

## Note

### A paper electrophoretic study of ion-pair formation

#### XI. The behaviour of optical isomers in optically active electrolytes

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(Received July 18th, 1975)

In previous parts of this series we pointed out several mechanisms which can lead to an interaction between cations and anions in paper electrophoresis. In addition to the usual protonation and complexing equilibria, we also showed that ion-pair equilibria can be used to effect separations in electromigration. Isolated trials were reported of electromigration of racemic mixtures of cobalt(III) complexes in an optically active counter-ion as electrolyte (for example,  $\text{Co}(\text{ox})_3^{3-}$  in *l*-strychnine<sup>1</sup> and  $\text{Co}(\text{dip})_3^{3+}$  and  $\text{Co}(o\text{-phen})_3^{3-}$  in (+)-camphor-10-sulphonic acid<sup>2</sup>). The trials were all entirely negative, *i.e.*, a single narrow band or spot of the migrating ion was observed without any separation of the optical isomers. We felt that this was remarkable as we had chosen systems which on crystallization separate the two optically active forms of the cobalt(III) complex. We assumed that there already exists some interaction between the anion and the cation before actual crystal formation. Thus we were very interested when Yoneda and Miura<sup>3</sup> reported the electrophoretic separations of the optical isomers of  $\text{Co}(\text{en})_3^{3+}$  using a low-potential paper electrophoretic technique and an electrolyte consisting of tartrate and aluminium chloride. An explanation was also given for the necessity of the aluminium chloride, *i.e.*, it influences the hydration of the ions present. These authors suggested that no separation was possible in absence of aluminium chloride. We have now extended this work to other cobalt complexes and examined some of the variables involved.

#### EXPERIMENTAL AND RESULTS

A CAMAG high-voltage electrophoresis apparatus was used in all experiments and, unless stated otherwise, Whatman No. 1 paper strips were used with 30 min runs at 1000 V and a circulating water temperature of 6-8°. The spots were detected by spraying the papers lightly with an aqueous solution of ammonium polysulphide.

Fig. 1 shows the electropherograms obtained with a solution of disodium *d*-tartrate as electrolyte at various concentrations and pH values. It is evident from Fig. 1a that a separation of  $\text{Co}(\text{en})_3^{3+}$  into two spots occurs only at higher pH values (when

\* The following abbreviations are used: ox = oxalate; en = ethylenediamine; dip = dipyridyl; *o*-phen = *ortho*-phenanthroline.

(a)

