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## Note

### Separation of optically active cobalt complexes by centrifuged column chromatography

D. T. HAWORTH, A. U. PAEGLIS and S. L. WENZEL

Department of Chemistry, Marquette University, Milwaukee, Wisc. 53233 (U.S.A.)

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The study of the separation of the optical isomers of tris(ethylenediamine)-cobalt(III) iodide by use of a centrifuged chromatograph column was suggested by our earlier thin-layer chromatography (TLC) work<sup>1</sup>. In that work we reported on the technique and conditions for microcrystalline cellulose-coated TLC plates containing varying amounts of *d*-quartz using an aqueous solvent system containing both aluminum chloride and the *d*-tartrate ion for the successful cobalt cation separations. The present work demonstrates that chromatograms of superior resolution of the optical isomers can be obtained by utilizing centrifugal force to accelerate the migration of the optical antipodes through closed columns of densely packed gels prepared with microparticulate adsorbents and containing an asymmetric environment of *d*-quartz.

#### EXPERIMENTAL

##### Column preparation

A slurry of 6% water standardized packets of microparticulate silica was prepared using various solvents consisting of toluene, water-methanol-acetone mixtures, petroleum ether (b.p. 30-60°) and *n*-hexane. A standard aliquot of the 3 ml slurry was used for column preparation with centrifuging for 6 to 10 min at 1500 *g* using a Sorvall Centri-Chrom Centrifuge (GLC-1). The 3-mm glass columns were silanized before use, dimethyldichlorosilane-toluene (2:100) washed with methanol, then water. For columns containing *d*-quartz, a 10-ml aliquot of the microparticulate silica slurry was used.

##### Solvent systems

The solvent system (3 ml) consisted of various aqueous mixtures of Na.K *d*-tartrate, and/or AlCl<sub>3</sub>.

##### Sample preparation

The loading disc (3 mm) method was used<sup>2</sup>. Samples of 1-8  $\mu$ l of a 4% (w/v) aqueous solution of *d*- and *l*-Co(en)<sub>3</sub><sup>3+</sup> cations\* as well as a 50:50 and a racemic mixture

\* en = ethylenediamine.

of the same concentration were evaporated onto the loading discs. Direct visualization of the brown-orange bands was used or detection by spraying with 10% (w/v)  $\text{Na}_2\text{S}$  was employed.

#### Time

A centrifuge time of 18 min was generally used. A standard of 3000 rpm (1500 g) was employed with a HL-4 rotor.

## RESULTS AND DISCUSSION

For the various solvents used for column preparation, petroleum ether and *n*-hexane were found to be most acceptable. The petroleum ether gave a good consistent slurry support for the *d*-quartz and it had a relatively long solvent life with no noticeable solvent front with the aqueous solvent system. However, there was some difficulty in suspending the slurry (1 h stirring and settling occurred after two to three days). The slurry could be resuspended by stirring. Hexane has many of the same characteristics as petroleum ether for column preparation and it was easier to suspend and maintain the slurry. Toluene gave an easily decomposed column of short life. The slurry was also relatively unstable with thickening after two to three days. Aqueous solvent systems gave a noticeable "solvent front" with a delineation of color and density between developed and undeveloped columns. With the water-methanol-acetone solvent mixtures, it was found that none was able to support *d*-quartz in the slurry. The column length varied slightly (4.6 to 5.0 cm) due to the small dissolution of column head sample. The data are reported as distance the species travels divided by column length (cm) vs. time of centrifugation (min).

The aluminum chloride, *d*-tartrate solvent that was suggested by our TLC (0.2 M  $\text{AlCl}_3$ , 0.3 M Na,K *d*-tartrate) study showed that in the absence of *d*-quartz in the column this solvent system affords a fair separation of the optical isomers of  $\text{Co(en)}_3^{3+}$  with the *d*-isomer traveling farther than the *l*-isomer. With this solvent system containing *d*-quartz excellent separations of *d,l*-isomers were obtained. The extent of separation was dependent on the amount of *d*-quartz and time of centrifuging. Using a solvent system consisting of only 0.2 M  $\text{AlCl}_3$  no separation was obtained; however, a sharp band (4.5/5.0 at 15 min) was observed. With a 0.3 M Na, K *d*-tartrate system, broadening of the band occurred with no separation, 2.9-3.2-5.0 at 18 min. This again testifies to the necessity of both constituents to be present in the solvent system and to their interaction with the isomers in the solvent flow<sup>1-3</sup>. No sample transport was observed for the solvent systems water, toluene and or petroleum ether.

Columns containing *d*-quartz which passed a 140- $\mu\text{m}$  mesh were prepared in the range of 1.5 to 8.0% (w/v) slurry. The separations of the  $\text{Co(en)}_3^{3+}$  isomers increased up to a practical limit of 5.0%, where a suspension saturation seemed to be reached. Over 5.0%, the life of the column decreases, which tends to make the columns very brittle and granular. Direct visualization of the bands is suggested as the sodium sulfide developer further accelerates column deterioration. Bands on the extruded column faded after about 2 to 3 h. Fig. 1 shows how the increased concentration of *d*-quartz will affect the separation for columns containing 1.5, 3.5 and 5.0% *d*-quartz. The data summarized in Table I indicate that with increased concentration of *d*-quartz, the *l*-isomer travels less along the column while the *d*-isomer distance remains

