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## Note

### Column and thin-layer chromatography of azophenol dyes and their cobalt(III) complexes

#### Adsorption chromatography with lipophilic Sephadex and silica gel

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In the preparation of cobalt(III) complexes of azophenols for use as analogs of cobalt(III) azotyrosine complexes of enzymes<sup>1,2</sup>, chromatography on lipophilic Sephadex was found to be the most effective means for preparative separation of the desired product from starting material and side products. In addition, analytical thin-layer chromatography (TLC) on silica gel was found useful in the preparation of mixed ligand complexes with bidentate azophenols and ethylenediaminediacetic acid (EDDA).

#### EXPERIMENTAL

The preparations of the ligands and complexes have been described previously<sup>1,2</sup>. All other chemicals were reagent grade and were used as supplied. The TLC plates were commercially prepared silica gel F<sub>254</sub> on aluminum (E. Merck, Darmstadt, G.F.R.), and were used without efforts to activate further.

In the purification of the ligand 2-tetrazolylazo-*p*-cresol, a column of 6 × 20 cm was able to separate a starting mixture of 0.5 g at a flow-rate of 30 drops/min, but the column had to be dedicated to this purpose, as a very slight pink tinge could not be removed from the Sephadex under conditions suitable to the stability of the chromatographic media.

In the elutions with methanol, it was important to evaporate the product mixture to dryness before dissolving in methanol to add to the column. In those separations, 200–250 mg of sample could be separated on a column 6 cm × 47 cm at a flow-rate of 3 drops/min. The eluent was collected with a fraction collector, and a run would normally take about two days. In the case of bis[2-thiazolylazo-*p*-cresolato]Co(III) it was necessary to add 1 drop of 12 *M* HCl to each 100 ml of methanol to maintain complete stability of the complex.

Standard TLC tanks were used with a solvent mixture of methanol–chloroform–acetic acid–12 *M* HCl (80:40:5:1.5). Preequilibration of the tank atmosphere seemed to have little effect on the separations. Spotting was done from methanol

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solutions. All components were colored; hence, no visualization technique was necessary, although all plates were checked under ultraviolet light (254 nm).

## RESULTS AND DISCUSSION

The results for the column separations are summarized in Table I. The purification of 2-tetrazolylazo-*p*-cresol was based upon a paper by Reeves *et al.*<sup>3</sup>, who separated mixtures of sulfonated azo dyes on Sephadex G-25. We found that for the unsulfonated azophenol, Sephadex LH-20 gave much sharper separations.

Because lipophilic Sephadex had been shown by Bachmann and Burnham<sup>4</sup> to be quite useful in separations of organic-soluble, highly aromatic systems, we thought it would be useful for our mixtures as well. Indeed, we were able to achieve more dramatic separations in terms of sample size tolerated by the column, and we suspect that our separations are based largely on adsorption, rather than gel filtration, as can be seen from Table I. Because of the large sample sizes which could be tolerated, the success with isocratic elution, and the great resistance of the Sephadex to channeling, we found it a much more satisfactory adsorbent for our purposes than either silica gel or alumina.

In the case of tridentate azophenols, tetrazolylazo-*p*-cresol and thiazolylazo-*p*-cresol, the color differences between bands were sufficiently great, so that fractions could be combined simply on the basis of visible spectra. However, all complexes of the bidentate azophenols, phenylazo-*p*-cresol and 2-(4-carboxyphenylazo)-4,5-dimethylphenol, were dark red or purple, which made this method of questionable value. Hence, the thin-layer system was developed, which permitted following the course of the synthetic reaction and the course of the column separations. The  $R_F$

TABLE I  
SEPARATIONS OF AZOPHENOLS AND COBALT COMPLEXES ON LIPOPHILIC SEPHADEX

Mixture	Components in order of elution	Mol. wt.	Color	Eluent
1	<i>p</i> -Cresol	107	Colorless	0.01 M KOH
	2-(Tetrazolylazo)- <i>p</i> -cresol	203	Orange	
	2,6-Bis(tetrazolylazo)- <i>p</i> -cresol	299	Purple	
2	Bis[2-tetrazolylazo)- <i>p</i> -cresolato]Co(III)	465	Blue	Methanol
	2-(Tetrazolylazo)- <i>p</i> -cresol	204	Yellow	
3	Bis[2-(thiazolylazo)- <i>p</i> -cresolato]Co(II)*	465	Red	Acidified methanol
	Bis[2-(thiazolylazo)- <i>p</i> -cresolato]Co(III)	495	Green	
4	2-Thiazolylazo- <i>p</i> -cresol	218	Yellow	Methanol
	Unidentified products**	—	Reddish	
5	(2-Phenylazo- <i>p</i> -cresolato) (EDDA)Co(III)	445	Dark red	Methanol
	2-Phenylazo- <i>p</i> -cresol	211	Yellow	
	Unidentified products**	—	Reddish	
	[2-(4-Carboxyphenylazo)-4,5-dimethylphenolato]-(EDDA)Co(III)***	502	Dark red	
	2-(4-Carboxyphenylazo)-4,5-dimethylphenol	269	Yellow	

\* Present only if there was insufficient oxidation of the reaction mixture.

\*\* Several bands, some before and some after the product.

\*\*\* Two bands, containing two isomers<sup>2</sup>.

TABLE II

TLC  $R_F$  VALUES OF MIXED LIGAND COMPLEXES OF COBALT(III) AND AZOPHENOLSThin layer, silica gel F<sub>254</sub> on aluminium (Merck); solvent, methanol-chloroform-acetic acid-12 M HCl (80:40:5:1.5).

Compound	$R_F$
2-Phenylazo- <i>p</i> -cresol	0.98
(2-Phenylazo- <i>p</i> -cresolato)(EDDA)Co(III)	0.55
2-(4-Carboxyphenylazo)-4,5-dimethylphenol	0.95
[2-(4-Carboxyphenylazo)-4,5-dimethylphenolato](EDDA)Co(III), band I from Sephadex column	0.53
[2-(4-Carboxyphenylazo)-4,5-dimethylphenolato](EDDA)Co(III), band 2 from Sephadex column	0.45

values found for products isolated and identified<sup>2</sup> are given in Table II. There were several other reaction products of differing  $R_F$  value present in all product mixtures before purification.

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