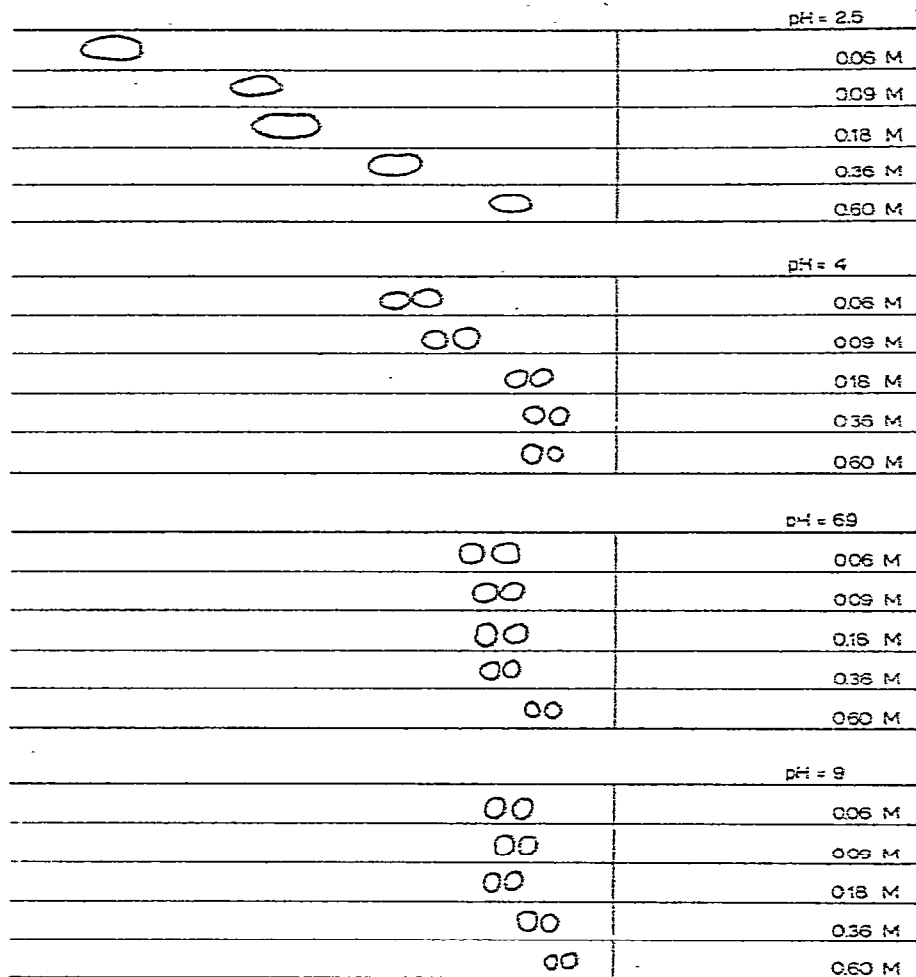


Fig. 1.

the tartrate ion is fully ionized, the  $pK$  values of tartaric acid being 3.06 and 4.5) and that the electrolyte concentration is not so critical. We would like to emphasize here that, contrary to what was reported by Yoneda and Miura<sup>3</sup> a separation also takes place in the absence of aluminium chloride. The optical isomers of  $\text{Co(en)}_3^{3+}$  were also separated on crystallization and the separated  $l$  and  $d$  forms were placed side by side with the mixture on the same electropherogram. Thus it was seen that the  $l$  form travelled faster in  $d$ -tartrate and the  $d$  form faster in  $l$ -tartrate, and that the velocities of the single optical isomers each corresponded to one of the two spots separated

(b)

