0.98 (0.96)	0.67 (0.65)	0.51 (0.26)	0.55 (0.37)	0.72 (0.56)	0.83 (0.67)

Except that the values were higher, the manner in which the R_F 's of the ions examined varied with the concentration of hydrochloric acid in the mixture was found to be similar to that observed in the ascending technique. This indicates that the mechanism of separation of these inorganic ions in centrifugal chromatography is essentially the same as that in conventional chromatography.

The best separation of the six ions examined is obtained (Fig. 3) with butanol-12 N HCl (1:1, v/v) in 45 minutes by centrifugal chromatography. Although the separation is better in this solvent than in the other solvents used, the ascending technique does not permit (Fig. 4) the separation of Co(II) and Zn(II).

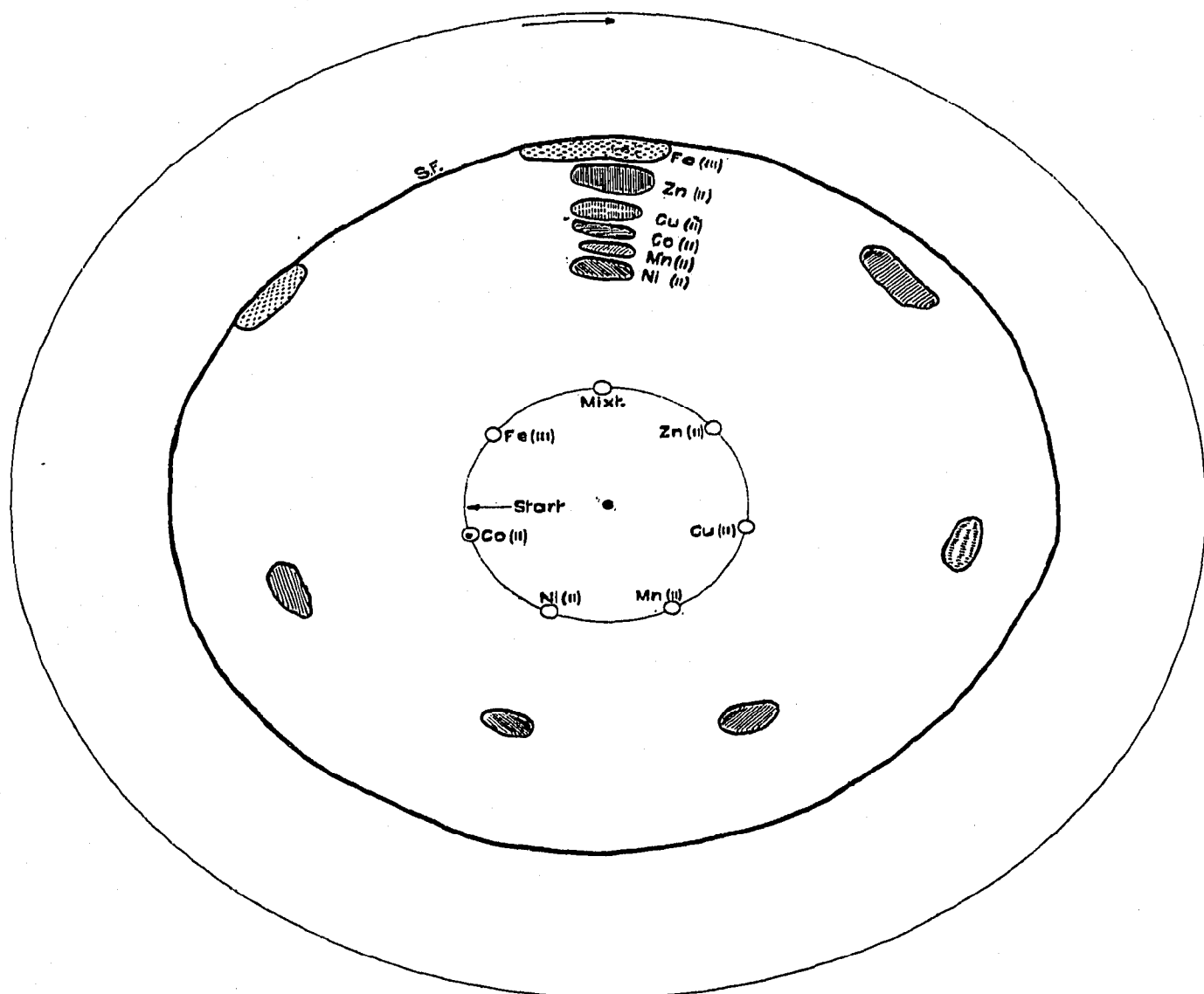


Fig. 3. Centrifugal chromatogram of Fe(III), Co(II), Ni(II), Mn(II), Cu(II) and Zn(II), and of a mixture of these ions. Paper: Ederol No. 202 (radius = 19 cm). Solvent: butanol-12 N HCl (1:1, v/v). Speed of rotation: 1200 rev./min. Time of development: 45 min.

