[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF ROCHESTER, AND THE EASTMAN KODAK COMPANY]

The Quantitative Titration of Amino Acids in Glacial Acetic Acid Solution[1]

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Introduction

The methods[2] generally used for the quantitative determination of amino carboxylic acids are either difficult or relatively inaccurate. In view of the acid–base relationships which have been found to obtain in acetic acid[3] it seemed likely that amino acids dissolved in glacial acetic acid would act as bases strong enough to be titrated with such acids as perchloric, sulfuric or hydrobromic.[4] To test this and also if possible to find suitable indicators to permit the visual observation of the end-point, the present investigation was undertaken.

Experimental

Materials.—All solutions were prepared in glacial acetic acid. The acetic acid used contained no non-volatile ingredient and less than 0.1% water. The amino acids were obtained from the Eastman Kodak Company and were used without further purification. The purity of all materials used was checked by analysis. The standard 0.1 *N* solutions of perchloric acid were prepared from the 72% aqueous solution, the exact amount of acetic anhydride required to react with the water being added. The concentration of the solution was checked by means of pure sodium carbonate. A tenth normal solution of guanidine acetate was used as a standard base.

Because of the high thermal expansion of acetic acid, weight rather than volume burets were employed.

Potentiometric Titration.—For the potentiometric titrations, a titration vessel similar to that described by Conant and Werner[3] was used. To carry out a titration, 0.1000 to 0.2000 g. of the amino acid was dissolved in approximately 30 cc. of glacial acetic acid. Chloranil and tetrahydrochloranil were added and the titration carried out in the conventional manner. It was found that a vacuum tube potentiometer[5] was much more convenient to set up and use than the electrometer recommended by Conant and Hall. The end-point was determined by plotting $\Delta E/\Delta V$ against V.

Colorimetric Titrations.—Conant and Werner's[3] spectrophotometric study of crystal violet served as a starting point in the selection of indicators. Crystal violet, α-naphtholbenzein and benzoyl auramine were found to be satisfactory indicators. Table I illustrates the agreement

TABLE I

Indicator	Acid color	Base color	0.1 *N* guanidine acetate, cc.
Crystal violet	Green	Blue	25.27
α-Naphthol-benzein	Green	Yellow	25.25
Benzoyl auramine	Yellowish	Blue-green	25.27

TABLE II

	Purity calcd. from N content	Percentage of theoretical found by: Colorimetric method Direct	Colorimetric method Back-titration	Potentiometric method Direct	Potentiometric method Back-titration
Glycine	99.7	100.23	100.64	99.40	
	100.0	100.24	100.71	99.52	
		100.27	100.45	99.90	
		100.31	100.45	100.04	
		100.31		100.12	
dl-α-Amino-*n*-valeric acid	99.7	97.94	97.85	98.06	
		98.15	98.14	98.15	
dl-β-Phenyl-alanine	98.6	98.98	99.14	99.47	
	98.7	99.14	99.22	98.83	
		99.14		99.14	
				98.83	
l-Tyrosine	100.0		99.96		100.78
			100.02		100.87
			100.30		99.97
l-Cystine	98.7		98.31		99.40
			98.66		99.40
			98.54		99.60
					99.64
d-Glutamic acid			99.90		100.10
			100.25		100.10
			100.37		
			100.02		
l-Aspartic acid	100.0		100.72		100.53
			100.99		100.86
d-Arginine picrate		99.91			
		99.25			
d-Lysine picrate	99.7	100.02	99.84	100.54	
		100.14	100.14	100.65	
		100.45	100.23		
l-Proline	99.2	99.42	99.35	99.34	
		99.61	99.56	99.56	
		99.63	99.52		
l-Tryptophan	100.5	100.40	100.31	99.44	100.45
		100.50	100.54	100.08	

(1) Presented before the Biological Division of the American Chemical Society at Cleveland, September 10–14, 1934; based on a thesis presented by Mr. Branchen to the Graduate School of the University of Rochester in partial fulfilment of the requirements for the Degree of Master of Science.

(2) Sörensen, *Biochem. Z.*, **7**, 45 (1908); Willstätter and Waldschmidt-Leitz, *Ber.*, **54**, 2988 (1921); Linderström-Lang, *Z. physiol. Chem.*, **173**, 32 (1928); Van Slyke, *Ber.*, **43**, 3170 (1910).

