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POTENTIOMETRIC TITRATION OF PEPTIDES AND THE STARTING MATERIALS AND INTERMEDIATES OF THEIR SYNTHESIS.

I. ACIDIMETRIC DETERMINATION OF N-ACETYLAMINO ACIDS

A. Ya. Veveris and B. A. Stintse

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The potentiometric titration of N-acetylamino acids and their sodium salts in nitromethane—acetic anhydride (2:1) with a nitromethane solution of perchloric acid has been investigated. A procedure has been developed for the quantitative determination of N-acetylamino acids in the presence of acetic acid in aqueous solutions. A differentiation determination of N-acetylamino acids and their salts in the presence of sodium acetate has been carried out.

The development of technological methods of peptide synthesis requires an improvement in the methods of analytical control, including those that are intended for determining the quantitative composition of the starting materials, the auxiliary reagents, and the intermediates of the various technological processes. Our preceding investigations [1, 2] have shown that extremely reliable information on the quantitative content of substances can be obtained from the results of potentiometric acid-base titration. The use of modern technology for the performance of potentiometric titration permits the consumption of samples undergoing analysis to be restricted to a few milligrams with the retention of an adequately high accuracy and reliability of the results.

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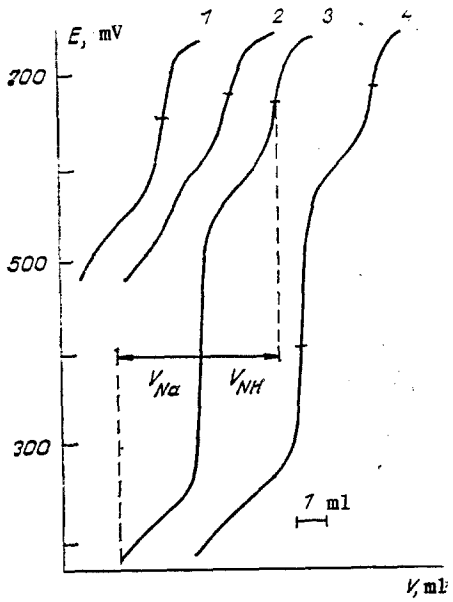

Fig. 1. Curves of potentiometric titration with a 0.1 M nitromethane solution of perchloric acid in acetic anhydride–nitromethane (1:2) of: 1) N-acetyl-D,L-valine; 2) an aqueous solution of N-acetyl-D,L-methionine; 3) the sodium salt of N-acetyl-D,L-serine; 4) a technological solution of a mixture of the sodium salt of N-acetyl-D,L-phenylalanine with sodium acetate and Acetic acid.

The aim of the prsent work was to develop a procedure for the quantitative determination of N-acetyl-D,L-amino acids (AAAs) in the presence of acetic acid, or of the sodium salts of AAAs in the presence of sodium acetate. The urgency of the problem is due to the substantial difficulties in monitoring a number of technological operations in the production of optically pure amino acids. In particular, the known method of alkalimetric titration [3] has proved to be unsuitable (because of the close acidic properties of the components) for the separate determination of AAAs and acetic acid in aqueous solutions. Difficulties have also arisen in the analysis of the sodium salts of the AAAs as technical products, which contain, in addition to sodium acetate, up to 25-30% of water.

The proposed procedure envisages the use of the acidimetric analysis of the proton-accepting capacity of the secondary amino group in nonaqueous solutions. Preliminary experiments showed that, by analogy with the preceding work [2], as titrant it is possible to use nitromethane solutions of perchloric acid, and the optimum titration medium in a mixture of nitromethane (NM) with acetic anhydride (AcA) in a volume ratio of 2:1. The potential jumps appearing on the titration curve (Fig. 1) in the 100-150 mV interval permit the conclusion that the secondary amino groups of AAAs possess a higher proton-accepting capacity in the medium selected than the same groups of N-tert-butoxycarbonyl derivatives of amino acids, when the corresponding potential jumps on the curves do not exceed 30-40 mV [2]. The results of quantitative determinations (Table 1) show the possibility of the use of acidimetric analysis for N-benzoyl derivatives of amino acids, as well. The appearance of slight distortions on the titration curves (Fig. 1, curve 2) where weighed samples of AAAs were heated with AcA (apparently because of the reaction between AcA and the NH group) have no appreciable influence on the correctness of the results of analysis (see Table 1).

