

greater extent in view of the longer boiling periods and higher acid concentration involved.

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### Positive Bromine Ions ?

IN a recent communication<sup>1,2</sup>, we described experiments in which bromine migrated towards the cathode from acid HOBr solution across paper-collodion membranes. We interpreted this migration as evidence of 'positive bromine ions' ( $\text{Br}^+$  or  $\text{H}_2\text{BrO}^+$  ions) that were present in these acid solutions. Further experiments revealed, however, that there was a considerable electro-endosmosis across the membranes in the direction of the cathode, and it became necessary to reconsider our results.

We avoided the use of membranes by placing two inverted U-tubes between the beaker containing the HOBr solution and those containing sulphuric acid and acting as electrode compartments. The U-tubes were filled with organic fluids lighter than water and able to react with active bromine. A solution of fluoresceine or carotene in butanol served for this purpose, and in both cases the anodic U-tube changed colour, whereas the cathodic one did not. Pure butanol also reacted with active bromine, and analysis revealed here, too, that migration was directed towards the anode. In each case the bromine accumulated to a greater extent in the anodic U-tube than in a control tube filled with the same reagent and immersed in the HOBr solution but without current passing through it, while the reaction at the cathodic side was less than that in the control tube, being, in fact, imperceptible.

This shows that we were able to detect the presence of  $\text{OBr}^-$  anions even in our 25 per cent sulphuric acid solution, where, using the new dissociation constant of HOBr as determined by Silov<sup>3</sup>, the  $\text{OBr}^-$  concentration was only  $10^{-10}$ . As no reaction whatever was observed on the cathode side, we may safely assume that the concentration of positive bromine ions, if such do exist, must be less than  $10^{-11}$  in our N/10 HOBr solution. We also tried to use such compounds as indicating reactants which do not react with HOBr unless it is acidified, hoping to exclude the reaction of the  $\text{OBr}^-$  ion. Refractometric measurements with xylene and with ethylbenzoate showed no significant effect, and a green solution of nitrosobenzene in butanol failed to give any colour reaction either. The voltage was about 300 V. D.C. and the current varied according to the conductivity of the organic fluid from a few microamperes to milliamperes. Thus, there is no direct electrochemical evidence for the existence of positive bromine ions.

We happened to prove, however, that fluoresceine migrates towards the anode in solutions more acid than pH 3.5, and eosine behaves similarly. We suggest that the ring oxygen atom may be responsible for this effect, acting as centre of an oxonium ion.

A detailed account of our results will appear in *Magyar Kémiai Folyóirat*.

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Budapest. Jan. 16.

<sup>1</sup> Gonda-Hunwald, K., Gráf, Gy., and Körösy, F., *Magyar Kémiai Folyóirat*, **56**, 203 (1950).

<sup>2</sup> Gonda-Hunwald, K., Gráf, Gy., and Körösy, F., *Nature*, **166**, 68 (1950).

<sup>3</sup> Silov, A., *Zsurnal fiziceszki. Chimii*, **24**, 702 (1950).

### Conservation of Amino-acid Chromatograms

IN filter-paper partition chromatography, the ninhydrin reaction has been used almost exclusively for the identification and quantitative assay of amino-acids. One of the chief disadvantages of the blue pigment formed in this reaction is its rapid fading on filter-paper chromatograms. This makes it difficult to construct permanent or semi-permanent reference maps from which not only the type of amino-acid, but also the approximate quantity of the unknown mixture, can be deduced.

It was found that the blue pigment could be rendered a great deal more stable by coupling it with a metal. This observation was made independently in our two laboratories and has led to a study of the metal derivatives of the blue pigment. All amino-acids form red pigments, except proline and oxyproline which form a yellow metal complex. These findings are summarized in the table. In addition to the metals mentioned in the table, nickel salts also yield red pigments very similar to cadmium.

Amino-acids	Zn <sup>++</sup>	Cd <sup>++</sup>	Co <sup>++</sup>	Cu <sup>++</sup>
Proline	yellow	yellow	brown	yellow ochre
Oxyproline	grey	yellow	light brown	
Alanine	} brick red	} port wine red	} chestnut	} salmon
Glycine				
Serine				
Threonine				
Phenylalanine	red	red ochre	brown	red ochre
Tryptophane	red	orange	brown	red ochre
Other amino-acids	red	port wine red	chestnut	salmon

Apparently the red complex salt is formed from two molecules of diketohydrindylidene-diketohydrindamine (the blue pigment) with one atom of the metal being held between the two nitrogen atoms. Hence it would be correctly described as triketohydrindylidene-2-amino-1-oxy-indenon(3)-metal, for which a structural formula has been suggested elsewhere<sup>1</sup>.

In practice, the red complex of the blue pigment is produced by dissolving a salt of one of the metals in methanol or ethanol and spraying each blue spot on the chromatogram with the reagent; on evaporation of the solvent, the corresponding red, reddish-brown or salmon-coloured pigment is formed. The following reagent, which keeps indefinitely, has been found useful: to 1 ml. of a saturated solution of copper nitrate add 0.2 ml. of 10 per cent (v/v) nitric acid and make up to 100 ml. volume with 95 per cent ethanol. This makes approximately a 0.8 per cent solution of copper nitrate at room temperature.

The accompanying photograph illustrates the results which can be obtained. A strip of filter-paper was saturated with a weak solution of alanine, dried, sprayed with 0.1 per cent ninhydrin and heated at 37° C. for 30 min. to develop the blue colour. A centre section of this strip was cut out and put aside, and the remainder was sprayed with the copper reagent. The cut-out section was now replaced and the strip photographed (Fig. 1). The