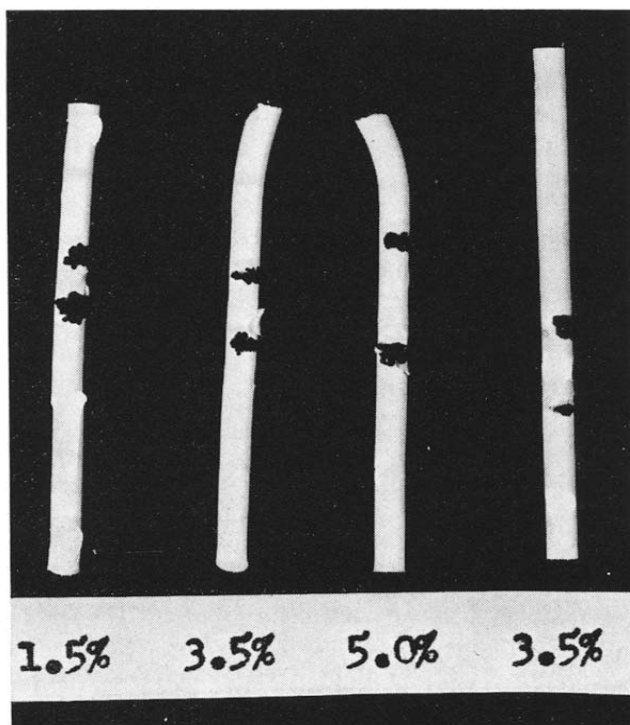


Fig. 1. Effect of per cent *d*-quartz and centrifuging time on the resolution of the optical isomers of  $\text{Co(en)}_3^{3+}$ . Centrifuging time 18 min, except for far right column, 12 min.

almost constant. Also the distance between the two bands increases (0.6 cm to 1.4 cm) with increased *d*-quartz. With a decrease in centrifuge time from 18 min to 12 min using 3.5% *d*-quartz columns, the  $\text{d-Co(en)}_3^{3+}$  band did not travel as far and the bands were closer together: 0.9 cm (12 min) versus 1.4 cm (18 min).

The position of each band on the column was independent as to whether the sample was applied from a racemic mixture of  $\text{Co(en)}_3^{3+}$ , a synthetic mixture containing 50% (w/w) of each isomer or by direct application of solutions of each individual isomer. Separated bands were cut from a number of columns and eluted with water. After compensating for the *d*-tartrate ion in the eluent by removal of a similar section

TABLE I

CHARACTERISTIC SEPARATIONS FOR OPTICAL ISOMERS OF  $\text{Co(en)}_3^{3+}$

Per cent <i>d</i> -quartz (w/v)*	Centrifuging time (min)	Band measurement l,d column length (cm)
1.5	18	2.3, 2.9-4.8
3.5	18	1.9, 3.1-4.8
5.0	18	1.5, 2.9-5.0
3.5	12	1.5, 2.4-5.0

\* Petroleum ether column solvent, 0.2 M *d*-tartrate, 0.13 M  $\text{AlCl}_3$ , 6-min column preparation time, 4.5- $\mu\text{l}$  sample per column.

from a chromatographed blank column, the faster band (*d*-isomer) had an optical rotation of  $\alpha_D = +87^\circ$  (obs.),  $+89^\circ$  (ref. 4) and the slower band (*l*-isomer) had an optical rotation of  $\alpha_D = -87^\circ$  (obs.),  $-89^\circ$  (ref. 4). These 4% (w/v) aqueous solutions were applied in 1- to 8- $\mu$ l range. Samples containing more than 160  $\mu$ g (4  $\mu$ l) gave a third band close to the sample head, which indicated overloading. Good detection, without development, was observed for each isomer down to 1  $\mu$ l (40- $\mu$ g sample).

Using the solvent system described above, one clear broad band for 1% and 2% (w/v) mixtures of the *d*- and *l*-isomers of *cis*-dinitrobis(ethylenediamine)cobalt(III) cations,  $[\text{Co}(\text{NO})_2(\text{en})_2]^+$ , was obtained. It was further observed that the distance travelled was slowed by an increase in *d*-quartz concentration (3.9/4.8 at 15 min for 3.5%, 3.0/4.18 at 15 min for 5.0%) suggesting that the *d*-quartz interacts with the isomers; however, no separation was obtained.

On changing the solvent system from an optically active anion (*d*-tartrate) to one containing an optically active cation, the separation of the optical antipodes of a complex inorganic anion was thought to be possible. This was borne out when we observed the resolution of the *d*- and *l*-isomers of potassium (ethylenediaminetetraacetato)cobalt(III) dihydrate,  $\text{K}[\text{Co}(\text{EDTA})] \cdot 2\text{H}_2\text{O}$ , on a 5% *d*-quartz column using 0.15 *M* aluminum chloride and 0.03 *M* quinine sulfate solvent system (2.8, 3.1/4.9 at 20 min). The bands (4  $\mu$ g) are easy to detect because of their deep purple color. Separated bands were isolated from the chromatogram and after elution with water and correction for the quinine ion from a blank chromatogram, the faster band (*d*-isomer) gave an optical rotation ( $\alpha_D$ ) of  $-0.02^\circ$ ,  $-0.03^\circ$  (ref. 5) and the slower (*l*-isomer) band gave an optical rotation of ( $\alpha_D$ ) of  $+0.02^\circ$ ,  $+0.03^\circ$  (ref. 5). Because of the low solubility of quinine sulfate, stronger solutions cannot be prepared.

We have shown that the *d*-tartrate ion or quinine sulfate with aluminum chloride can successfully be used in an aqueous solvent system to resolve enantiomorphous pairs by centrifugation. This resolution is much improved by the incorporation of *d*-quartz into the column to provide an asymmetric environment.

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