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SEPARATION OF OPTICAL ISOMERS BY ION-EXCHANGE CHROMATOGRAPHY USING COPPER(II) IONS AS COMPLEX-FORMING AGENTS

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SUMMARY

On the basis of the differences between the stability constants of complexes of D- and L-aminodiols with copper(II) ions, an ion-exchange chromatographic method using copper(II) ions as complex-forming agents was developed for the quantitative separation of pairs of optical isomers. The stability constants of some complexes were determined. The distribution coefficients of two enantiomers were examined by static batch and elution methods. The optimal conditions of the separation and the composition of the eluent are discussed.

INTRODUCTION

The known methods for the separation of optical isomers are based mainly on reactions involving the formation of diastereoisomers. Using these reactions, ion-exchange and other chromatographic methods have been developed[1-3].

We have found that ion-exchange chromatography, in which the principle of separation is the difference between the stability constants of the two enantiomers formed with the same metal ion, can also be used for separations in a few cases. A chromatographic method has been developed for the separation of optical isomers (D- and L-forms) of racemic *threo*-1-(*p*-nitrophenyl)-2-amino-1,3-propanediol (D- and L-aminodiols) using copper(II) ions as complex-forming agents.

In order to determine the complex formation constants of D- and L-aminodiols, pH titrations were carried out. The stability constants of copper(II) complexes were determined by a modified graphical method of Leden[4]. We found that there are deviations in the values of the stability constants of the corresponding complexes.

In order to obtain information on the distribution properties of the two enantiomers between a cation-exchange resin and solutions containing copper and sodium ions, distribution experiments were carried out using the static batch method. We found that D-aminodiol complexes are more strongly bound than those of the L-isomer to the resin in the copper form if there is no copper in solution, corresponding to the order of the stability constants of the complexes. However, the distribution coefficients were found to be very high for separation. From the results of distribution measurements carried out with ion exchanger in the sodium form, it was found that the order of the distribution coefficients of the D- and L-aminodiols changes with

increasing concentration of copper(II) ions and the separation of optical isomers can be carried out under more favourable conditions if a resin in the mixed sodium and copper form is used instead of simply the copper form. For the separation, an eluent containing 0.14 mole of sodium acetate and 0.01 mole of copper(II) chloride was chosen.

In order to obtain information on the necessary size of the column, the plate height of the column was first calculated from the elution curve obtained by an elution experiment using Glueckauf's equation[5]. The required column length for quantitative separation were calculated using the following equation[6]:

$$L > 2\pi h \left[\frac{\left(\dfrac{D_2 + a}{D_1 + a}\right) + 1}{\left(\dfrac{D_2 + a}{D_1 + a}\right) - 1} \right]^2 \tag{1}$$

where D_1 and D_2 are the volume distribution coefficients of the two optical isomers, a is the void fraction, h the plate height and L the required length of the column.

EXPERIMENTAL AND RESULTS

Reagents
       In all experiments, analytical-grade reagents were used.

Ion-exchange resin
       Dowex 50-X8 resin (100–200 and 50–100 mesh) was used after extraction with methanol in Soxhlet apparatus.

Instruments
       The pH was measured with a Radelkis pH meter (Budapest, Hungary), and the quantitative determination of the bases was carried out with a Spektromom 202 spectrophotometer (MOM, Budapest, Hungary).

Determination of the protonation and complex formation constants of D- *and* L-*aminodiols*
       Volumes of 45 ml of 2×10^{-2} and 5×10^{-2} M alcoholic stock solutions of D- and L-aminodiol were titrated with 0.25 and 0.1 N sodium hydroxide solutions in the absence and presence (10^{-3} M) of copper(II) ions. The pH was measured using glass and calomel electrodes, and the concentration of the free copper(II) ions was determined with an ion-selective electrode[7]. During the titrations, nitrogen gas was bubbled through the solutions. From the titration curves, the protonation and complex formation constants were calculated. We found that the protonation constants of the two enantiomers are identical. (log $K = 7.97 \pm 0.02$ and log $K = 7.94 \pm 0.02$). It was found that the optical isomers form, in addition to regular 1:1 and 1:2 complexes, also protonated and mixed hydroxo-complexes with copper(II) ions. The logarithmic values of the stability constants are given in Table I.

