

activity by longer incubation with the substrate and it is convenient to compare directly samples in adjacent channels run under identical conditions. The evidence for two electrophoretically distinct forms of histidase in a crude extract is based on the two bands appearing after the histidase activity stain, the standard spectrophotometric assay of unstained bands after elution, and the conversion of histidine to urocanate when gel portions from unstained bands are incubated with histidine. There is a progressive conversion of histidine to a product which corresponds to urocanate in  $R_F$  value, color reaction to diazotized sulfanilic acid and UV quenching ability on paper chromatograms.

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### **A reaction for identification of $Al^{3+}$ , $Ga^{3+}$ , $In^{3+}$ , and $Tl^{+}$ separated by paper chromatography**

Many reactions have been used for the identification of the periodic group III-A elements on paper chromatograms<sup>1-4</sup> but most of them are not common to the whole group of ions. In the present work, a reagent is proposed which forms colored complexes with  $Al^{3+}$ ,  $Ga^{3+}$ ,  $In^{3+}$  and  $Tl^{+}$ , allowing detection of all the above cations separated on a paper chromatogram.

Oxidized hematoxylin has been investigated by one of us<sup>5</sup> in the chromatographic identification of Ge(IV). The same reagent has now been found to give colored spots with all the ions mentioned above. Work involving the detection of group III-A elements in ores can be simplified by the use of this single detectant after separation by paper chromatography.

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### Experimental

**Samples.** Solutions of the individual ions were prepared from the pure metal by dissolving in  $\text{HNO}_3$  and diluting with distilled water to obtain a concentration of 1 mg/ml.

**Reagent.** For the chromatographic identification of the ions, a solution of oxidized hematoxylin prepared according to NEWCOMBE *et al.*<sup>6</sup> was used.

**Procedure.** Aliquots (5–10  $\mu\text{l}$ ) of the different ionic solutions and their mixture were spotted on strips of Whatman No. 1 paper and the analysis was performed by the ascending technic. Seven different compositions of the solvent system isobutanol–HCl–water, described in Table I, were tested in order to find the best conditions for the complete separation of the cations (Table II). The organic upper layer was always used. Also the systems methyl ethyl ketone ( $\text{C}_4\text{H}_8\text{O}$ )–2% HCl (10:90), 0.1 M in  $\text{NH}_4\text{Cl}$ ;  $\text{C}_4\text{H}_8\text{O}$ –2% HCl (20:80), 0.1 M in  $\text{NH}_4\text{Cl}$ ; and  $\text{C}_4\text{H}_8\text{O}$ –2% HCl (30:70), 0.1 M in  $\text{NH}_4\text{Cl}$  were tried.

TABLE I

COMPOSITION OF ISOBUTANOL–HCl–WATER SOLVENT SYSTEMS AND THE MOLARITY OF HCl IN THE ORGANIC PHASE

| Solvent | Isobutanol | HCl | Water | HCl molarity<br>in the organic<br>phase |
|---------|------------|-----|-------|---|
| 1       | 65         | 15  | 120   | 0.266                                   |
| 2       | 75         | 15  | 105   | 0.342                                   |
| 3       | 90         | 15  | 95    | 0.386                                   |
| 4       | 70         | 20  | 110   | 0.443                                   |
| 5       | 80         | 20  | 100   | 0.450                                   |
| 6       | 70         | 25  | 105   | 0.543                                   |
| 7       | 70         | 30  | 100   | 0.788                                   |

TABLE II

$R_F$  VALUES OF PERIODIC GROUP III-A IONS WITH ISOBUTANOL–HCl–WATER SOLVENTS

| Ion              | Solvent |      |      |      |      |      |      |
|------------------|---------|------|------|------|------|------|------|
|                  | 1       | 2    | 3    | 4    | 5    | 6    | 7    |
| $\text{Al}^{3+}$ | 0.02    | 0.02 | 0.02 | 0.04 | 0.04 | 0.03 | 0.01 |
| $\text{Ga}^{3+}$ | 0.01    | 0.03 | 0.03 | 0.15 | 0.08 | 0.08 | 0.20 |
| $\text{In}^{3+}$ | 0.13    | 0.16 | 0.14 | 0.23 | 0.25 | 0.24 | 0.12 |
| $\text{Tl}^{+a}$ | 0.00    | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

<sup>a</sup>  $\text{Tl}^+$  does not migrate because it is very little soluble in dilute HCl.

After each run the paper was dried overnight in a stream of air at room temperature, then sprayed with the reagent and immediately after exposed to  $\text{NH}_3$  vapors in order to eliminate the last traces of acid from the solvent. The group III-A cations were revealed as intense violet or pink-violet spots.

Decreasing amounts of the samples were tested in order to find the detection limit, which was 0.6  $\gamma$  for  $\text{Ga}^{3+}$ , 0.7  $\gamma$  for  $\text{Al}^{3+}$ , 3.3  $\gamma$  for  $\text{In}^{3+}$ , and 3.0  $\gamma$  for  $\text{Tl}^+$ .

*Results and discussion*

The best separation was achieved with the organic phase of the mixture isobutanol-HCl-water (70:20:110) for which the corresponding  $R_F$ 's are given in Table III. Tailing was observed with all four cations in the solvents containing methyl ethyl ketone. On the other hand, compact spots were obtained in all the different isobutanol systems used.

TABLE III

$R_F$  VALUES OF IONS WITH THE SOLVENT SYSTEM ISOBUTANOL-HCl-WATER (70:20:110), SOLVENT No. 4

| <i>Ion</i>       | $R_F$ | <i>Ion</i>       | $R_F$ |
|------------------|-------|------------------|-------|
| Al <sup>3+</sup> | 0.04  | Fe <sup>3+</sup> | 0.11  |
| Ga <sup>3+</sup> | 0.15  | Sn <sup>2+</sup> | 1.0   |
| In <sup>3+</sup> | 0.23  | Sn <sup>4+</sup> | 0.92  |
| Tl <sup>+</sup>  | 0.00  | Sb <sup>3+</sup> | 1.0   |
| Zr <sup>4+</sup> | 0.01  | Bi <sup>3+</sup> | 0.58  |
|                  |       | Ge(IV)           | 0.35  |

Interference by other elements was investigated. Among 25 cations, Fe<sup>3+</sup>, Sn<sup>2+</sup>, Sn<sup>4+</sup>, Sb<sup>3+</sup>, Bi<sup>3+</sup> and Ge(IV) form colored complexes with hematoxylin, but they do not interfere in the chromatographic identification of periodic group III-A ions, since they have  $R_F$  values quite different from these, as can be seen in Table III. The only real interference is Zr<sup>4+</sup>, which presents the same behavior as Tl<sup>+</sup> ( $R_F = 0.01$ ) and should, therefore, be absent from the solution.

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