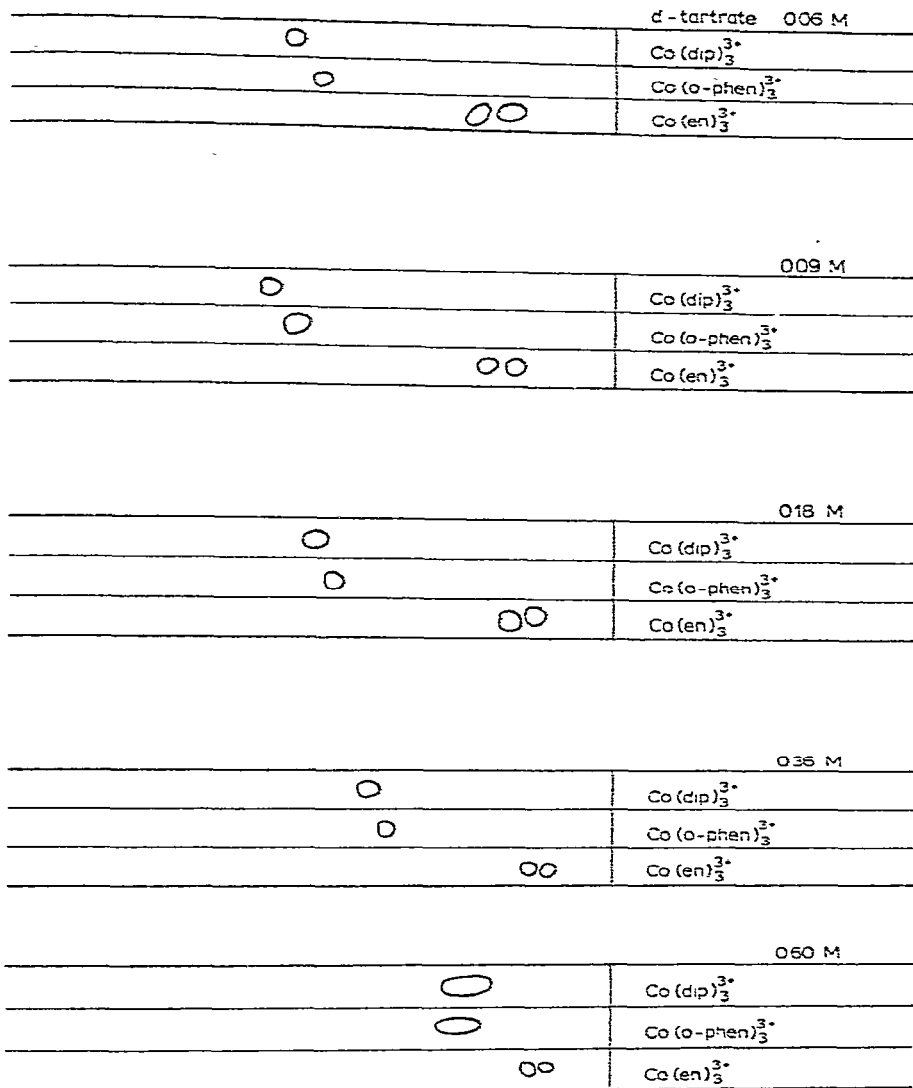


Fig. 1. Electropherograms of cobalt(III) complexes on Whatman No. 1 paper in *d*-tartrate solutions at 1500 V for 1 h at 8°. (a), Racemic  $\text{Co}(\text{en})_3^{3+}$  at various pH values and concentrations; (b),  $\text{Co}(\text{dip})_3^{3+}$ ,  $\text{Co}(\text{o-phen})_3^{3+}$  and  $\text{Co}(\text{en})_3^{3+}$  placed side by side in various concentrations of *d*-tartrate at pH 6.9.

from the mixture. Fig. 1b shows on the other hand that no separation of the isomers of  $\text{Co}(\text{dip})_3^{3+}$  and  $\text{Co}(\text{o-phen})_3^{3+}$  occurred. Analogous results were obtained with *l*-tartrate instead of *d*-tartrate as electrolyte.

Work with disodium *d*-malate at various concentrations and pH values yielded no separation of the isomers of any of the three complexes examined. Some typical electropherograms are given in Fig. 2 in which the slower movement of  $\text{Co}(\text{en})_3^{3+}$  compared to that of  $\text{Co}(\text{dip})_3^{3+}$  and  $\text{Co}(\text{o-phen})_3^{3+}$  is shown.

Finally Fig. 3 shows the electropherograms obtained in mixtures of aluminium

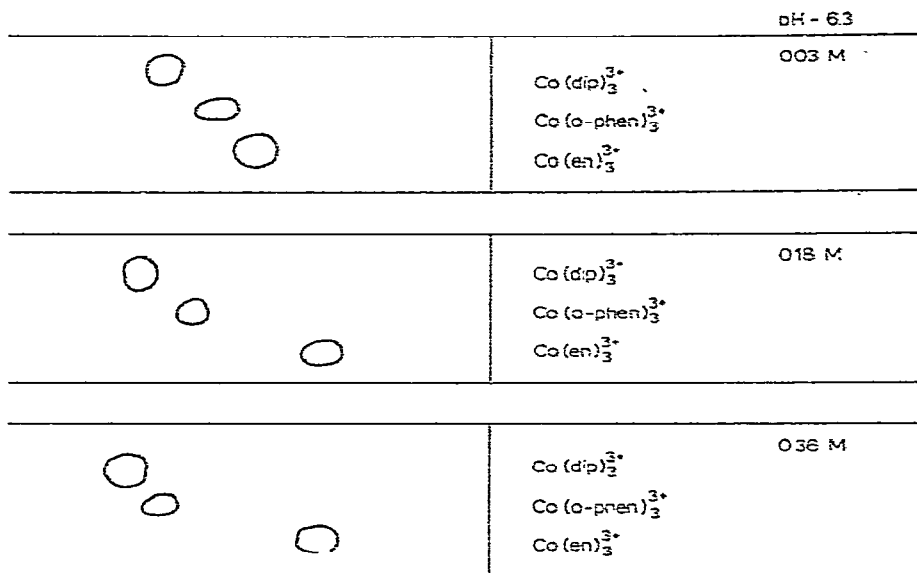


Fig. 2. Electropherograms of cobalt(III) complexes on Whatman No. 1 paper in *d*-malate solutions at 1000 V for 30 min at 6–8°.  $\text{Co}(\text{dip})_3^{3+}$ ,  $\text{Co}(\text{o-phen})_3^{3+}$  and  $\text{Co}(\text{en})_3^{3+}$  were placed side by side in various concentrations of *d*-malate at pH 6.3.

