Polarographic determination of Amino acids

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The polarographic determination of amino acids as copper complex has already been introduced by Jones,¹⁾ Martin²⁾ and others: This method is based on the fact that soluble copper-amino acid complex is formed by the addition of the copper phosphate suspension into amino acid solution. However, considerable errors are encountered in this method. The reason for this may be attributed to a relatively high solubility of copper phosphate and for lack of reproducibility, because they utilize such reaction as between liquid and solid. Such reaction process as mentioned above, therefore, should be avoided in order to reduce the errors. By thorough investigation the authors found a superior method which is employed a liquid-liquid reactoin. This method is used the solution of copper instead of the copper phosphate suspension, and then the pH of the solution is adjusted to 9.0, the precipitate is filtered off and the concentration of copper ion in the filtrate is determined polarographically.

Recommended procedure

Electrolysis solution is prepared by mixing the following solutions in turn:

Sample solution containing not more than

1×10^{-3} M amino acid.....below 30 ml.

0.05M Cu^{++} (*1).....1 ml.

Adjust the pH of the solution to 9.0 with NaOH using phenolphthalein as indicator.

(*2)

pH: 9.0 buffer solution (*3)10 ml.

Saturated potassium chloride solution.....5 ml.

0.5% soluble starch aqueous solution1 ml.

Make the above solution up to 50 ml with water and allow to stand for 60 minutes at room temperature. Filter the solution, transfer 20 ml of the filtrate into a 25 ml

*1. Any one of the following compounds can be used for the determination in this experiment :
 CuSO_4 , $\text{Cu}(\text{NO}_3)_2$ and CuCl_2 .

*2. Roughly neutralize the solution by 10% NaOH and then accurately neutralize by using N/10 NaOH till the solution turns to reddish.

*3. Method for preparing "pH 9.0 buffer solution"

N-NaOH..... 24.7 ml.

0.5M H_3BO_3 137.5 ml.

1M KCl 80 ml.

The pH of solution should be determined by using glass electrode.

volumetric flask, add 2 ml of 4N(NH_4OH , NH_4Cl) solution and 1 ml of saturated sodium sulfite solution and make the solution up to 25 ml with water. Transfer the solution into polarographic cell, and determine the wave of copper amine with the applied voltage between 0 and -0.8 volt vs. Hg.

In the case when used the potassium chloride as supporting electrolyte, the filtrate is transferred into polarographic cell, and determine the wave of copper-amino acid complex with the applied voltage between 0 and -0.8 volt vs. S.C.E.. Potassium chloride and NH_4OH - NH_4Cl are both suitable for the determination of amino acid, and these two electrolytes should be employed depending upon the purpose since they have characteristic properties respectively. In the case when potassium chloride as supporting electrolyte is used, the polarographic wave is due to the reduction wave of copper-amino acid complex, and when NH_4OH - NH_4Cl as supporting electrolyte is used, the polarographic wave is the reduction of $\text{Cu}(\text{NH}_3)_4^{++}$. For this reason, the use of potassium chloride is superior to NH_4OH - NH_4Cl in the study of copper-amino acid complexes individually, but the calibration curves individually to each amino acids should be employed. On the contrary, the use of NH_4OH - NH_4Cl applicable for the determination of all kinds of amino acids, because the polarographic wave of $\text{Cu}(\text{NH}_3)_4^{++}$ is obtained in this method, and the reaction ratio between amino acids and copper are already known in most of amino acids. For the reason, when there exist many kinds of amino acids, this method is much advantageous. The solubility of Cu^{++} is not recognized at all. This fact was assured in the experiment by using the recorder sensitivity of $0.002 \mu\text{A}/\text{mm}$. The blank current of Cu^{++} is recognized slightly unless potassium chloride is used. Here, the use of potassium chloride is effective to complete the precipitation of Cu^{++} to $\text{Cu}(\text{OH})_2$, and is useful as supporting electrolyte. The amino acids which can be determined by the present method are tryptophane, leucine, methionine and phenylalanine: however, the copper complex of phenylalanine can be dissolved N, N-dimethylformamide. Practically, 5 ml of N, N-dimethylformamide and 1 ml of 0.05M Cu^{++} are added to the sample solution to be determined and then pH is adjusted by NaOH solution to pH 9.0 using of phenolphthalein as indicator. The determination is performed by the same procedure as stated above. It is, however, impossible to determine tryptophane, leucine and methionine by the present method because of the slight solubility of the copper complex.

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- 1) T.S.G. Jones, Biochem. J., 42, No. 3 ix (1948).
- 2) A.J. Martin and R. Mittelman Biochem., 43, 353 (1948).