In the case of highly and colored mixtures of amino acids from protein hydrolysis, decolorization was effected by the addition of aluminium sulfate to the hydrolyzate made alkaline with barium hydroxide and subsequent filtration. The barium was removed as the sulfate.

The method has been successfully applied to mixtures of amino acids from casein, vignin, gelatin and vegetable albumin and to mixtures rich in the di-amino acids, as well as to individual amino acids, and shows that in a single esterification as high as 90% of the amino acid acidity may be esterified in the case of amino acid mixtures. Alanine was 96% esterified, lysine 82% and glutamic acid 85%. From the data given a tentative correction of 2 or 3% can be added to the per cent. of amino acid esterified.

From the results obtained in this investigation, it appears that there is no unesterifiable residue of amino acids. The reaction as carried out by the above method is apparently one in which an equilibrium is reached when an appreciable amount of amino acid is still unesterified.

This method will be of value in determining the best conditions under which the esterification of α-amino acids should be run, and in comparing the efficiency of the different methods of esterification.[1]

URBANA, ILL.

THE COMBINATION OF FRACTIONATION WITH SPECTROPHOTOMETRY IN PROXIMATE ORGANIC ANALYSIS.[2]

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In making the chemical examination of various commercial products it is often desirable to estimate with some precision certain colorless substances which are found in quantities of less than a few mg. in the sample available. The spectrophotometric method[3] for estimating dyes has the special advantages of being applicable with very small amounts of the coloring matters and requiring no quantitative preliminary purification of them. It has not been largely used for the determination of colorless organic compounds because but few of these substances can be converted easily and completely into colored derivatives by means of reagents that are themselves colorless or widely different in shade. A procedure of somewhat general applicability, however, consists in combining or condensing the substance to be estimated with a suitable compound

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[1] An investigation of the cause of the variation of the amount of esters formed during long continued esterification is being planned. The method will also be so modified as to allow the addition (or subtraction) of a correction factor which will include the under-titration as well as the saponification of the amino acid esters.

[2] Published by permission of the Secretary of Agriculture.

[3] For the development of quantitative spectrophotometry, see G. and H. Kruess, "Kolorimetrie und quantitativ Spektralanalyse," Hamburg, 1909.

to form a strongly colored derivative, this being separated then by fractionation with immiscible solvents[1] from other colored substances present in the reaction mixture.

To establish the identity of the colored derivatives several points on their absorption curves may be determined. Most of these substances change in hue or color intensity with acids and alkalies and possess such stability that their dissociation constants as weak acids or bases may be determined by the usual spectrophotometric method.

A few experiments made with test solutions and well known reagents[2] are described below.[3]

Sulfanilic Acid.—Ten cc. of the solution to be tested was treated with 1.0 cc. of a 2% solution of picryl chloride in glacial acetic acid, heated to boiling, and allowed to stand 5 minutes. Five cc. of a 1.4% solution of aniline hydrochloride was then added, the mixture again heated to boiling, allowed to stand 5 minutes, cooled, and acidified with 1.6 cc. of 5 *N* hydrochloric acid to bring its acidity to about 0.5 *N*. It was then shaken out successively in 3 funnels with 15 cc. portions of benzene, and the benzene portions washed by shaking with a 15-cc. portion of 0.5 *N* hydrochloric acid, this being passed through the funnels in the same order as the original solution, to which it was finally added. The acid solution was made to a definite volume with 0.5 *N* acid and the light absorption at 435 $\mu\mu$ determined.

Five trial solutions treated in this way contained respectively: (*a*) 0.0050 g., (*b*) 0.00150 g., and (*c*) 0.00050 g. dry sulfanilic acid; (*d*) 0.020 g. pure G. salt (disodium beta-naphthol disulfonate) and 0.020 g. pure sodium-1-naphthol-2-sulfonate; and (*e*) pure water only. The trinitro-diphenylamine sulfonic acid solution obtained from (*a*) was diluted to exactly 100 cc. with 0.5 *N* hydrochloric acid and 75 cc. titrated with 0.1 *N* titanium trichloride solution by Knecht and Hibberts' method, adding first an excess of sodium tartrate and a measured amount of Light Green S. F. Yellowish solution to serve as indicator. 2.47 cc. was required to reduce the nitro compound, corresponding to 0.00474 g. of sulfanilic acid in the original solution, or a recovery of 95%. The transmissive index or extinction coefficient[4] at 435 $\mu\mu$ was found to be 1.07. The solu-

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[1] *J. Ind. Eng. Chem.*, **5**, 26 (1913).

[2] Picryl chloride, Turpin, *J. Chem. Soc.*, **59**, 714 (1881); Dinitrophenylhydrazine, Purgotti, *Gaz. chim. ital.*, **24**, 555 (1899); Borsche, *Ann.*, **357**, 180 (1907); diazobenzene sulfonic acid, Ehrlich, *Z. klin. Med.*, **5**, 285 (1885).