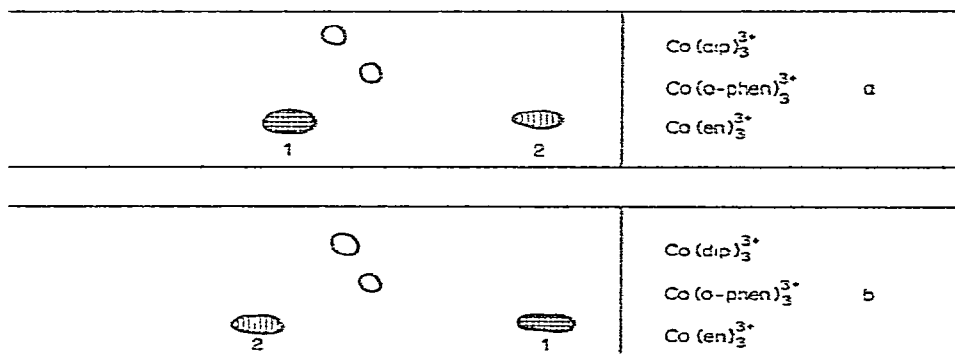


Fig. 3. Electropherograms of cobalt(III) complexes in 0.12 M aluminium chloride–0.18 M disodium tartrate at pH 2.6. Other conditions as in Fig. 2. (a), *d*-Tartrate; (b), *l*-tartrate.  $\text{Co}(\text{dip})_3^{3+}$ ,  $\text{Co}(\text{o-phen})_3^{3+}$  and  $\text{Co}(\text{en})_3^{3+}$  were placed side by side. 1 = *d*- $\text{Co}(\text{en})_3^{3+}$ ; 2 = *l*- $\text{Co}(\text{en})_3^{3+}$ .

chloride with *d*- or *l*-tartrate as was reported by Yoneda and Miura<sup>3</sup>. Comparing these results with those in Figs. 1 and 2, it is evident that the separation of the optical isomers is much better in the aluminium chloride–tartrate mixtures. The distances between the spot centres were *ca.* 60 mm, while in tartrate without aluminium chloride the distances were 7–9 mm. Furthermore the sequence of the *d* and *l* forms is the opposite of that found in tartrate. We stress here that neither  $\text{Co}(\text{dip})_3^{3+}$  nor  $\text{Co}(\text{o-phen})_3^{3+}$  separate into two spots in the presence of aluminium chloride. It is readily shown by paper electrophoresis that aluminium(III) complexes with tartrate under the conditions of these experiments and that a complex aluminium tartrate anion is presumably responsible for the separation effect observed. With the aid of the above results,

we can throw some light on the separation effects observed.

It seems that for effective separation of optical isomers it is not sufficient that a racemic mixture of a complex cation is in an optically active environment, but that outer-sphere complexes must be formed in this case probably involving hydrogen bonding. The isomers of  $\text{Co}(\text{dip})_3^{3-}$  and  $\text{Co}(\sigma\text{-phen})_3^{3+}$  do not separate as they do not form this type of outer-sphere complex with tartrate. Also, extensive exploratory experiments (not reported here in detail) to separate the isomers of  $\text{Co}(\text{en})_3^{3+}$  in electrolytes containing optically active amino acids and attempts to separate racemic mixtures of amino acids in optically active tartrate as electrolyte were entirely without success. In addition, the anion must have certain geometrical properties; if these are not fulfilled when outer-sphere complexing takes place, as is the case with *d*-malate, no separation will be obtained. Optically active anionic complexes, such as aluminium tartrate, may, due to their shape, have a much higher separation effect than the simple tartrate anion. This is well known from separations by crystallization where the antimony tartrate anion is often employed for resolving racemic mixtures. We can also now explain why Yoneda and Miura<sup>3</sup> reported no success with tartrate alone. Under the conditions of low-voltage paper electrophoresis, the resolution could not have been such as to separate in tartrate alone without simultaneously marring the separation due to diffusion in long runs.

Our results permit some generalizations concerning the possibilities of paper electrophoresis for the separation of optical isomers. As discussed above, the vicinity of optically active counter-ions is not sufficient to produce a separation effect, and an interaction of the outer-sphere type (with some geometric specificity) must be formed. This rather limits the scope of this technique as such complexes cannot move very quickly and presumably the best separation effects will occur near the "isoelectric point" of the system where separation is poor. Separations of optically active isomers will thus be the exception rather than the rule.

In paper electrophoretic studies of the interaction of inorganic anions with metal ions of charge  $1+$ ,  $2+$  and  $n+$  we have already noted<sup>4</sup> that, in the range of concentrations used in electrophoretic studies (*i.e.*, 0.1–1 *N*), the interactions are quite different to those observed in saturated solutions during the formation of a precipitate. The situation seems to be analogous for separations of optical isomers, for, while *l*-strychnine can separate the isomers of  $\text{Co}(\text{ox})_3^{3-}$  and (+)-camphor-10-sulphonic acid can separate the isomers of  $\text{Co}(\text{dip})_3^{3+}$  by crystallization, no interactions are noted within the limits of detectability in paper electrophoresis. This work may also give food for thought to biologists working with optically active drugs. From our investigation, interactions in solution seem unlikely: if there is an effect due to only one optical isomer, it should be a reaction taking place on a suitable surface.

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