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The Preparation of Permanent Turbidimetric Standards for Use in the Determination of Small Amounts of Chloride

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Permanent turbidimetric standards are useful in the determination of small amounts of chloride. They are of particular value in the control of the purity of Analar and B.P. chemicals, since they avoid the repeated and costly preparation of fresh standards; and they may be used for other turbidimetric tests, e.g. the determination of proteins by precipitation with sulphosalicylic or trichloroacetic acid.

We feel that such standards may be of value to workers in biochemical and other fields, and the purpose of this paper is to describe the preparation of permanent standards of cloudy Perspex which we have found useful in the routine determination of chloride, and which have been employed by King (1950) for urinary and C.S.F. protein estimations.

The method of preparation of the standards involves, in the first place, the production of a clear interpolymer of 50 % methyl methacrylate and 50 % styrene. In the second stage of the process, this interpolymer is dissolved in methyl methacrylate monomer; this monomer is then polymerized under conditions which involve uniform precipitation of the styrene/methyl methacrylate interpolymer.

METHODS

Interpolymer. A polymethyl methacrylate-polystyrene interpolymer is prepared as follows: 200 g. of redistilled methyl methacrylate monomer are heated with 200 g. of redistilled styrene monomer and 1·6 g. benzoyl peroxide catalyst with stirring in a conical flask (600 ml.) at 70° under reflux, until the viscosity of the syrup is approximately 4 poises. This operation takes about three-quarters of an hour. The syrup is cooled and then evacuated free from air by means of a water pump. The cold syrup is poured into a cell formed by two glass plates (30·5 × 30·5 × 0·6 cm.) separated from one another by rubber tubing (6 mm. diam., 1 mm. wall) and bound by screw clips (Fig. 1). After filling and binding, the cell is maintained at 75° for about 6 hr. in an air oven. A steady current of air is circulated through the oven throughout this period. After cooling, the polymer sheet is detached from the glass plates, ground and bottled.

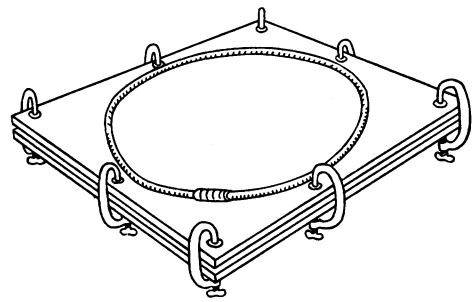

Fig. 1. Cell for forming interpolymer.

Standards. Turbidimetric standards are prepared in the following way: a known weight of the ground polymer (see below) is dissolved in 50 ml. of redistilled methyl methacrylate monomer by heating under reflux with stirring for 1 hr. at 50° in the apparatus shown in Fig. 2. 1·1 ml. of a

solution of 0·5 g. of benzoyl peroxide in 10 ml. of methyl methacrylate monomer is then added. The temperature is raised to 70–85° and maintained there until a syrup of viscosity 4 poises is produced: this operation takes approximately three-quarters of an hour. The syrup is cooled, evacuated free from air and poured into soda-glass test tubes (25 × 1 cm.). These tubes are then lowered at a rate of 25 cm. in 12 hr. into an oil bath maintained at 75°, using the apparatus shown in Fig. 3. The purpose of carrying out the final polymerization by this method is to avoid, as far as possible, contraction bubbles in the final product.

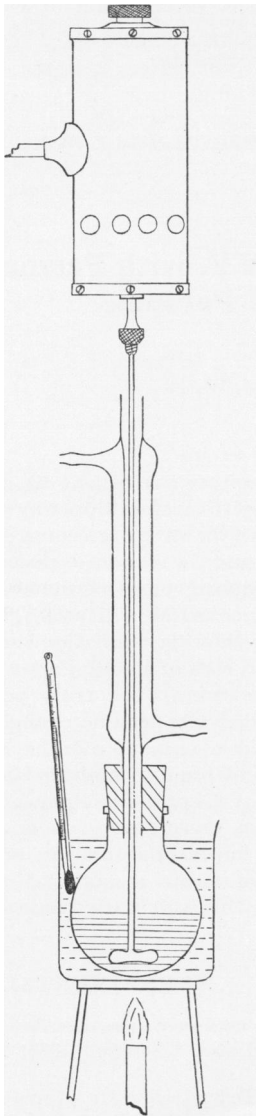

Fig. 2. Apparatus for preparation of interpolymer-monomer syrup.

When fully immersed the tubes are allowed to remain in the oil bath for a further period of 4 hr., in order to obtain the desired turbidimetric standards. The glass tubes are broken, and the prepared standards cut into suitable lengths (5–7·5 cm.) and fixed in tightly fitting glass tubes (1 cm. diam.).

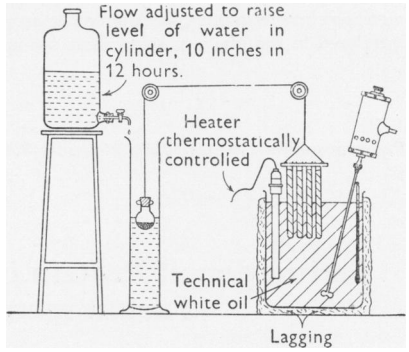

Fig. 3. Apparatus for preparation of standards.