TABLE I

LOGARITHMIC VALUES OF OVERALL STABILITY CONSTANTS

Composition of complexes	L-Aminodiol	D-Aminodiol
[Cu(LH)]	10.19 ± 0.02	10.07 ± 0.02
[CuL]	5.75 ± 0.01	6.07 ± 0.01
[CuL$_2$]	9.45 ± 0.02	10.51 ± 0.02
[CuL(OH)]	11.45 ± 0.02	13.93 ± 0.02
[CuL(OH)$_2$]	21.29 ± 0.04	23.93 ± 0.04

Determination of the distribution coefficients of D- *and* L-*aminodiols*

The determination of the distribution coefficients was carried out on room temperature in solutions containing 50% of methanol.

The solution containing the optically active base and the required salt or acetate buffer was equilibrated with ion-exchange resin in the copper form. After equilibration, the concentration of the base was determined by spectrophotometry and the distribution coefficients were calculated (before the spectrophotometric determinations, ammonia was added to the solution and the copper–ammine complex formed was removed by ion-exchange resin in the ammonium form). The distribution coefficients were calculated from the original and the final concentration values, and the results are given in Table II.

TABLE II

DISTRIBUTION COEFFICIENTS

Cation-exchange resin: 0.5 g of Dowex 50-X8 in the copper form.

Composition of solution	Distribution coefficients	
	D-Aminodiol	L-Aminodiol
10^{-3} mole of D-(or L-)aminodiol, pH 6.75	890	718
10^{-3} mole of D-(or L-)aminodiol and 10^{-2} mole of copper(II)ions, pH 4.30	151	198

Distribution measurements were also carried out using resin in the sodium form and a constant concentration of sodium ions in which different amounts of copper(II) chloride were added to the solution. After equilibration, the solutions were analyzed as above and the distribution coefficients calculated. It was found that the difference between the distribution coefficients of D- and L-aminodiols is the most favourable if a solution containing of 0.01 mole of copper(II) ions and 0.14 mole of sodium ions is used.

Elution experiments

In the elution experiments, a 190 × 6.5 mm ion-exchange column containing resin in the sodium form, prepared from Dowex 50-X8 cation-exchange resin, was used. The column was first equilibrated with the eluent solution by passing *ca.* 400

ml of eluent through it. The eluent consisted of sodium acetate and acetic acid buffer of pH 5, which contained 0.14 mole of sodium ions, 0.01 mole of copper(II) chloride and 50% of methanol.

After equilibration of the column, the methanolic solution of the optical isomers (0.20 ml) was placed on the column and elution was carried out with an eluent flow-rate of 22.0 ml/h. The effluent was collected in 2-ml fractions. The absorbance of the fractions was measured spectrophotometrically at 275 nm after addition of ammonia and removal of copper(II) ions with ion-exchange resin in the ammonium form[8].

From the elution curves obtained, the plate height and the required plate number for quantitative separation were calculated.

Separation of D- *and* L-*aminodiols*

According to the calculation, a column 51 cm long was prepared. The composition of the eluent was the same as in the elution experiments. The feeding of the column and the continuous detection of the separated species in the effluent were carried out with an apparatus of our own design (Fig. 1). In the apparatus after the chromatographic column, ammonia solution was added continuously to the effluent and the solution was passed through a column containing resin in the ammonium form, thus removing copper(II) ions. The absorbance of the effluent from the latter column was measured spectrophotometrically at 275 nm using a flow cell. Under these conditions, the two optical isomers were resolved to the extent of 93% (Fig. 2).
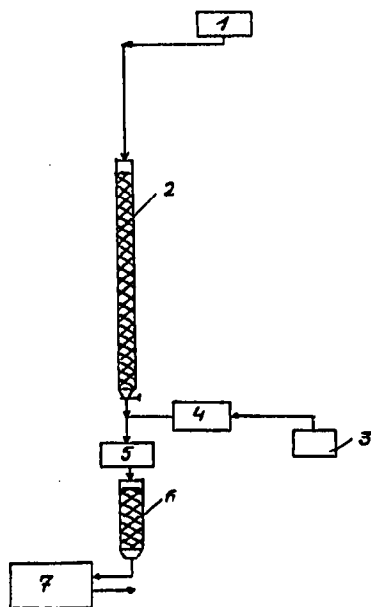

