

paring DNP-derivatives nor those used in hydrolyzing DNP-peptides hinder the resolving power of the glass paper technique. Upon completion of dinitrophenylation of a sample in bicarbonate or trimethylamine buffers, the reaction mixture may be spotted directly on the glass paper without preliminary extraction or purification of DNP-amino acids. Solutions of DNP-amino acids in 6 *N* HCl or in the various mixtures containing glacial acetic, formic and perchloric acids used in digesting DNP-peptides or DNP-proteins can be applied directly to the glass paper without seriously altering  $R_F$  values or quality of DNP-amino acid resolution. The three solvent systems presented here, an acidic, a basic and a non-aqueous acidic, offer wide selectivity and versatility in separating DNP-derivatives.

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## The use of ninhydrin in the detection of cationic complexes of cobalt, nickel and chromium

In the course of the preparation<sup>1-4</sup> and purification of metal coordination compounds prior to the assessment of their biological properties<sup>5</sup>, several methods of detection on paper and thin-layer chromatograms were tried. Of a number of reagents used, ninhydrin was found to give characteristic stains with the cationic complexes of cobalt, nickel and chromium.

All the complexes excepting compound No. 10 (Table I) were dissolved in water, applied to paper strips and developed by the descending method in a solvent system made up of *n*-butanol-acetic acid-water-pyridine (30:6:20:24, v/v). After staining with 0.2% ninhydrin in acetone, the spots were visualised by heating the paper strips for 10-15 min at 80-90°. The water-labile compound No. 10 was suspended in acetone to which water was added drop by drop to get a fine suspension.

In spot tests carried out on strips of paper the cationic complexes listed in Table I reacted sensitively to ninhydrin, the range of detectability being 0.2-1.0 µg.

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TABLE I

SPOT TESTS OF CATIONIC COMPLEXES OF COBALT, NICKEL AND CHROMIUM WITH NINHYDRIN

No.	Compound <sup>a</sup>	Colour of spot	Sensitivity ( $\mu\text{g}$ )
1	$[\text{Co}(\text{en})_2\text{Cl}_2]\text{Cl}$	Deep brown-red	0.5
2	$[\text{Co}(\text{en})_3]\text{Cl}_3$	Purple-red (brownish)	0.5
3	$[\text{Cr}(\text{en})_3]_2(\text{SO}_4)_3$	Deep violet (bluish)	0.5
4	$[\text{Co}(\text{NH}_3)_6]\text{Cl}_3$	Orange-brown (purplish)	1.0
5	$[\text{Ni}(\text{en})_3]\text{Cl}_2$	Very deep-violet	0.2
6	$[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Br}_3$	Orange-brown (purplish)	1.0
8	$[\text{Co}_4(\text{en})_6(\text{OH})_6]\text{Cl}_6$	Dull red-brown	0.5
9	$[\text{Cr}_4(\text{en})_6(\text{OH})_6]\text{Cl}_6$	Deep violet-brown	0.5
10	$[\text{Ni}(\text{NH}_3)_6]\text{Cl}_2$	Light orange-yellow	1.0

<sup>a</sup> (en) = ethylenediamine.

Ethylenediamine-containing complexes of cobalt and nickel responded more intensely to the ninhydrin test than the corresponding ammonia-containing complexes of cobalt and nickel. The stains were stable for several months on paper chromatograms and 5–15  $\mu\text{g}$  of the compounds, excepting compounds 8 and 9, were detectable without any trailing effect. Compounds 4 and 6 containing ammonia had, however, to be used in 20–35  $\mu\text{g}$  amounts on account of the lower order of sensitivity. Compound No. 5, the nickel–ethylenediamine complex, was by far the most reactive of this series. Some typical results are summarised in Table II.

Presumably ninhydrin reveals the compounds studied by reacting with ammonia or ethylenediamine. In independent experiments it was found that ninhydrin

TABLE II

CHROMATOGRAPHIC DETECTION OF COBALT, NICKEL AND CHROMIUM AND THEIR CATIONIC COMPLEXES WITH NINHYDRIN

No.	Compound	Shade <sup>a</sup>	$R_F$ value	Amount taken ( $\mu\text{g}$ )
1	$[\text{Co}(\text{en})_2\text{Cl}_2]\text{Cl}$	Violet-brown (intense and deep shade)	0.33	8
2	$[\text{Co}(\text{en})_3]\text{Cl}_3$	Pink-violet (deep shade)	0.21	8
3	$[\text{Cr}(\text{en})_3]_2(\text{SO}_4)_3^b$	Violet (deep shade)	0.168	8
4	$[\text{Co}(\text{NH}_3)_6]\text{Cl}_3$	Distinct yellow (weaker and lighter shade)	0.165	15
5	$[\text{Ni}(\text{en})_3]\text{Cl}_2$	Violet (very deep and intense)	0.21	5
6	$[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]\text{Br}_3$	Yellow pinkish (very light shade as No. 4)	0.184	15
8	$[\text{Co}_4(\text{en})_6(\text{OH})_6]\text{Cl}_6^c$	Pink-brown	0.20	8
9	$[\text{Cr}_4(\text{en})_6(\text{OH})_6]\text{Cl}_6^c$	Violet-pink (intense and deep)	0.23	8
10	$\text{CoCl}_2^d$	Pink-yellow	0.77	40
11	$\text{NiCl}_2^d$	Yellow (very light shade)	0.78	40
12	$\text{Cr}_2(\text{SO}_4)_3 \cdot \text{K}_2\text{SO}_4$	Green-gray (very light and dull)	0.20	60

<sup>a</sup> The shades of the detected compounds on the chromatograms were in some cases slightly different from those of the spot tests.

<sup>b</sup> Compound 3 contained a minor impurity corresponding to an  $R_F$  value of 0.11 in a brown-violet shade.

<sup>c</sup> Compound 8 showed a weak streaking from the point of application up to the major zone while compound 9 gave a prominent streaking both below and above the major zone.

<sup>d</sup> The shades of the detected spots became very prominent on storage.

could react with simple salts of cobalt, nickel and chromium. These ions reacted with ninhydrin relatively less sensitively. It would seem, therefore, that the net colour produced by ninhydrin is the result of reaction with the nitrogen-containing compounds—ammonia or ethylenediamine—as well as direct reaction with the metals that constitute the complexes. The relative difference in the intensity and shade of stains produced by ethylenediamine-containing complexes and ammonia-containing complexes could be used for distinguishing these two groups of metal complexes. The presence of metals (Ni, Co, Cr.) may be responsible for the stability of the stains of all the metal complexes detected as against the transient stains given by ninhydrin with amino acids. In the latter case, stability can be conferred on the stains by incorporating metallic cations<sup>6,7</sup> into the ninhydrin reagent.

In view of the fact that ninhydrin can stain a number of nitrogenous and non-nitrogenous compounds as demonstrated by the present study as well as by a number of recent reports<sup>8-14</sup>, the results of the use of ninhydrin for the specific detection of amino acids and amines have to be very cautiously interpreted.

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