[3] The optical measurements were made with a König-Martens spectrophotometer provided with a heavy brass carrier by means of which the absorption cell could readily be adjusted to allow the full and symmetrical illumination of the slits. One of the compartments of the double cell was filled with the colored solution, the other with the pure solvent (or blank) allowing the transmissivity of the dissolved colored substance to be determined directly. Monochromatic light from a quartz mercury lamp (Cooper-Hewitt, Type Y) was used when the solution under examination showed relatively high absorption for one of the strong mercury lines.

In the calculation it is assumed that within the range of the concentrations employed the specific absorptive power of the colored substance may be considered to be independent of the volume of the solvent. In other words the ratio between the concentrations of 2 solutions is taken to be the same as that between their extinction coefficients or transmissive indices.

[4] Bunsen and Roscoe (*Pogg. Ann. Physik.*, **101**, 248 (1857)) defined the extinction coefficient of an absorbing medium as the reciprocal of the thickness of the layer required to reduce the intensity of the incident light to $^1/_{10}$ its original value.

tions from (*d*) and (*e*) were practically colorless and were discarded. Those from (*b*) and (*c*) were diluted to 100 cc. and 50 cc., respectively, and gave indices of 0.35 and 0.25. Assuming proportionality these correspond, respectively, to 0.00155 g. and 0.00055 g. of sulfanilic acid.

Acetone.—A reagent was prepared by dissolving 0.10 g. of 2,4-dinitro-phenylhydrazine in 1.0 cc. of warm pyridine and adding 10 cc. of conc. hydrochloric acid. Ten cc. of the aqueous solution to be tested was treated with 5 cc. of the reagent and the mixture allowed to stand 15 minutes in a stoppered bottle. It was then transferred to a separatory funnel with about 7 cc. of 2 *N* hydrochloric acid and shaken out with 3 portions (19 to 20 cc. each) of carbon tetrachloride, these being shaken out successively in a second funnel with a 20-cc. portion of 2 *N* hydrochloric acid. The carbon tetrachloride was then diluted to exactly 60 cc. in a graduated cylinder, and its transmissive index at 435$\mu\mu$ determined and compared with that obtained with a standard carbon tetrachloride solution of pure acetone-dinitrophenyl-hydrazone. Because of slight oxidation or of some impurity in the reagent a very faint coloration was obtained with blanks, being due to a substance similar in its solubilities to the hydrazone.

Ten cc. of a solution containing 0.000508 g. of redistilled commercial C. P. acetone (about 98%, d_4^{25} 0.7862) gave by this procedure a carbon tetrachloride solution having a transmissive index of 0.651. A blank carried through by the same procedure gave 0.053 and a solution of 0.0100 g. of pure acetone-dinitrophenyl-hydrazone in 100 cc. of carbon tetrachloride gave 0.852. 0.000472 g. of acetone was, therefore, recovered from the acid solution.

β-Naphthol.—A diazo solution was prepared by mixing at ordinary temperature 12 cc. of 0.2 *N* sodium sulfanilate, 10 cc. of 0.1 *N* sodium nitrite solution, 20 cc. of *N* hydrochloric acid and sufficient water to make 50 cc. Ten cc. of the aqueous solution to be tested was treated in succession with 5 cc. of the diazo solution, 3 cc. of a 0.1 *N* solution of purified G-salt (disodium beta-naphthol disulfonate), 3 cc. of 5 *N* sodium carbonate solution, and 3 cc. of glacial acetic acid. It was then shaken out in 3 funnels with 25-cc. portions of amyl alcohol and these were washed in the same order with 3 20-cc. portions of *N* acetic acid solution. The amyl alcohol portions were each diluted with 50 cc. of gasoline and the dye completely removed by shaking out with several small portions of water. The aqueous solution thus obtained was treated with 6 cc. of glacial acetic acid and water to bring its volume to 100 cc. A second solution (*b*) was treated by a similar procedure, but omitting one funnel and one washing portion of acid and making up the sulfobenzene-azo-β-naphthol solution obtained to 25 cc. The transmissive indices were determined at 481$\mu\mu$–485$\mu\mu$ and compared with that obtained with a standard solution of pure dry Orange II in *N* acetic acid solution. Solution (*a*) contained 0.00050 g. of β-naphthol; recovered 0.00046 g. Solution (*b*) contained 0.000050 g.; recovered 0.000043 g.

Summary.

Examples are given of a procedure for the estimation of small amounts of organic compounds which consists in converting them into colored derivatives, these being separated from the excess of the reagents and from any other colored substances present by fractionation with immiscible solvents and finally estimated spectrophotometrically.

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