In an analysis of the sodium salts of AAAs the titration curves each have two potential jumps (Fig. 1, curve 3). In the analysis of comparatively pure samples the corresponding consumption of titrant, V_{Na} and V_{NH} are practically equal to one another. This equality (divergences of up to 0.1 ml are permitted) may serve as one of the criteria for evaluating the purity of preparations. Determinations of artificial mixtures have shown that when sodium acetate is present as an impurity $V_{Na} > V_{NH}$, and the difference between these volumes is proportional to the amount of sodium acetate impurity. The opposite inequalities ($V_{NH} > V_{Na}$) were observed in the titration of two-component mixtures of AAAs with their sodium salts.

Samples of AAAs and their salts analyzed by the acidimetric method were used for the preparation of aqueous solutions (with given concentrations) and of artificial mixtures. The results of the determinations are given in Table 2. To obtain comparative results, the concentrations of the AAAs in water were determined in parallel by potentiometric titration with a 0.1 M aqueous solution of potassium hydroxide in water–dimethyl sulfoxide (1:2) as medium.

The preliminary results of the acidimetric titration showed the necessity for making changes in the original method. If the solutions to be analyzed were subjected to titration immediately after the addition of the organic solvents AcA and NM to a weighed sample of an

TABLE 1. Results of the Quantitative Determination of N-Acetyl-amino Acids, Their Sodium Salts, and Some Artificial Mixtures

Substance analyzed	Taken, mg	Found, mg	Relative error, %
N-Acetyl-L-methionine	68.8	68,1	—1,0
N-Acetyl-D,L-alanine	79,5	78,4	—1,4
N-Acetyl-D,L-valine	98,3	99,2	+0,9
N-Acetyl-D,L-serine	90,6	91,8	+1,3
N-Acetyl-D-aminophenylacetic acid	83,6	84,9	+1,6
N-Benzoyl-L-tyrosine	86,2	85,7	—0,6
N-Benzoyl-L-valine	87,7	86,8	—1,0
Sodium salt of N-acetyl-D,L-serine	110,8	109,3 (from V_{Na})	—1,4
		111,8 (from V_{NH})	
Sodium N-acetyl-D,L-aminophenyl-acetate	116,0	115,2 (from V_{Na})	—0,7
		117,3 (from V_{NH})	+1,1
Sodium acetate + sodium salt of N-acetyl-D,L-serine	38,5	38,0	—1,3
	88,7	88,1	—0,7
Sodium acetate + sodium N-acetyl-D,L-aminophenylacetate	24,7	25,2	+2,0
	96,5	95,0	—1,6
Sodium salt of N-acetyl-D,L-serine+ N-acetyl-D,L-serine	64,4	63,7	—1,1
	58,3	59,4	+1,9
Sodium salt of N-acetyl-D,L-valine + N-acetyl-D,L-valine	51,9	51,6	—0,6
	70,5	69,6	—1,6

aqueous solution, titration curves with ill-defined potential jumps were obtained which make it difficult to determine the corresponding volumes V_{NH}. Apparently, even small amounts of water added with the weighed samples have an averse effect on the results of the titration of the secondary amino group, and the binding of water by AcA takes place slowly at room temperature in spite of the fairly considerable (10- to 25-fold by volume) excess of AcA.

The results given in Table 2 were obtained after the preliminary keeping of the weighed samples of solutions to be analyzed with AcA at 60°C for 1.5 h and are characterized by good reproducibility and reliability. Since an increase in the amount of water in solution being analyzed leads to a lengthening of the time of analysis, in determinations with lower concentrations of AAAs the weight of the samples was not increased but titrants with lower concentrations (0.025-0.05 M) were used.

The procedure developed also has practical value for the separate analysis of mixtures consisting of 3-4 components. In particular, technological solutions of mixtures of D,L-valine + N-acetyl-D,L-valine + acetic acid; D,L-aminophenylacetic acid + sodium N-acetyl-D,L-aminophenylacetate + sodium acetate, and D,L-methionine + N-acetyl-D,L-methionine + sodium salt of N-acetyl-D,L-methionine + sodium acetate have been analyzed by a combination of acidimetric and alkalimetric titrations.

