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Analytical Studies on the Oxidation of S-containing Amino Acids with Pentavalent Vanadium Reagent

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A simple, quick and convenient method has been developed for the micro estimation of S-containing amino acids. The sample 1–5 mg is allowed to react with 2 mL of 0.3 N ammonium metavanadate(V) reagent and 10 mL of 10 N H_2SO_4 was added before the reaction helps in detecting the end point. The unconsumed reagent can be accurately titrated with 0.025 N ferrous ammonium sulphate solution using N-phenyl anthranilic acid as indicator. Standard deviation as well as coefficient of variation was calculated for reproducible and accurate result. The accuracy of the method is within ± 1%.

INTRODUCTION

Sulphur containing amino acids are of great importance in the biochemical process of animals. A number of procedures have been developed for the determination of sulphur containing amino acids.[1–9] In the present paper we describe a method for the determination of S-containing amino acids at the mg level using ammonium pentavanadate(V) as the good oxidizing reagent.[10–13] The present method is better than the existing methods and does not require a catalyst and sophisticated instrumentation.

EXPERIMENTAL

Ammonium pentavanadate (0.03 N solution) was prepared by dissolving 3.5 g of ammonium pentavanadate in 10 mL of 10% sulphuric acid in a 100 mL measuring flask and made up to the mark with distilled water.

Ferrous ammonium sulphate (0.025 N solution) was prepared by dissolving 2.4508 g (AnalaR BDH) of ferrous ammonium sulphate in distilled water in a 250 mL measuring flask and 10 mL of sulphuric acid was added to check the hydrolysis. The solution was standardized by titration with standard potassium dichromate solution (0.02 N) using diphenylamine sulphonic acid as indicator. Solid N-phenyl anthranilic acid was dissolved in 3 mL of 5% sodium carbonate and the solution was diluted up to 150 mL with distilled water.

Sample solution—Stock solutions of each of the S-containing amino acids were prepared in distilled water. 100 mL of the sample was taken in a 100 mL measuring flask and the solution was raised to the mark with distilled water.

General procedure

Aliquots containing 1–5 mg of the sample were placed in a 100 mL Erlenmyer flask and 2 mL of 0.03 N solution of ammonium pentavanadate was added followed by the addition of 10 mL of 10 N sulphuric acid. The reaction mixture

was shaken gently and heated on a boiling water bath for a prescribed reaction time. After the reaction was over the mixture was cooled to room temperature and titrated with 0.025 N Fe(II) solution using N-phenyl anthranilic acid solution as indicator. A blank experiment was also run under identical conditions using all the reagents except the sample.

The amount of the sample was calculated by the following expression:

$$\text{mg of the sample} = \frac{M(B - S)N}{n}$$

where

M = Molecular weight of the sample,
N = Normality of Fe(II) used titrate the blank experiments.
B = Volume of Fe(II) used titrate the blank experiments.
S = Volume of Fe(II) used titrate the sample experiments.
N = Number of mols of the V(V) reagent consumed permol of the sample.

RESULTS AND DISCUSSION

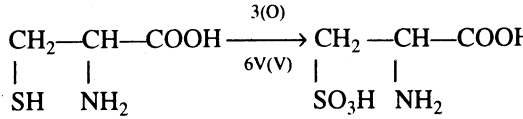
With recommended procedure the determination of cysteine, cystine and methionine has been successfully achieved on 1–5 mg of sample within an accuracy of ± 1% (Table-1) in most of the cases.

TABLE -1
DETERMINATION OF CYSTEINE, CYSTINE AND METHIONINE WITH 0.3 N V(V).

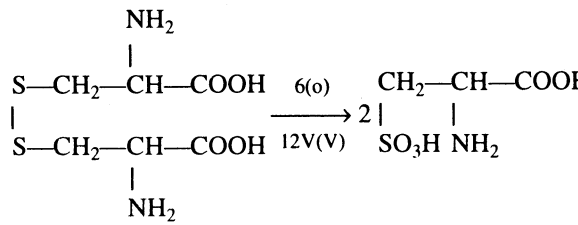
Sl. No.	Sample	Amount taken (mg)	Reaction time (min)	Amount recovered (mg)	Stoich-iometry	Error*	SD	CV
1.	Cysteine	1.0040	10	0.9980	6	–0.60	0.0042	0.4194
		5.0190		5.0425		+0.47	0.0012	0.0238
		10.0300		9.9721		–0.58	0.0420	0.4167
2.	Cystine	1.0050	10	0.9996	12	–0.54	0.0043	0.4289
		5.0105		5.0387		+0.54	0.0239	0.4759
		10.0210		10.0685		+0.47	0.0018	0.0179
3.	Methionine	1.0040	10	1.0098	8	+0.58	0.0051	0.6071
		5.0250		5.0528		+0.55	0.0003	0.0059
		10.0400		10.0982		+0.58	0.0559	0.5357

The effect of variables such as volume and concentration of the reagent sulphuric acid, temperature and reaction time were studied. It was found that the recommended concentration of V(V) reagent is suitable to achieve quantitative reaction. The addition of 10 mL of 10 N H_2SO_4 before the reaction helps in detecting the end point. It was also observed that the reaction was incomplete at room temperature (27°C) and direct heating of the reaction mixture gave inconsistent results. Accurate and constant results were obtained when the reaction was carried out on a water bath. Normally the reaction is completed within 10 minutes.

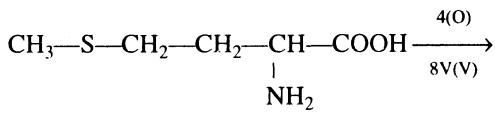
Considering the oxidation reaction of S-containing amino acids and the number of equivalents of V(V) consumed for a particular sample the following course of reaction may be suggested for the oxidation of cysteine, cystine and methionine.



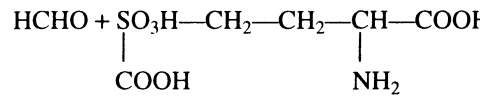
Cysteine Cysteic acid



Cystine Cysteic acid



Methionine



Homocysteic acid

It was found that the presence of easily oxidisable organic compounds like alcohols, phenols, aromatic hydrocarbons and thioureas interfere in the determination while the other amino acids like glycine, alanine are unaffected by the V (V) reagent.

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