(3) Hall and Conant, THIS JOURNAL, **49**, 3047 (1927); Conant and Hall, *ibid.*, **49**, 3062 (1927); Hall and Werner, *ibid.*, **50**, 2367 (1928); Conant and Werner, *ibid.*, **52**, 4436 (1930); Hall, *ibid.*, **52**, 5115 (1930).

(4) See Kolthoff and Willman, *ibid.*, **56**, 1014 (1934). This work was published near the completion of our own.

(5) Ellis and Kuhl, *Rev. Sci. Insts.*, **4**, 131 (1933); Hill, *Science*, **73**, 529 (1931).

obtained in the titration of 25.0 cc. of approximately 0.1 *N* perchloric acid solution.

Since the benzoyl auramine reacts slowly with acetic acid, it should be freshly prepared each week.

Colorimetrically, the amino acids were titrated by dissolving 0.1000 to 0.2000 g. of the sample in approximately 30 cc. of glacial acetic acid, adding a few drops of indicator solution and carrying out the titration in the usual manner. Some care is required in the use of crystal violet because of the number of colors observed. When added to a basic solution such as guanidine acetate, crystal violet acquires a purple color which changes through blue, on the addition of perchloric acid, to a greenish-blue at the end-point. All of the colorimetric data were obtained by means of crystal violet. The results are given in Table II.

Discussion of Results

The potentiometric titration curves of the amino acids are given in Figs. 1 and 2. Curves for acids insoluble in acetic acid (Fig. 2) were obtained by dissolving the amino acid with an excess of 0.1 *N* perchloric acid and back-titrating

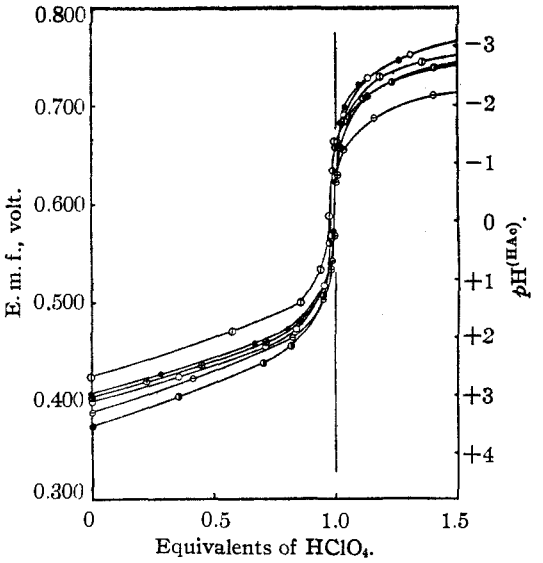

Fig. 1.—●, Glycine; ⊙, α-amino-*n*-valeric acid; ⊖, *dl*-β-phenylalanine; ⓓ, *d*-lysine picrate; ◑, *l*-proline; ⊕, *l*-tryptophan.

with guanidine acetate. As these titration curves are characteristic of strong acids and bases, sharp end-points should be obtainable with suitable indicators. With the three indicators tested above, results were secured which surpassed in accuracy and precision those obtained potentiometrically. This is a natural consequence of the factors involved in the accurate determination of potentials in non-aqueous solutions of very low conductivity.

The data presented in Table II are expressed as percentages of the theoretical. It is of interest to note that whereas arginine and lysine are diacid bases in acetic acid solution, only one of the nitrogen atoms in tryptophan is capable of functioning as a base. Picric acid has no detectable effect on the accuracy of the titration.

Table III illustrates the precision obtainable in the titration of a sample of glycine when using weight burets and crystal violet indicator. Obviously, the same precision as ordinarily accepted in aqueous systems can be realized.

TABLE III

Percentages of glycine calculated from titration of 0.2000-g. sample with 0.1000 *M* perchloric acid using crystal violet indicator

100.23	100.31	100.31	100.24	100.27

Arith. av. = 100.27%

Deviation = ±0.03% or ±3 parts in 10,000

When working with anhydrous acetic acid, the importance and effect of the ionic strength[3] of the system on the titrations must be borne in mind.

