Evaluation of the Sakaguchi Reaction for Quantitative Determination of Arginine.* (22318)

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Sakaguchi(1) reported a color reaction specific for arginine which several investigators have since attempted to adapt for quantitative use. Weber(2) suggested that under certain conditions the reaction could be modified to give a quantitative colorimetric method. The procedure of Weber was further modified by Jorpes and Thoren(3) and Fisher and Wilhelmi(4), who attempted to reduce the rapid fading of color noted by Weber. MacPherson (5,6) attempted to eliminate color fading by adding urea prior to the development of color and by using a high concentration of chromogenic reagent (α-naphthol) and developing reagent (potassium hypobromite). In our hands, this procedure gave a highly colored blank with no improvement in color stability. Sakaguchi(7) reported that if 8-hydroxyquinoline was used in place of α-naphthol, a more stable color was produced. However, we have been able to detect little difference in the stability of colors formed with either chromogenic agent.

We have evaluated the published methods and modifications of these methods and have derived a procedure for estimating arginine which is highly satisfactory from the points of view of sensitivity and duplicability. Levels of arginine as low as 2 γ can be determined quite accurately.

Experimental. Reagents. Arginine hydrochloride—10 γ arginine/ml; prepared as needed from stock solution containing 605 γ arginine hydrochloride/ml, kept at 5°. This solution is stable for about 1 month. *Sodium hydroxide*—10% solution in water. *8-Hydroxyquinoline*—0.02%; prepared by diluting a 0.2% solution in ethanol with distilled water. *Sodium hypobromite*—1%; prepared by diluting 1 g liquid bromine to 100 ml with 5% sodium hydroxide. This is stable for 1 month in the cold and dark. *Urea*—40% solution in water. *Procedure*—All reagents and solutions should be cooled in ice bath before beginning the determination. To a tube containing 5 ml of the test solution (0—30 γ arginine) are added 1 ml of 0.02% 8-hydroxyquinoline and 1 ml of 10% sodium hydroxide. The solutions are mixed thoroughly and replaced in the ice bath for 2 minutes. Then 0.2 ml of 1% sodium hypobromite is added rapidly to develop the color. After mixing and within 15 seconds, 1 ml of 40% urea is added to destroy excess hypobromite and prevent rapid fading of color. One minute after addition of the hypobromite, 5 ml of cold distilled water are added, the solutions mixed, and the absorption is read at 490-510 mμ. It is important that the sample be read within 5 minutes after development of color. Also the readings should be made at the same time interval after color development in each set of analyses.

Standard Curve. A set of 4 tubes is prepared containing 0, 10, 20, and 30 γ of arginine. The blank is developed first and is used to bring the colorimeter or spectrophotometer to zero. The other tubes are then developed in the order given above and results read and plotted.

Results. A linear relationship exists between optical density of the color developed and concentration of arginine in the range of 0-50 γ of arginine per tube; however, it is recommended that 30 γ be the upper limit when using ordinary colorimeters.

Fig. 1 shows a typical standard curve prepared as described above. This curve is reproducible from one experiment to the next within $\pm$0.5%. In Fig. 2 is presented the

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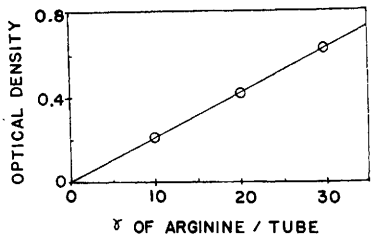

FIG. 1. Typical standard curve for arginine.

absorption spectrum of the color produced in the reaction. It can be seen that the maximum absorption occurs near 506 mμ. Reasonably good quantitative results can be obtained in the range 490-510 mμ, however.

Sakaguchi(1,7) and Poller(8) have studied the nature of compounds which interfere with this color reaction. Glycine appears to give the most interference among the amino acids. Glycine was found to cause a slight color intensification when present in small amounts (up to 100 γ/developing tube) in a solution with arginine. When the concentration of glycine in the developing tube reaches 150 γ or above, however, color fading is noted. In either case if a standard curve is established with a specific amount of glycine, arginine can still be determined.

Our work indicates that arginine may be determined in the presence of mixtures of amino acids. Those we have investigated were amino acid mixtures similar to casein and egg albumin hydrolysates(9). Thus, recovery experiments using amino acid mixtures containing known amounts of arginine indicate that accurate results are easily obtained.

In this laboratory a number of buffers commonly used in biochemical studies (tris(hydroxymethyl)aminomethane, glycine, acetate, phosphate, pyrophosphate, and bicarbonate) were also investigated for possible interference. Only glycine and tris(hydroxymethyl)-aminomethane ("tris") were found to interfere to a measurable extent. It should also be noted that the level of sodium hypobromite is quite critical since an excess tends to cause decomposition of the color produced. Of the

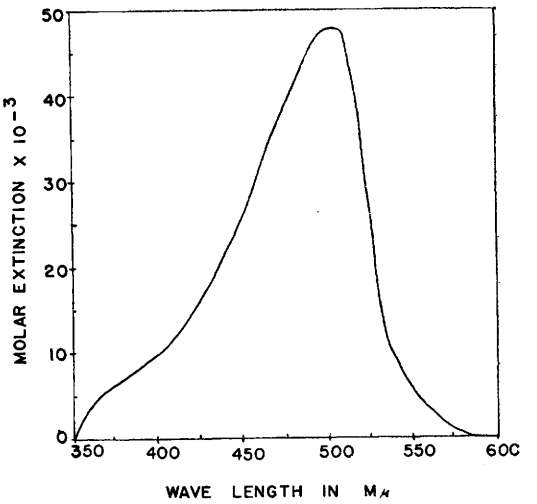

FIG. 2. Absorption spectrum of the colored product formed with arginine.

series of hypobromite concentration studied, 0.2 ml of 1% sodium hypobromite was found to give full color development and negligible color fading if read within 5 minutes.

Summary. The Sakaguchi reaction has been studied to obtain a reliable quantitative method for the colorimetric estimation of arginine. The best conditions for this method have been investigated and reported. The method has been found to be entirely satisfactory for determining arginine over the range of 0-30 γ. Duplicability of the method is ± 0.5%, (Standard deviation).

1. Sakaguchi, S., *J. Biochem.*, Japan, 1925, v5, 25.
2. Weber, C. J., *J. Biol. Chem.*, 1930, v86, 217.
3. Jorpes, E., and Thoren, S., *Biochem. J.*, 1932, v26, 1504.
4. Fisher, R. B., and Wilhelmi, A. E., *ibid.*, 1938, v32, 606.
5. MacPherson, H. T., *ibid.*, 1942, v36, 59.
6. ———, *ibid.*, 1946, v40, 471.
7. Sakaguchi, S., *J. Biochem.*, Japan, 1950, v37, 231.
8. Poller, K., *Ber. deut. chem. Ges.*, 1926, v59, 1927.
9. Block, R. J., and Bolling, D., *The Amino Acid Composition of Proteins and Foods, Analytical Methods and Results* (2nd ed.) Thomas, Springfield, Ill. (1951).

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