Fig. 1. Chromatographic apparatus for the separation of D- and L-aminodiols. 1 = Eluent; 2 = cation-exchange column of Dowex 50-X8 (mixed sodium–copper form; $L = 51.0$ cm, $\varnothing = 0.7$ cm); 3 = ammonia solution; 4 = peristaltic pump; 5 = mixing coil; 6 = cation-exchange column of Dowex 50-X8 (ammonium form); 7 = spectrophotometer.
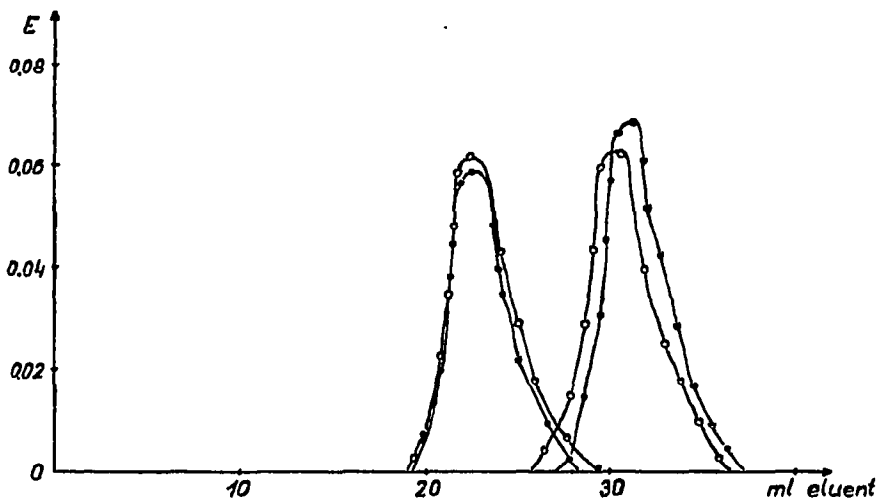
Fig. 2. Separation of D- and L-aminodiols. Eluent: ○, 0.01 M copper(II) chloride, 0.14 M sodium acetate, 50% methanol; ●, 0.01 M copper(II) chloride, 5×10^{-4} M D-aminodiol, 0.14 M sodium acetate, 50% methanol.

In order to increase the resolution, the earlier experiments were repeated under similar conditions but with an eluent that contained, in addition to 0.01 mole of copper(II) ions, also $5 \cdot 10^{-4}$ mole of D-aminodiol. In this manner we achieved a virtually quantitative separation of 0.11 mg of racemic aminodiol mixture.

DISCUSSION

A significant difference was found between the stability constants of D- and L-aminodiol complexes with copper(II) ions. The cause of this rare phenomenon may be explained by the fact that the compound has two asymmetric centres, and the groups coupling to asymmetric centres take part in the formation of the complexes. Other workers[9] also found that the optically active isomers of some structurally similar compounds (adrenaline) form complexes of different stabilities with copper(II) ions.

Under the optimal experimental conditions, where the chromatographic separation was achieved, the following two dominating reactions may be assumed:

$$R–Na + LH^+ \rightleftharpoons R–LH + Na^+ \tag{2}$$

$$Cu^{2+} + LH^+ \rightleftharpoons CuL + H^+ \tag{3}$$

Eqn. 2 is the ion-exchange reaction, while eqn. 3 represents complex formation in the solution phase. According to calculation, complex formation inside the resin phase plays only a moderate role.

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