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## Note

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### Detection of inorganic cations on acid pherograms using ferric thiocyanate and fluorescein

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It has been shown by Gross<sup>1</sup> that many inorganic cations may be characterized by their electrophoretic mobilities on paper impregnated with an aqueous solution of formic acid, and his data indicate that some mixtures, especially of alkali metal and alkaline earth metal ions, should be rapidly separable in this electrolyte. Only a limited number of reagents is available, however, for the sensitive detection of these classes of metal ions on pherograms, although a wider selection is available for the heavy metals. Gross used bromophenol blue and ninhydrin<sup>2</sup> for the detection of the alkali metals and 8-hydroxyquinoline as a general reagent for the alkaline earth and other metal ions.

The results of the present work show that two other common reagents, ferric thiocyanate and fluorescein, are useful for the detection, on acid pherograms, of a wide range of inorganic cations, including those of the alkali and alkaline earth metals.

## EXPERIMENTAL

### *Materials.*

Inorganic salts were commercial samples of analytical-reagent grade.

### *Electrolytes*

(a) An aqueous solution 0.75 *M* with respect to formic acid<sup>1</sup>. (b) An aqueous solution 1 *M* with respect to acetic acid and 0.75 *M* with respect to formic acid<sup>3</sup>.

### *Spray reagents*

(a) Ferric thiocyanate stock solution consisted of a mixture of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (1 g) and KCNS (1.08 g) in 100 ml water. For use as a spray reagent this solution was diluted 8-fold with ethanol-water (50:50) mixture. (b) Fluorescein (0.2%) in ethanol. (c) Bromophenol blue (0.05%) in ethanol.

### *Apparatus and procedure*

The enclosed strip paper electrophoresis apparatus is described in detail else-

where<sup>4</sup>. Whatman No. 4 paper was used and solutions (usually 0.1 *M*) of the salts transferred to strips pre-wetted with electrolyte using a platinum loop delivering 0.7  $\mu$ l.

Electrophoresis was allowed to proceed for 20 min at about 22 V/cm after which the pherograms were heated in an oven at 100° until just dry.

Spray reagents were then applied rapidly and evenly to both sides of pherograms. Those treated with ferric thiocyanate were returned to the oven and heated for no longer than was necessary to just dry them.

Pherograms treated with fluorescein were suspended in air at room temperature for about an hour and then viewed in the light of a UV lamp emitting at 2540 Å.

## RESULTS AND DISCUSSION

The blood-red colour of acidic solutions of ferric thiocyanate is due to undissociated  $\text{Fe}(\text{CNS})_3$ . This complex is stable at pH 2.5 and below, but it breaks down with incipient precipitation of  $\text{Fe}(\text{OH})_3$  as the solution is raised to pH 3.5, at which level the solution becomes pale orange-yellow. The marked colour change which this reagent undergoes with change of pH is the basis of the present method of detecting metal and ammonium ions on pherograms run in acid electrolytes. The dried papers retain sufficient acetic and/or formic acid to maintain the applied spray reagent in the form of its red complex, and on re-drying, the paper background becomes deep pink.

Like the alkali and alkaline earth metal ions present on papers, the ions of many heavy metals probably exert a buffering action on the residual electrolyte, raising the pH at least to a point where the ferric thiocyanate complex decomposes, the metal ions then appearing as pale creamy-yellow spots contrasting strongly with the background. Heavy metal ions acting in this way include  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Al}^{3+}$ , but others like  $\text{Ag}^+$  and  $\text{Hg}^{2+}$ , also detectable as a pale spot or streak (as in the case of  $\text{Ag}^+$ ), probably destroy the red iron complex by forming insoluble thiocyanates or more stable complexes.

The cations are easily located on pherograms run under standard conditions as spots containing 0.07  $\mu$ mole of each (applied in one loopful of 0.1 *M* solutions), the lower limit of detection of most lying in the range 0.01–0.02  $\mu$ mole.

Fluorescein is somewhat less sensitive as a reagent for the detection of the cations, but spots containing 0.07  $\mu$ mole of each are located readily after treating papers with the reagent as described under Experimental and viewing them in the light of the UV lamp. Ions of alkali metals and (with the exception of  $\text{Be}^{2+}$ ) those of alkaline earth metals, together with  $\text{NH}_4^+$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Al}^{3+}$ , are visible as yellow fluorescent spots against a dull fluorescent ground. Among ions of lower atomic weight,  $\text{Be}^{2+}$  is unique in appearing as a brown spot and, in this respect, it resembles heavy metal ions like  $\text{Ag}^+$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{Bi}^{3+}$ . The brown spots of the latter vary characteristically in intensity from one metal to another. Some changes may occur as pherograms "age", the most notable of which is an overnight browning of the initially yellow fluorescent spots of the heavy metal ions,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Al}^{3+}$ .

Bromophenol blue gives strong blue spots against an initially pale yellow ground, not only with the alkali metal and alkaline earth metal ions, but also with ions of many of the heavy metals, e.g.  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$  and

$\text{Pb}^{2+}$ , and this appears to confirm that heavy metal ions also exert a buffering action on the background of acid electrolyte remaining on dried papers.

With one exception, the present work confirms the order of mobilities of the cations as reported by Gross<sup>1</sup>. Of the two alkaline earth metal ions,  $\text{Ca}^{2+}$  and  $\text{Ba}^{2+}$ , he found  $\text{Ca}^{2+}$  to have the higher mobility in the formic acid electrolyte; under present conditions,  $\text{Ba}^{2+}$  is found to separate narrowly as the leading spot when these two ions are applied in admixture.

Artefacts consistently occur on pherograms treated with any of the three spray reagents. They take the form of spots appearing at positions normally occupied by  $\text{Na}^+$  and  $\text{Ca}^{2+}$  and are (a) coloured red against the deep pink background generated by the ferric thiocyanate reagent, (b) dark (non-fluorescent) spots on the dull fluorescent ground of fluorescein-treated papers or (c) bright yellow spots against the initially pale yellow ground due to bromophenol blue.

The artefacts are due to impurities<sup>5,6</sup> of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  in papers being radially displaced from the starting-points of pherograms at the moment of application of the aqueous solutions of experimental salts. They are therefore analogous to another artefact, recently described in detail<sup>7</sup>, which is due to an impurity of  $\text{Cl}^-$  in papers.

An inverted form of the above artefact due to the  $\text{Na}^+$  impurity was reported by Takács<sup>5</sup> who effectively concentrated the impurity along an initially dry starting-line of a pherogram by allowing electrolyte to close the gap from each side by capillary attraction in order to complete the circuit prior to an electrophoretic run. He thereby obtained a positive reaction for  $\text{Na}^+$  (and for  $\text{Mg}^{2+}$ , also) on his developed pherogram, in contrast to the present artefactual "negative" response which is caused by local depletion of impurities.

#### REFERENCES

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