CLXXI. NOTE ON THE EFFECT OF AMMONIUM SULPHATE AND OTHER SALTS ON THE COLORIMETRIC ESTIMATION OF PHOSPHORUS.

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IN view of the present extensive use of the Briggs colorimetric method for the estimation of phosphorus, certain difficulties which have been encountered in the application of this method to solutions containing high concentrations of salts are worthy of record.

EFFECT OF AMMONIUM SULPHATE.

During the course of investigations at present in progress, it was desired to estimate, by the Briggs [1922] method, the phosphorus content of small quantities of solutions containing proteins. For the removal of the latter, half saturation with ammonium sulphate was employed, but the results were quite irregular, in nearly all cases low values being obtained. The cause of the irregularity was the high concentration of ammonium sulphate. This salt, in concentrations above 1.4*N*, markedly decreases the velocity of colour production in solutions containing the amounts of phosphorus which are those normally estimated by this process.

The effect of measured amounts of ammonium sulphate in solutions in which definite quantities of phosphorus were present was investigated. After the addition of Briggs' reagents in the usual way, the colour produced in these solutions at the end of half-an-hour was compared with a standard similarly treated, but without the addition of the ammonium sulphate. Plotting these half-hour values, calculated as alleged phosphorus content (= depth of colour relative to standard) against the concentration of ammonium sulphate, the curve of Fig. 1 is obtained.

It is at once evident that the colour is markedly depressed as the concentration of ammonium sulphate increases, although when below a certain value—in this series, 1.4*N*—the colour attained in half-an-hour is actually deepened by the addition of the ammonium salt. This may be due to the lavender-blue tint which is seen in all solutions containing ammonium salts in more than minimal quantities when treated according to the Briggs technique.

There is, however, no error produced by the small amounts of ammonium sulphate that may result from the micro-incineration method usually employed for determining the total quantity of phosphorus in presence of small quantities of protein. Taking caseinogen as containing 0.8 % P and 16 % N, and supposing quantities are chosen so that 0.05 mg. of phosphorus is present in a total vol. of 25 cc. when the colorimetric determination is made, the concentration of ammonium sulphate derived from the nitrogen of the protein molecule will not exceed 0.003*N*, a figure considerably lower than that found by experiment to be the least affecting the determination. No less than 2 g. of protein need to be oxidised for the ammonium sulphate produced to interfere seriously with colour production.

Fig. 1. Curve showing depth of colour after half-an-hour with constant amount of PO_4 and different concentrations of ammonium sulphate. (0.2 mg. P in 50 cc. total volume.)

Although the rate of colour production in presence of ammonium sulphate is slowed, the final depth of colour attained after standing for a lengthy period is practically unaffected. This is clearly shown in Fig. 2 which explains itself.

It will be seen that with a concentration of 1.4*N* ammonium sulphate, the colour production at the end of half-an-hour is only about 80 % of that which the amount of phosphate present should produce.

Since, however, the colorimetric estimation is made against a standard which is itself slowly changing, the curves in Fig. 2 will be somewhat distorted. Such distortion, however, is confined to the readings made within the first

quarter of an hour, since the standard solution has developed at least 93 % of its colour value at the end of half-an-hour.

In view of the observation of Briggs that sulphuric acid when present in excess exerts an inhibiting action upon colour formation, it was suspected that the retardation produced by ammonium sulphate might in reality be due to the sulphate ion. That this is not so is evidenced by the fact that neither magnesium nor zinc sulphate has any disturbing effect, although sodium sulphate depresses colour formation to a slight extent. Neither can the effect be due to the ammonium ion, for solutions treated with ammonium nitrate exhibit no irregularity.

Since the diminution in rate of colour production is not due to the NH_4 ion, nor to the SO_4 ion, it is possible that it may be the result of some specific effect of un-ionised ammonium sulphate.

Fig. 2. Velocity of colour production in presence of different concentrations of ammonium sulphate. (0.05 mg. P in 25 cc. total volume.)

It has been found that ammonium sulphate is without effect once the colour has developed ($\frac{1}{2}$ or 1 hr.); hence the inhibition is upon the *progress* of the reaction and is not merely a disturbance of the ionic equilibrium of the solution. Neither can it be due to an interference with the preliminary reaction—

Ammonium Molybdate + Phosphate $\rightarrow$ Ammonium Phosphomolybdate
for maintenance of the two reactants at a temperature of 65° for one hour before the ammonium sulphate is added in no way influences the retarding effect of the latter.

EFFECT OF ANTICOAGULANTS.

One of the disadvantages attending the use of the Bell-Doisy [1920] technique was that when applied to plasmas containing excess of anticoagulant, the colour production was interfered with. Denis and von Meysenbug [1922] investigated the case of sodium citrate and potassium oxalate with reference to this method.

In his first paper Briggs [1922] points out that his technique is not affected by the concentrations of these salts usually employed.

The present author has found that ammonium oxalate, sodium oxalate, sodium sulphate, sodium fluoride and sodium citrate all interfere with colour production if their concentrations be sufficiently great. The limiting concentrations at which interference first occurs have been determined for these salts and are given below.

Concentration of Phosphorus—0.05 mg. in 25 cc.

Anticoagulant	Concentration at which interference occurs	Approximate concentration usually present
Sodium oxalate	0.008 <i>N</i>	0.002 <i>N</i>
„ citrate	0.008 <i>N</i>	0.003 <i>N</i>
„ sulphate	0.8 <i>N</i>	0.109 <i>N</i>
„ fluoride	0.01 <i>N</i>	0.007 <i>N</i>

Following the usual practice of adding nine parts of blood to one part of sodium fluoride (3 % solution) and then using the quantities recommended by Briggs [1924], the concentration of fluoride in the final solution will not exceed 0.007*N*; thus no interference will result. The concentration is, however, rather dangerously near the strength at which interference first occurs.

It will be apparent that although the colorimetric method is eminently suited for the estimation of small quantities of phosphorus, there are causes of error which unless guarded against are liable to render the results inaccurate.

REFERENCES.

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