SOME NOTES ON THE PHOTOELECTRIC COLORIMETRY OF NESSLERIZED SOLUTION

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Recently the colorimetry of nesslerized solution became a familiar method for the clinico-chemical determination of nitrogen. Since I felt it very convenient in routine laboratory work, I have attempted to examine the accuracy and reliability of this colorimetry by utilizing Erma's photoelectric spectrophotometer, the effective width of the spectral band of which is 10 mμ to 35 mμ ranging in wave-length from 400 to 700 mμ.

METHODS

As the fluid to be nesslerized, 0.01–0.03 mg N/12 ml aqueous solution of ammonium sulfate was used, for such solutions seemed to be the optimum for the colorimetry. Nessler's solution was prepared according to Koch and McMeekin (1924)[2]; 2.0 ml of the potassium mercury iodide being mixed with 10.0 ml of 10 g/dl aqueous solution of sodium hydroxide shortly before use. A total of 1.0 ml of this Nessler's solution was added to 12.0 ml of the aforementioned N solutions which had been taken in a test tube, by means of a pipette drop by drop, shaking the test tube after each addition.

After waiting for a period of time sufficient to develop the orange yellow color of the mixture to the full extent, its optical density E was measured with the photoelectric colorimeter using a cuvette of test tube type, the inside diameter of which was 20 mm. The blank solution was a mixture of 12 ml of water added by 1 ml of Nessler's solution.

The optical density E is expressed in the following equation, where I_o and I are the intensities of light after passing through a certain depth (20 mm in this experiment) of the solvent (blank solution) or the colored solution (nesslerized solution), respectively.

$$E = log_{10} I_o - log_{10} I$$

RESULTS AND DISCUSSION

Fig. 1 shows the light absorption curves of the solutions colored according to the method described above. The optical density of the solutions was measured at different wave-length covering the whole range of visible light, after allowing to stand still from 15 to 30 minutes at room temperature of 15 C. At a glance we

* Under the direction of Prof. S. Shibata.

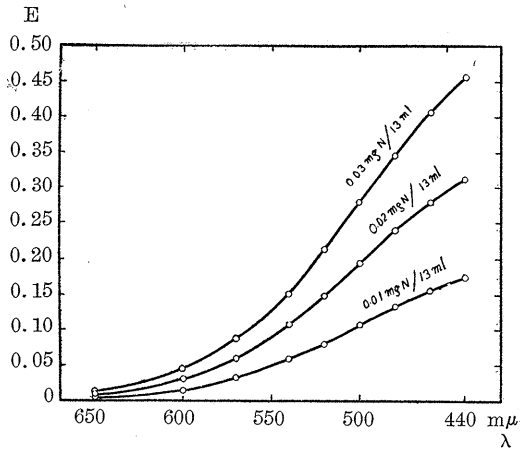

Fig. 1 Absorption curve of nesslerized solution
(E : optical density; λ : wave-length of light)

will notice the simple curved lines which ascend with decreasing wave-length from the longer to the shorter, without showing any special windings suggestive of light absorption bands. If we examine the correlation between the nitrogen concentration C and optical density E at the wave-length of 440, 460, 480, 500, 520, 540, 570, 600 and 650 mμ, we get a linear equation:

$$E=KC+m \qquad 1.$$

(K and m are constants which are determined by the wave-length of light), so far as the N concentration of colored solutions remains within the range from 0.01 to 0.03 mg N/13 ml. This is clearly shown in figure 2. Since m never becomes zero in the equation 1, as is revealed by the straight lines which do not pass through the origin of co-ordinates (ordinate, optical density; abscissa, N concentration of solution), Lambert-Beer's law does not hold for this colorimetry. Furthermore, equation 1 has two undetermined constants, K and m. These peculiarities let us adopt the special colorimetric procedures which have been deviced by Shibata (1951)[3], as follows: one measures the optical density of the unknown solution together with those of two standard solutions, one of which is expected to be more concentrated, and the other more diluted than the unknown, and the reads are made of the concentration of the unknown solution in comparison with the straight line connecting two points which represent the standard solutions on the optical density-concentration graph. As will be seen in Fig. 2, the shorter the wave-length used for colorimetry, the steeper is the inclination of the optical density-concentration curve, so that the slightest differences in nitrogen concentration can be caught by using the light of a wave-length shorter than 500 mμ. For the photoelectric colorimeter used in this study the wave length of 460 mμ or the filter No. S47 will be statisfactory. At a wave-length less than 450 mμ, the intensity of the ligth after passing through the solutions becomes too weak to allow accurate estima-

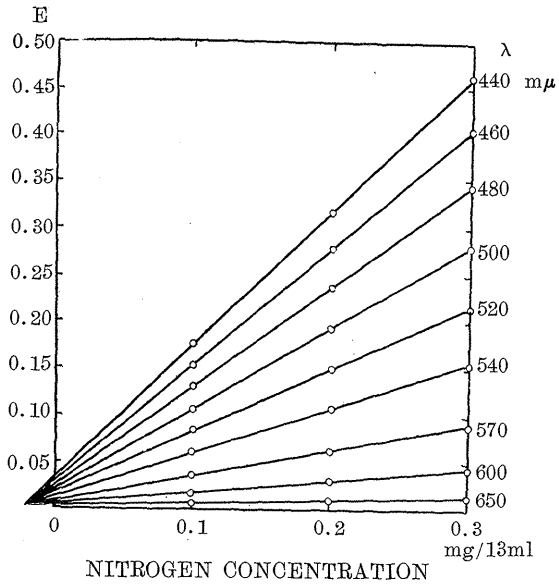

Fig. 2 Calibration curve of nesslerized solution
(E : optical density; C : nitrogen concentration of solution;
λ : wave length of light utilized)

tions of their optical density.

