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Note

Paper chromatography of azo-derivatives of chromotropic acid

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Mono- and bis-azo derivatives of chromotropic acid are widely used as reagents in the photometric determination of various elements. The available reagents almost invariably contain impurities; these take the form of reactants and by-products formed during the course of their syntheses. The impurities influence significantly the choice of analytical conditions and other important analytical investigations such as the determination of stability constants and composition of metal complexes. A rapid and simple procedure for testing the purity of the reagents is therefore required, and a paper chromatographic technique is suitable for this purpose.

In our investigations of the application of chlorophosphonazo III [3,6-bis(4-chloro-2-phosphophenyl azo)-chromotropic acid] to the photometric determination of alkaline-earth and other metal ions, we have needed to purify the reagent and a paper chromatographic technique is appropriate in following its process of purification. A few procedures for azo derivatives of chromotropic acid have been recorded in the literature^{1,2}, but an attempt to apply the developing solvents in these procedures to test for chlorophosphonazo III was unsuccessful, because they gave only a very diffuse, ill-defined spot on the chromatogram. Thus, a new developing solvent was required. A developing solvent composed of pyridine-28% conc. ammonia-1-pentanol has proved to be most suitable for testing for chlorophosphonazo III and the other azo-derivatives of chromotropic acid.

EXPERIMENTAL

Apparatus

A Toyo Roshi, Type C, glass chamber was used with a paper holder containing Whatman 3 MM paper. The solvent, pyridine-28% conc. ammonia-1-pentanol, was made up as follows: solvent system 1: (10:10:0); solvent system 2: (10:10:2); solvent system 3: (10:10:5); solvent system 4: (10:10:8).

Dyes

The azo-derivatives of chromotropic acid investigated in the present study and their manufacturers are as follows: arsenazo I, Aldrich (Milwaukee, Wisc., U.S.A.), BDH (Poole, Great Britain), Dotite (Kumamoto, Japan); arsenazo III, Aldrich, BDH, Dotite, Merck (Darmstadt, G.F.R.); chlorophosphonazo III, Dotite; methyl-

sulphonazo III, Aldrich, BDH, Dotite; nitrosulphonazo III, Aldrich, BDH, Dotite; 2-(*p*-sulphophenylazo)-1,8-dihydroxynaphthalene-3,6-disulphonic acid (SPADNS), Aldrich, BDH, Dotite; sulphonazo III, Aldrich, BDH, Dotite, Merck.

Preparation of test solution

25 mg of each dye is weighed into a 5-ml volumetric flask and dissolved in water. If necessary, 2 or 3 drops of 2 *N* ammonia are added.

Procedure

2- μ l test solutions are applied as spots on the starting line of the chromatogram by means of a 2- μ l micropipette. The size of the chromatogram is 50 \times 400 mm, the distance of the starting line from the edge of the paper being 80 mm. The dyes are spotted on the starting line at intervals of 10 or 20 mm. The chromatogram is attached to a paper holder (Fig. 1) and then placed in the chamber containing about 10 ml of the developing solvent. The chromatogram is normally allowed to stand for 3 h to permit equilibration of the vapour within. Development is conducted by a descending technique for 4 h, adding 25 ml of the developing solvent the solvent reservoir of the holder. The apparatus and solvent systems are placed in an air-conditioned room at a temperature of $20 \pm 1^\circ$. After development, the chromatogram is taken out of the chamber and air-dried.

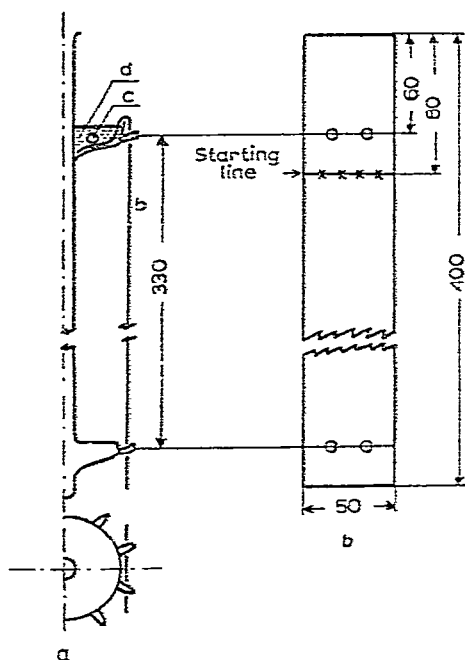


Fig. 1. Paper holder and chromatogram: a, paper holder; b, chromatogram; c, a weight (a ring of glass rod); d, developing solvent (solvent reservoir).

RESULTS AND DISCUSSION

Effect of "equilibration" of the chromatogram on development of the dyes.

The term "equilibration"³ is defined in the present study to imply that the chromatogram is placed in the chamber saturated with an atmosphere of the developing solvent for a definite time before commencing development. The effect of equilibration has been investigated for four dyes: chlorophosphonazo III, methylsulphonazo III, SPADNS and sulphonazo III. The results are given in Fig. 2. The spot of main component in chlorophosphonazo III tails back to the starting line when the chromatogram is developed without equilibration. A clearer spot is obtained after an hour of equilibration. A good separation of the main component and impurities in the dye is obtained after 19 h equilibration, but, in practice, the main component and impurities are identifiable after 2-4 h of equilibration, and the chromatogram is therefore allowed to stand for 3 h in routine work. This effect is not noticeable for other dyes investigated.