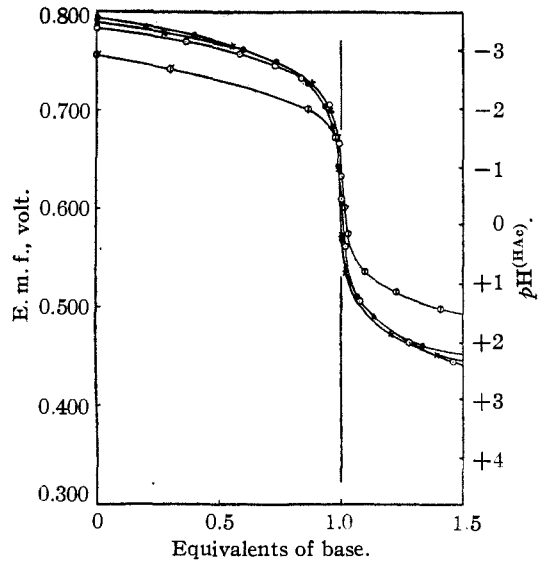

Fig. 2.—⊙, *l*-Aspartic acid; ●, *d*-glutamic acid; ⓓ, *l*-cystine; ×, *l*-tyrosine.

The acidity of the titrating acid and the *pK* value of the indicator are both greatly affected by relatively small changes in ionic strength, but as the ionic strength of the solution at the equivalent point is approximately the same as that under the conditions of standardization, no difficulty is experienced.

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Summary

1. A rapid and precise method for the quantitative determination of organic amino acids is presented.

2. For the titration of strong acids and bases in acetic acid systems, crystal violet, α-naphtholbenzein, and benzoyl auramine have been found to give results that are in accordance with those obtained potentiometrically with the chloranil electrode.

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[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF UPSALA]

The Salt Extractable Proteins of Wheat Flour. Ultracentrifugal Study

BY LAURA KREJCI[1] AND THE SVEDBERG

Gortner, Hoffman, and Sinclair[2] have investigated the peptization of wheat proteins by solutions of different salts. They have found that the amount of nitrogen extracted varies with the ions present in the extracting solution. The potassium halides in particular show a marked lyotropic series: normal potassium iodide extracts almost five times as much as normal potassium fluoride, and almost three times as much as normal potassium chloride. This paper is the report of an ultracentrifugal investigation of the protein matter extracted from wheat flour by solutions of these salts.

Potassium Fluoride, Potassium Chloride, Potassium Bromide and Potassium Iodide Extracts of Wheat Flour

The flour used for this investigation was made from Manitoba wheat specially ground in an experimental mill at the laboratory of Upsala Ångkvarn.

A comparison was made of the protein extracted by half normal solutions of potassium fluoride, potassium chloride, potassium bromide and potassium iodide. All the crude extracts contained much non-centrifugible light-absorbing material. To eliminate this each, with the exception of the fluoride, was precipitated one or more times by saturation with solid ammonium sulfate. The crude iodide extract could not be studied because of the high light absorption of the potassium iodide; the ammonium sulfate precipitates were therefore dissolved in 0.5 N potassium chloride. To provide a basis for comparison, half the precipitate from the bromide extract was dissolved in 0.5 N potassium bromide, half in 0.5 N potassium chloride.

(1) Fellow of the American Scandinavian Foundation.

(2) Gortner, Hoffman, and Sinclair, "Colloid Symposium Monograph," 1928, Vol. V, 179–198.

The results are shown in Table I. The sedimentation constants have been corrected for the density and viscosity of the salts present to a basis of sedimentation in pure water. Estimates are included of the amount of non-centrifugible material present after four hours of centrifuging. The protein is inhomogeneous in every case; the constants therefore represent average values which may be used to characterize the mixture of proteins present in each solution.

In almost every case precipitation with ammonium sulfate causes an increase in sedimentation constant and a decrease in non-centrifugible light absorbing material; the only exception is the fourth precipitation of the chloride extract. The change is more marked for the portion of the bromide extract dissolved in potassium chloride than for that dissolved in potassium bromide. It is also greater for the portion of the iodide extract which dissolved readily in potassium chloride than for the portion which dissolved only on longer contact with the solvent. This indicates that the ammonium sulfate precipitates of the heavier proteins dissolve readily in solutions of low salt content, while those of the lighter proteins require a higher salt content in the solvent, or a longer time of contact with the solvent, or both, to dissolve. The behavior of the four-times precipitated chloride extract can be explained by the fact that the three preceding precipitates had been dissolved in water, and adhering ammonium sulfate was the only source of salt in the solvent. The solutions were cloudy; this cloudiness doubtless consisted of protein matter which was insoluble in the dilute ammonium sulfate solution, but soluble in 0.33 N potassium chloride.

The sedimentation constant for the crude fluoride extract is 2.20; for the crude chloride extract, 2.38; and for the crude bromide extract