LXIV. NESSLERISATION AND AVOIDANCE OF TURBIDITY IN NESSLERISED SOLUTIONS.

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(Received February 25th, 1924.)

ONE of the main conclusions reached by Stanford [1923] in a recent paper, under the title given above, is stated as follows: "No condition could be discovered in which clear solutions could be obtained by 'direct Nesslerisation,' as suggested by Folin." In a preceding part of the paper, Stanford further writes as follows:

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's 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 oil drops of the dark brown colouring matter which it is proposed to estimate.

The direct nesslerisation methods have been taught to hundreds of students and are in daily use in scores of American hospital laboratories. The avoidance of turbidity was necessarily the chief difficulty that had to be overcome in the development of the process and it is rather naïve on the part of Stanford to assume that I "in a later paper" advised the removal of the coloured turbidity by means of the centrifuge. The centrifuging is recommended in connection with total nitrogen determinations to remove the silica often produced by the boiling mixture of phosphoric and sulphuric acids during the preliminary digestion. On page 133 of my laboratory manual [1922] occurs the following:

If the unknown nesslerised digestion mixture is turbid, centrifuge a portion, giving a crystal clear fluid above a white sediment (silica). If the sediment is coloured, the nesslerisation was not successful and the determination must be discarded.

On page 237, under directions for determining the non-protein nitrogen in blood, the following helpful hints are given:

Many seem to have trouble in obtaining perfectly clear solutions when nesslerising the digestion mixtures obtained with blood filtrates. The cause is lack of suitable alkalinity in the Nessler solution. The following data will help to overcome the difficulty.

Twenty cubic centimetres of normal hydrochloric acid may be titrated with the Nessler solution and if the solution is substantially correct, a good end point will be obtained at 11 to 11.5 cc. with phenolphthalein as indicator.

If an end point is obtained much below 11 cc., as at 9.5 cc., the Nessler solution is too alkaline and turbidity is likely to occur.

Turbidity due to excess of alkalinity may likewise be produced because the sulphuric-phosphoric acid mixture is too weak. If 5 cc. of the dilute acid (1 : 1) are further diluted 10 times (to 50 cc.), 10 cc. of the solution so obtained, when titrated with the Nessler solution and phenolphthalein as indicator, should give a fairly good end point at 9 cc. to 9.3 cc.

Again on page 285, under directions for preparing the Nessler reagent, is given the following:

In the absence of modifying circumstances, such as the presence of much acid or alkali this (Nessler) reagent should be added in the proportion of 10 cc. per 100 cc. of the volume to which the nesslerised solution is to be diluted. As a general rule the volumetric flask (or volumetric test tube) should be at least two-thirds full before adding the Nessler reagent. If attention is not given to this detail, turbid mixtures are obtained, and turbid solutions must never be used for colour comparisons.

When Stanford concludes that direct nesslerisation is unattainable, and that clear solutions can be obtained only by adding the Nessler solution "*drop by drop with constant shaking*," this proves only that he is using an unsuitable reagent. Under my directions for determining the ammonia in urine, page 129, are given the following directions for nesslerising 0.5 mg. of ammonia in a 100 cc. volumetric flask:

Transfer 10 cc. of Nessler's solution (see page 283) to a measuring cylinder. Now give the volumetric flask a vigorous whirl so as to set the solution spinning within the flask and add at once the whole of the Nessler solution in the cylinder....If the process of nesslerisation has been successful, a deep red but crystal clear solution is obtained. If it is not perfectly clear, throw it away and prepare a fresh standard. With a little experience no trouble is encountered in getting clear solutions.

The preparation of the Nessler reagent here referred to is described in the paper entitled: "A System of Blood Analysis" [Folin and Wu, 1919]. It is not the best reagent for each individual purpose, but has been so modified as to be fairly satisfactory for all the different nesslerisations involved in blood and urine analysis. It is least satisfactory for nesslerising the urea of urine after decomposing with Jack bean extracts.

The most disturbing impurity is magnesium in any form, and it is because of its magnesium content that tap water cannot be used for the preparation of solutions which are to be nesslerised¹.

¹ In the course of the past dozen years, I have repeatedly returned to the subject of colorimetric nitrogen determinations and have made various observations as to the applicability and limitations of Nessler's reagent. For the convenience of any one wishing to make further studies on this topic I venture to give the following specific references to the *Journal of Biological Chemistry*, vol. 11, pp. 493, 496; vol. 26, pp. 474-5, 478-80, 483, 497-98; vol. 38, pp. 111-12. Stanford evidently has not had access to this literature.

REFERENCES.

- Folin (1922). *Laboratory Manual of Biological Chemistry*. D. Appleton Co.
Folin and Wu (1919). *J. Biol. Chem.* 38, 89.
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