EXPERIMENTAL

Samples of N-acetylamino acids synthesized under laboratory conditions (N-acetylglycine, N-acetyl-D-aminophenylacetic acid, N-acetyl-L-methionine, and the sodium salts of N-acetyl-D,L-serine and N-acetyl-D,L-valine) obtained on industrial apparatus under production conditions were first examined by the methods of elementary and chromatographic analysis. The N-benzoyl-L-tyrosine and N-benzoyl-L-valine preparations (Reanal) were used without additional quality checking.

In the investigations and analyses, the equipment and the titration technique described in preceding papers [2, 4] were used, with burettes having working volumes of 10 and 25 ml.

Analytical Procedures. In the analysis of AAAs, their sodium salts, and artificial mixtures, weighed samples (between 0.06 and 0.12 g) were dissolved in 0.5-1 ml of glacial acetic acid (to accelerate the dissolution of the salts the acetic acid was heated to the boil), and then 10 ml of nitromethane and 5 ml of acetic anhydride were added. Potentiometric titration was conducted with a 0.1 M solution of perchloric acid in nitromethane. The corresponding volumes of titrant consumed were determined from the titration curves, and the ratios of V_{Na} and V_{NH} were estimated. A control experiment was carried out in parallel (a mixture of 0.5-1 ml of acetic acid with 5 ml of AcA and 10 ml of NM was titrated) and the consumption of titrant in this experiment was deducted from V_{NH}.

TABLE 2. Results of the Determination of the Concentrations of N-acetylamino Acids and Their Sodium Salts in Aqueous Solutions

Substance analyzed	Titration with perchloric acid		Comparative analysis by titration with alkali, %
	aqueous solution taken, mg	found, % (at n = 5, α = 0.95)	
N-Acetyl-D,L-serine	283,3	27,8±0,6	27,3
N-Acetyl-D,L-aminophenylacetic acid	311,6	14,5±0,5	14,7
N-Acetyl-D,L-valine	350,8	9,7±0,3	9,5
N-Acetyl-D,L-methionine	290,5	31,9±0,8	31,8
Sodium salt of N-acetyl-D,L-serine	251,7	18,6±0,5	—
Sodium salt of N-acetyl-D,L-phenylalanine	246,8	16,3±0,4	—
Sodium salt of N-acetyl-D,L-methionine + N-acetyl-D,L-methionine	339,2	18,6±0,6 9,7±0,5	— 9,2
Sodium N-acetyl-D,L-aminophenylacetate + N-acetyl-D,L-aminophenylacetic acid	304,3	16,8±0,6 15,6±0,3	— 15,3
Sodium acetate + sodium salt of N-acetyl-D,L-serine	401,4	5,7±0,3 20,2±0,8	— —
Sodium acetate + sodium salt of N-acetyl-D,L-valine	371,8	8,1±0,3 25,4±0,7	— —

In the analysis of aqueous solutions of AAAs, sodium salts, and their mixture, to each of the weighed samples (0.2-0.4 g) was added 5 ml of acetic acid, and the stoppered vessels, were kept in a thermostat at 60°C for 1.5 h. Then 10 ml of nitromethane was added and potentiometric titration was carried out with 0.08 M nitromethane solution of perchloric acid. If the consumption of titrant, V_{NH} or V_{Na}, were less than 1 ml, titration was repeated with a 0.025-0.030 M nitromethane solution of perchloric acid.

In the comparative analysis of aqueous solutions, samples weighing between 0.3 and 0.5 g were taken, and to each were added 5 ml of water and 10 ml of dimethyl sulfoxide (kh.ch. "[chemically pure"]). Titration was performed with a 0.1 M aqueous solution of potassium hydroxide until a well-defined potential jump appeared on the titration curve.

SUMMARY

Conditions for the potentiometric titration of N-acetyl and N-benzoyl derivatives of amino acids by a nitromethane solution of perchloric acid in the mixed solvent acetic acid—nitromethane (1:2) have been investigted. The possibility has been shown of the separate analysis of two-component mixtures of the sodium salts of N-acetylamino acids with N-acetylamino acids or sodium acetate. A procedure has been developed for determining the concentrations of N-acetylamino acids and their sodium salts in aqueous solutions.

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