Fig. 2. Effect of equilibration of the chromatogram on development of the dyes. Dyes: 1 = chlorophosphonazo III, 2 = SPADNS, 3 = sulphonazo III and 4 = methylsulphonazo III. Developing solvent: solvent system 3.

Effect of the mixing ratio of 1-pentanol in the developing solvent on the chromatographic separation

In the developing solvent, the pyridine-28% conc. ammonia ratio is kept constant at 10:10, while 1-pentanol is varied from 0 to 8 in ratio. As can be seen in Fig. 3, increasing the ratio of 1-pentanol progressively reduces the rate of movement of all the dyes investigated. From the stand point of testing the purity of the dyes, a favourable mixing ratio of 1-pentanol has been investigated for individual dyes.

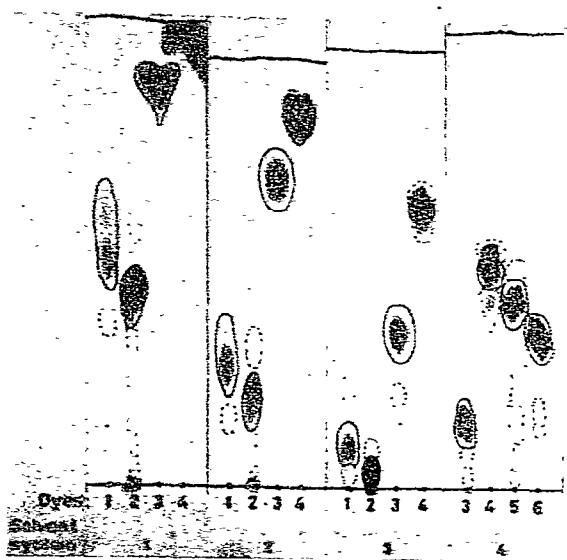


Fig. 3. Effect of the solvent composition on development of the dyes. Dyes: 1 = arsenazo I, 2 = arsenazo III, 3 = SPADNS, 4 = nitrosulphonazo III, 5 = methylsulphonazo III and 6 = sulphonazo III.

In chlorophosphonazo III, solvent system 2 is most favourable for the testing of impurities having lower R_F values than that of the main spot, while solvent system 3 is most favourable in distinguishing the two spots of impurities having higher R_F values (see Fig. 4). Since it seems that there is an analytical significance in the latter

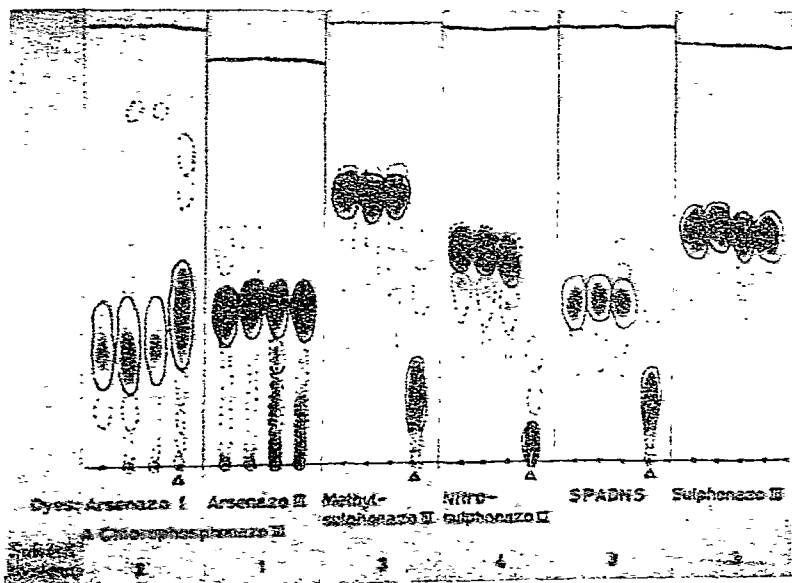


Fig. 4. Paper chromatography of azo derivatives of chromotropic acid from various manufacturers. Individual samples of each dye are arranged in the order described above.

impurities, solvent system 3 is used in routine work. As can be seen in Fig. 3, solvent system 2 is favourable for arsenazo I, while solvent system 1 is favourable for arsenazo III. Solvent system 3 is favourable for SPADNS and sulphonazo III. Solvent system 4 is favourable for methylsulphonazo III and nitrosulphonazo III.

Test of purity of the dyes from various manufacturers

Six azo-derivatives of chromotropic acid purchased from three or four manufacturers have been investigated by the procedure described above. The results are given in Fig. 4 and the observations are as follows: arsenazo III: the spot of arsenazo I as an impurity is observed in two samples (refer to Fig. 3); arsenazo I, SPADNS and sulphonazo III: a few faint coloured spots of impurities are observed; methylsulphonazo III and nitrosulphonazo III: similar patterns of spots of impurities are observed in each sample.

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