The advantages of centrifugal chromatography are thus: (1) better resolution in a fraction of the time (10-15 times faster) required by other chromatographic methods; (2) possibility of studying the separation of many substances at the same time and under the same experimental conditions.

SUMMARY

Centrifugally accelerated paper chromatography has been applied to the separations of inorganic ions. A study has been made of the various factors which favour good and reproducible fractionation. A comparative study of the separation of Fe(III), Co(II), Ni(II), Mn(II), Cu(II) and Zn(II) by this procedure and by ascending chromatography is reported. The developing solvent consisted of butanol-HCl

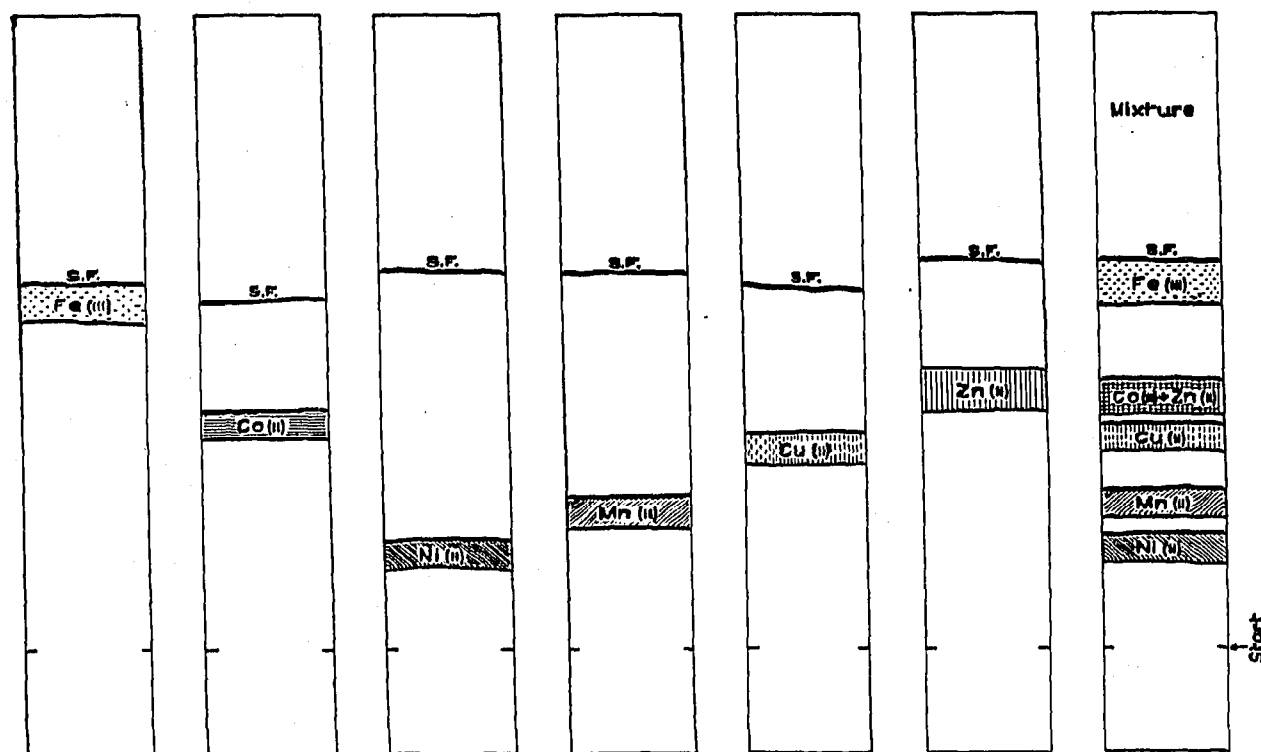


Fig. 4. Chromatograms of Fe(III), Co(II), Ni(II), Mn(II), Cu(II) and Zn(II), and of a mixture of these ions, obtained by ascending development. Paper: Ederol No. 202 (3.5 cm wide strips). Solvent: butanol - 12 N HCl (1:1, v/v). Time of development: 7 h.

(1:1, v/v) mixtures in which the concentration of the HCl was varied in the concentration range from 1 to 12 N. The best separation of all the six ions was obtained with the solvent butanol-12 N HCl using centrifugal chromatography only.

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