The turbidity produced varies with the concentration of the interpolymer in the product, but in our experience not in a definite and reproducible manner. By dissolving amounts of polymethyl methacrylate-polystyrene interpolymers, ranging from 0·15 to 0·42 g., in 50 ml. methyl methacrylate monomer we have prepared a range of ten standards very suitable for chloride tests. These standards cover the range 0–3·8 ml. 0·01 N-HCl when used according to the method given below, i.e. using 1 g. of a substance for analysis the standards would cover the range 0–0·135% chlorine as chloride.

Comparison. We are indebted to Prof. E. J. King for his advice on the method of matching AgCl suspensions with turbidimetric standards. When the standards are placed in a specially constructed viewing rack (Fig. 4) and illuminated by light from a window behind the operator, the turbidimetric comparisons are made very readily.

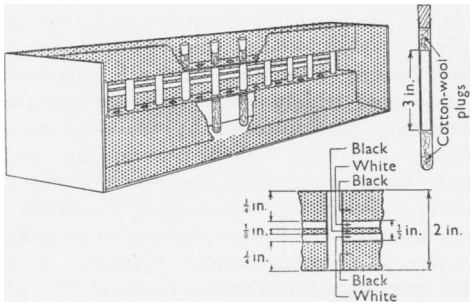

Fig. 4. Viewing rack for turbidimetric standards.

Calibration. It is, of course, necessary to calibrate against known AgCl suspensions. We have calibrated our standards by comparison with AgCl prepared as follows (B.P., 1948): a definite volume of 0·01 N-HCl and 1 ml. of HNO_3 (70%,

w/w) are measured into a 50 ml. flask. The solution is diluted to 50 ml. with distilled water, and 1 ml. of 5% (w/v) $AgNO_3$ is added. The turbid solution is set aside for 5 min., and then transferred, for viewing purposes, to tubes similar to those containing the permanent standards.

In our experience a standard prepared from 0·42 g. polymethyl methacrylate-polystyrene interpolymer and 50 ml. methyl methacrylate monomer corresponds to an AgCl suspension prepared in the above manner from 3·8 ml. 0·01 N-HCl.

SUMMARY

1. The preparation of turbidimetric standards, made of Perspex, has been described.

2. The turbidities of these standards are permanent, and form a useful basis of comparison for silver chloride, protein and other analytical procedures which depend on the production of finely dispersed precipitates.

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Estimation of Protein in Urine and C.S.F. with Permanent Turbidimetric Standards of Perspex

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Several methods exist for the determination of the protein of urine, C.S.F. and other body fluids, including those of Esbach (1874), Exton (1925), Kingsbury, Clark, Williams & Post (1926) and Hiller, McIntosh & Van Slyke (1927). That of Kingsbury *et al.* is probably the simplest and the most widely used. Although it cannot be considered a precise method, it is nevertheless sufficiently accurate for clinical purposes. The results usually required for urinary and C.S.F. protein need be only approximate, and the assessment of the turbidity produced by mixing urine or C.S.F. with sulphosalicylic acid against convenient turbidimetric reference standards such as those of Kingsbury *et al.* makes a quick and very useful procedure for clinical laboratories. These standards, which consist of a permanent suspension of formazin in gelatin, were investigated by King & Haslewood (1936), and modified to allow the use of a small volume of the urine or C.S.F. The formazin standards are widely used; they are satisfactory within the limits imposed by the consistency of turbidity formation when protein and sulphosalicylic acid are mixed, and the ability of the observer to select the turbid standard which most closely matches the test mixture of urine or C.S.F. with the sulphosalicylic acid. Plimmer & Lowy (1945) found that even with a photoelectric photometer the agreement between the results of this procedure and those obtained by Kjeldahl was only about ± 5%. With the visual matching of test with standards only about ± 10% accuracy is obtained, but this is sufficient for the purpose.

Although the formazin standards have been easy to use, and have yielded acceptable results, they have not been as permanent as could have been wished. Despite careful stoppering and sealing of the test tubes in which they are contained, evaporation takes place, and the standards degenerate. With the object of securing more permanent standards the author approached Mr J. Haslam, who had already prepared for chloride estimation turbidimetric standards of solid rods of cloudy Perspex. It is believed that these standards are truly permanent in the sense that there has been no change in their optical characteristics over many months; and it is believed that they will remain unaltered indefinitely. The comparison of the protein-sulphosalicylic acid turbidities against these standards is as easy as it is against the formazin standards, and it is believed that they constitute a more satisfactory point of reference for this widely used procedure.

EXPERIMENTAL

The standards used were those described in the accompanying paper by Haslam & Squirrell (1950). The procedure is as follows:

Procedure. To 3 ml. of 3% (w/v) sulphosalicylic acid in a test tube of the same internal diameter as those which contain the standards is added 1 ml. of urine, C.S.F. or other albuminous body fluid (e.g. ascitic fluid). The turbidity is compared after 5 min. with those of the standards. If the degree of turbidity is too great for comparison a suitable dilution of the fluid is made with water, and the test is repeated. It is convenient to make the comparison by viewing the test and standards against a strip of white cardboard with a transverse black line (0·5 cm.) which is mounted in the rack as illustrated in Fig. 4 of Haslam & Squirrell (1950). The viewing is best made with the operator