CIII. NESSLERISATION, AND THE AVOIDANCE OF TURBIDITY IN NESSLERISED SOLUTIONS.

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THE brown coloration which is produced when very dilute solutions of ammonium salts are treated with Nessler solution has been used as a means of detecting and estimating ammonia in water analysis for many years, and more recently has found extensive employment in biochemical investigations, where the quantity of material available so frequently necessitates colorimetric analytical methods.

The quantities of substance which are measured in this kind of work are most conveniently reckoned in hundredths of a milligram. To avoid the constant repetition of this clumsy phrase, I venture to coin the word "centi-milligram," conveniently written cmg.

In the case of every comparison of coloured substances there is a particular concentration at which the comparison is most easily made. This will depend, of course, on the thickness of the layer of liquid which is to be examined, and it varies with different colours, and to some extent with different individuals. Our comparisons are conducted in the dilution colorimeter [Stanford, 1923, 1] using a 10 mm. layer. In these circumstances the most satisfactory results are obtained when the concentration is in the neighbourhood of 1 cmg. of nitrogen per cc.

Nesslerisation differs also, however, from the other common colorimetric processes in that it is peculiarly liable to give rise to solutions which are *turbid*, and so useless for colorimetric comparison. This is well known, but until now its cause does not seem to have been discovered. It is stated in Clowes and Coleman's well-known handbook that the Nessler solution must always be added to the ammoniacal solution, and never in the reverse order, or a turbid solution will result. As will be shown, this statement is not correct. In the past few years Folin has described several rapid methods for the estimation of nitrogen or urea nitrogen in products of animal origin "by direct nesslerisation," that is to say, the reaction product is treated with Nessler solution and compared with a nesslerised standard. Cole [1920] has stated that he was unable to repeat these experiments because of the turbidity of the resulting solutions. The trouble seems also to have occurred to Folin himself, for he says, in a later paper, "if the solution be turbid, centrifugalise

a portion of it." A strange counsel, seeing that the turbidity is due to minute oily drops of the dark brown colouring matter which it is proposed to estimate.

After very numerous repetitions of Folin's procedure I could only confirm Cole's statement, but in further investigating the matter I was able to discover the cause of the occurrence of turbidity, and the means of avoiding it. The question really divides itself into two. On the one hand there have to be considered the processes which occur when a pure solution of ammonium sulphate or ammonium chloride is treated with Nessler solution, and on the other hand what occurs when, say, the product of a urine digestion is treated with Nessler reagent.

I have not been able to discover any conditions in which such impure products may be nesslerised to give a clear solution, and it is for that reason that I devised the method for the removal of ammonia from such liquids which is described in the following paper [Stanford, 1923, 2]. Some points with regard to such solutions will be referred to below.

Method of nesslerisation to obtain clear solutions. A clear solution will always be obtained (1) if the concentration of ammonia is below a certain limit (see below) and (2) if the addition of the Nessler solution be made *drop by drop with constant shaking*.

It does not matter whether the reagent is added to the ammonia or *vice versa* provided the addition be carried out in this way, but if the ammonia solution be added to the reagent, the latter should be diluted if possible beforehand.

It ought to be mentioned that two Nessler solutions are in common use, one of which is about twice as concentrated as the other. All the statements in this paper refer to the more dilute solution (about 22 g. KI per litre), but they hold good also for the stronger solution.

Ammoniacal solutions to be nesslerised should not contain more than 2 or 3 cmg. of nitrogen per cc. Turbidity can be avoided when the solutions are a good deal stronger than this, but it is not desirable to try, for a concentration of 1 cmg. of nitrogen per cc. gives a coloration convenient for colorimetric comparison in a 10 mm. layer.

The following experiments demonstrate the whole matter quite clearly:

(1) A solution containing 1 cmg. N per cc. (as $(\text{NH}_4)_2\text{SO}_4$) nesslerises clear when treated with one-tenth of its volume of Nessler solution added drop by drop with constant shaking.

(2) Repeat No. 1 without shaking and a turbid solution results.

(3) Repeat No. 1. The clear solution when treated with a few drops of 20 % sodium hydroxide becomes turbid at once, and the same thing happens if an excess of Nessler reagent be added.

(4) Repeat No. 1, but add to the ammonium sulphate solution a little urease solution (Folin) first; the nesslerised liquid will be turbid even though the reagent be added in drops and with shaking.

Ageing of nesslerised solutions. It is well known that the colour of a nesslerised solution alters rather rapidly with lapse of time. It was hoped that when the solutions were obtained with the precautions above mentioned this alteration might not occur, but unfortunately this is not the case. It is, therefore, still necessary to nesslerise solutions intended for comparison as nearly simultaneously as possible.

SUMMARY.

(1) Pure solutions of ammonium salts always give clear solutions when the Nessler reagent is added drop by drop with constant shaking.

(2) The ammonia solution should not contain more than 3 cmg. of nitrogen per cc., and the Nessler reagent should be one-tenth of the volume of the solution to be nesslerised.

(3) Excess of alkali or of Nessler reagent makes the clear solution turbid, as also does the addition of traces of urease.

(4) No conditions could be discovered in which clear solutions could be obtained by "direct nesslerisation" as suggested by Folin.

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REFERENCE.

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