Titrimetric Microdetermination of L-Cysteine Hydrochloride Separately and in the Presence of Other Natural Amino Acids

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There is no simple direct titrimetric determination of cysteine separately and in the presence of other natural amino acids without its preliminary separation. Cysteine has been estimated using N-bromo-succinimide (1), colorimetrically (2), by titration of amino proups in a medium of mixed non-aqueous solvents (3), by ampherometric titration (4), polarographically in aqueous solutions (5), by iodometric titration (6) and by the reaction of bromine (7).

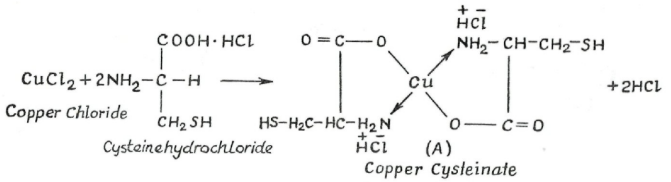
Most of these methods are lengthy, tedious and time consuming and hence do not represent a successful method for the direct estimation of cysteine in micro amounts in the presence of other natural amino acids. On the other hand physical instruments viz. the amino acid analyser are very costly. This paper reports a complexiметric procedure of a direct titration of traces of L-cysteine hydrochloride separately and in the presence of other natural amino acids with a standard solution of copper chloride using 4-(-2 Thiazoly-azo) resorcin (TAR) as indicator without its preliminary separation from the reaction mixture.

Principle

It was observed that L-cysteine hydrochloride reacts with copper chloride quantitatively in citrate buffer (8) at pH value 6.0. On the other hand 4-(-2 Thiazoly-azo) resorcin TAR combines with copper ion resulting in a pink coloured complex. If a solution of copper chloride is added from a microburette in a mixture of L-cysteine hydrochloride, buffered at pH value 6.0 along with TAR dye as indicator which is yellow in colour, then no TAR + Cu complex is formed till all the cysteine hydrochloride present in the mixture has completely reacted with copper ion. After this reaction, the excess of copper chloride reacts with TAR to form a pink coloured complex. This sharp change from yellow to pink colour has been used to observe the end point. The titrations were carried out easily with 0.001 M, 0.01 M, 0.02 M, 0.03 M and 0.04 M of L-cysteine hydrochloride employing 0.001 M, 0.01 M, 0.02 M 0.03 M and 0.04 M of copper chloride solutions respectively and

gave almost quantitative results. The presence of other natural amino acids in the reaction mixture does not interfere in this titration.

The stability of the reaction at pH value 3.0 to 10.5 in different buffers was investigated. However, the best result was observed in citrate buffer at pH value 6.0. The pH of the reaction mixture after the titration was determined by Leeds and Northrup pH meter. It was observed that with the increase in the amount of L-cysteine hydrochloride taken, the pH value at the end point decreases which is probably due to the reaction given below:



Experimental

Standard L-cysteine hydrochloride and copper chloride solutions were prepared by dissolving the exactly weighed amount in doubly distilled water. 5 mg of 4-(-Thiazoly-azo) resorcin (TAR) was dissolved in absolute alcohol and made up to 100 ml.

Citrate Buffer: 8.5 ml of 0.1 M solution of citric acid and 41.5 ml of 0.2 M solution of sodium citrate were mixed and diluted to a total of 100 ml with doubly distilled water to maintain the pH value 6.0.

All the chemicals were of analytical reagent grade qualitiy.

Procedure

One ml of L-cysteine hydrochloride of 0.001 M, 2.0 ml of citrate buffer of pH value 6.0 and 0.1 ml of TAR dye were taken in a beaker. The colour of reaction mixture was yellow. To this reaction mixture a standard solution of 0.001 M of copper chloride was added by a microburette, till a sharp change in colour (from yellow to pink) was observed at the end point. Different amounts of L-cysteine hydrochloride were similarly titrated as shown in table one.

Further micro-amounts of L-cysteine hydrochloride were estimated in the presence of different natural amino acids as a mixture of lysine monohydrochloride DL-leucine, DL-threonine, DL-methionine, hydroxyproline, L-asparagine, DL-aspartic acid, DL-alanine, DL-valine, L-arginine hydrochloride, DL-isoleucine, proline, DL-norleucine, DL-phenylalanine, cystine, L-histidine hydrochloride, L-tyrosine, DL-tryptophan, glutamic acid, glycine and DL-serine. It was found that these amino acids even when present together did not interfere in the titration as shown in tables 2 and 3.

Result and Discussion

Table 1 indicates that L-cysteine hydrochloride can be estimated quantitatively by direct complexiметric titration with a standard solution of copper chloride employing 4-(-2 Thiazoly-azo) resorcin (TAR) as indicator in a citrate buffer medium at pH value 6.0. Tables 2 and 3 show that there is no interference of the other natural amino

Table 1
Titration of 0.001 M of L-cysteine hydrochloride Against 0.001 M copper chloride.

Cysteine hydrochloride 0.001 M (ml)	Copper chloride 0.001 M (ml)	Amount of cysteine hydrochloride in mg	
		Actual	Calculated
1.00	0.50	0.1755	0.1755
2.00	1.00	0.3510	0.3510
3.00	1.50	0.5265	0.5265
4.00	2.00	0.7020	0.7020
5.00	2.50	0.8775	0.8775

Table 2
Titration of L-cysteine hydrochloride in the presence of varying quantities of other natural Amino Acids Against copper chloride.

Mixed amino acids solution (ml)	Cysteine hydrochloride 0.04 M (ml)	Copper chloride 0.04 M (ml)	Amount of cysteine hydrochloride in mg	
			Actual	Calculated
0.50	1.00	0.50	7.02	7.02
1.00	1.00	0.50	7.02	7.02
1.50	1.00	0.50	7.02	7.02
2.00	1.00	0.50	7.02	7.02
2.50	1.00	0.50	7.02	7.02
3.00	1.00	0.50	7.02	7.02

Table 3
Titration of Varying Quantities of L-cysteine hydrochloride in the presence of other Amino Acids Against copper chloride.

Mixture of natural amino acids (ml)	Cysteine hydrochloride 0.01 M (ml)	Copper chloride 0.01 m (ml)	Amount of cysteine hydrochloride in mg	
			Actual	Calculated
1.00	1.00	0.50	1.7550	1.7550
1.00	1,50	0.75	2.6325	2.6325
1.00	2.00	1.00	3.5100	3.5100
1.00	2.50	1.25	4.3875	4.3875
1.00	3.00	1.50	5.2650	5.2650

acids in the estimation of L-cysteine hydrochloride even when these amino acids are present together in ten times higher concentration than L-cysteine hydrochloride. It was found by quantitative results that copper forms 1 : 2 chelated complex with cysteine hydrochloride as shown in figure A which is confirmed potentiometrically. Further cysteine hydrochloride can be titrated with copper acetate or copper chloride employing either 1-(Pyridyl-2'-azo) naphthol (PAN) or 4-(Pyridyl-2-azo)-resorcin (PAR) as indicator with quantitative results comparable with those obtained with the dye TAR.

Since the complex between copper chloride and cysteine hydrochloride is formed in the ratio of 1 : 2, the calculations were done accordingly by multiplying the values obtained by two.

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Summary

A simple titrimetric method for the estimation of traces of L-cysteine hydrochloride separately and even in the presence of other natural amino acids is reported. The cysteine hydrochloride solution from 0.001 M to 0.04 M has been titrated with standard copper chloride solution employing 4-(2- Thiazoly-azo) resorcin (TAR) as indicator. Copper forms 1 : 2 complex with cysteine hydrochloride.

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