In general, the color does not develop completely when Nessler's solution is added at one time to the solution to be nesslerized, for the solution thus colored either becomes turbid or its optical density fluctuates to a great extent, resulting in unreliable colorimetry.

Dropwise addition seems better, except in the cases of nesslerization of acid solutions, such as in the case of Kjeldahl's digestion where a beautiful clear orange-yellow coloration was developed by simple at-a-time addition of Nessler's solution.* When the nitrogen concentration of the solution to be nesslerized is higher than 0.03 mg/12 ml, it should be diluted with distilled water to 0.01~0.03 mg/12 ml before nesslerization, since 1 ml of Nessler's solution is not sufficient to develop the complete coloration of a solution whose nitrogen content surpasses 0.05 mg N/12 ml.

Folin and Wu (Hawk et al., 1947)[1] have pointed out that 10 parts of Nessler's solution should be added to 100 parts of the solutions to be nesslerized, and that the Nessler's solution should have the alkalinity to the degree that a volume 11.0 to 11.5 ml of this solution is to be required to neutralize 20 ml of 1 N HCl, if titrated with phenolphthalein as indicator. According to our experiences, however, a perfect coloration was developed at alkalinity within the limits of 100±20% of

* The white turbidity which appears when Nessler's solution is poured drop by drop into the acid solution is not a matter of trouble, because it turns transparent by further addition, though the coloration of this solution is not so beautiful as in the case of at-a-time addition.

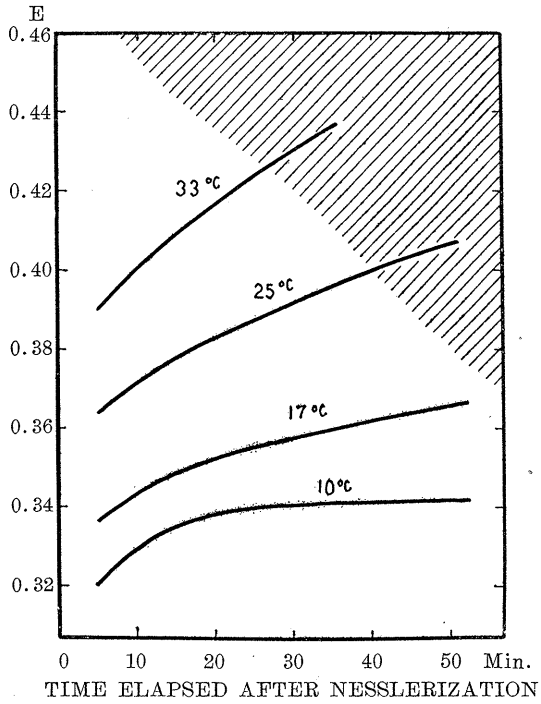

Fig. 3 The influence of the room temperature and the time elapsed after the nesslerization upon the intensity of the color of the solution. The shaded area represents the zone where colored solution is apt to become turbid (photometry at 460mμ; E: optical density

that indicated by these authors.

As shown in Fig. 3, the color of nesslerized solution becomes intense as the temperature rises, at which the solutions are allowed to stand for the definite period. At low temperature, however, the changes in color intensity with the lapse of time is not significient, as for example, at 10°C (see Fig. 3). In such instances, a relatively long period of time must elapse before the color develops to its full extent. Without waiting for a sufficient period of time, therefore, statisfactory results can not be obtained, as the following example indicats:

Observations on 0.025 mg/13 ml solution, nesslerized at 13°C, wave-length: 460 mμ.

Optical density at 10–15 minutes ——— 0.341, 0.348, 0.347
30 minutes ——— 0.349, 0.349, 0.348
40 minutes ——— 0.351, 0.350, 0.350

On the basis of these observations, it seemed better to determine the optical density at the times which vary according to room temperature, as indicated below:

at 10°C —— 30–50 minutes after addition of Nessler's solution.

at 15°C —— 15–30 minutes
at 20°C —— 10–20 minutes
at 25°C —— 7–15 minutes
at 30°C —— 5–10 minutes

The turbidity which occasionally take places in the nesslerized solutions, especially in those of the urease-treated deproteinized blood serum, can be avoided either by adding a smaller amount of Nessler's solution added to 12 ml of the fluid to be nesslerized or by adding one or two drops of 20 g/dl aqueous trichloracetic acid solution immediately after development of the color. For similar purpose, Looney[3] (Shibata 1951) recommended gum gutti solution but the present study did not verify its beneficial effect.

Summary

The Nessler coloration is so delicately influenced by various factors, namely by the procedures in adding Nessler's solution, the room temperature, the time elapsed after nesslerization, and so forth. Therefore, the use of the calibration curve would be misleading rather than helpful for the colorimetry of nitrogen-substance-containing fluid. In this colorimetry, it is not difficult to limit the error within a range of 1%, so long as the operator takes special care of the factors influencing the color-development. Thus, the colorimetry of nesslerized solution may be considered to be one of the most excellent means for the clinico-chemical determination of nitrogen.

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