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DETERMINATION OF CYSTEINE IN PRESENCE OF CYSTINE

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Selenium dioxide has been used for the determination of cysteine in presence of cystine. The orange color produced is measured at 400 nm. This method is specific for cysteine while cystine does not give any color. Microgram quantities have been determined quite precisely and accurately.

INTRODUCTION

Cysteine is an essential amino acid. It is very important constituent of various foods and food products. It has also got numerous uses in medicinal and other preparations, like, hair grooming preparation, hair dressing, shampoos and various other cosmetic preparations. This compound finds special use in the treatment of cancer.

Many colorimetric, electrometric and titrimetric methods are available for the quantitative determination of cysteine [1–2]. Number of oxidizing agents have been used in titrimetric methods, but these methods suffer from certain drawbacks, for instance, either the method itself is complicated and time-consuming or the oxidizing agents used are not stable. Anson [3] used sodium tetrathionate to determine the SH contents of various proteins but it is unstable. Porphyrindin and iodosobensoate [4–5] have also been used at pH 7 but end-points are not sharp. Stringent conditions were required when iodoacetamide [6] was used. Reid, Slage and Sampe [7] used a similar procedure employing bromine water but as bromine water is not a stable standard solution so due to this defect the procedure was not helpful. Another method for the determination of dialkyl sulfides involving oxidation of the sulfide to sulfoxide by means of standard bromide–bromate solution have been described by Edsberg and Siggia [8]. This method suffers from the disadvantage of having an end-point difficult to distinguish and thus not helpful [9] to even slightly coloured compounds. Iodine, iodate, perbenzoic acid, ferricyanide and hydrogen peroxide have also been used as oxidizing agents [10–14]. Thibert and Sarwar used N-bromosuccinimide [15] for the determination of sulfur amino acids. However, the need of developing some simple and accurate method for the determination of cysteine has got equal importance because most of the methods described here are volumetric and not specific.

In this investigation an attempt has been made to develop a simple and accurate spectrophotometric method for the determination of cysteine. This method is based on the development of orange color when selenium dioxide was added to an alkaline solution of cysteine. As the determination of cysteine in presence of its counterpart cystine is of great importance, this color is not given by cystine. This forms the basis of specific determination of cysteine.

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EXPERIMENTAL

Apparatus

All the absorbance measurements were made with a Beckman DB- spectrophotometer, using 1-cm cells. Pipettes graduated at 0.01. ml intervals were used.

Reagents

All reagents used were of analytical garde. (i) *L*-Cysteine Hydrochloride: Standard solutions were prepared by dissolving an appropriate amount of *L*-cysteine hydrochloride in distilled water and standardized [15]. (ii) Selenium Dioxide: A 1%-solution was prepared in distilled water (iii) Polyvinyl Alcohol (BDH): A 2%-solution was prepared in distilled water (iv) Sodium Hydroxide: A 5%-solution was prepared in distilled water.

PROCEDURE

To 0.4 ml (0.32 mg) test solution was added 2 ml

(2% PVA solution followed by 1 ml 1% SeO_2 solution and 1 ml NaOH (5%). The contents were then allowed to stand for couple of minutes for development of color. After this the volume was made to 10 ml and the contents were shaken well. Then the absorption of the light orange color was measured at 400 nm against reagent blank using 1-cm cells.

Calibration Graph. A calibration graph (Fig. 5) was prepared by taking different amounts of standard cysteine solutions. The unknowns were determined from this graph.

Order of Mixing the Reagents. The order of mixing the reagents has got particular importance. During a systematic study of color reaction it was observed that to a test solution of cysteine PVA should be added first and then SeO_2 folowed by NaOH. However, the color was developed even if PAV was added after SeO_2 and NaOH but then the suspension was not very fine. The good results were only possible with the sequence described above.

RESULTS AND DISCUSSION

Effect of pH. The color reaction is fairly stable at pH 8–13.6 (Fig. 1).

The color intensity decreases with the fall or increase in pH.

Effect of Time on Absorbance. After mixing the reactants at room temperature the color reaction becomes stable in 5 min, and remains stable for 4 hr. After this the suspension starts settling down (Fig. 2).

Effect of Temperature on Absorbance. The color intensity remains constant from 18°–90° (Fig 3).

Effect of Reagent Concentration on Absorbance. The optimum amount of SeO_2 has been determined, 10 mg/10 ml for 80 μg of cysteine, for maximum color development (Fig 4).

Effect of PVA. Polyvingly alcohol stabilize the color reaction. If it is not added fine suspension gets precipitated and thus no satisfactory results could be obtained.

Effect of Diverse Materials. Presence of glucose sodium benzoate, sodium sulphite and cystine has no adverse effect on the absorbance, even when present upto very large amount (20-folds); but ascorbic acid does effect to a large extent. Ascorbic acid gives color without the addition of NaOH.

It can be seen from Table 1 that the results obtained under the conditions described above, are precise and accurate. Small amounts of cysteine can be determined with good accuracy. The method is quite useful when it is desired to determine cysteine in presence of cystine, which usually is present, because with mild oxidations of cysteine the product is cystine. There are very limited methods

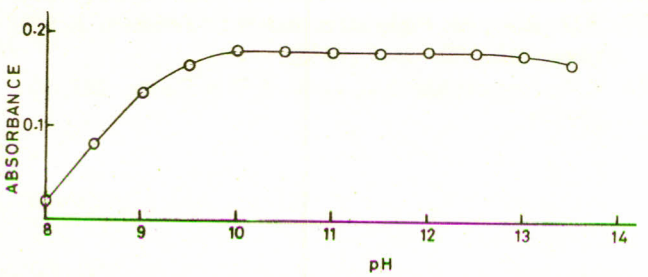


Fig. 1. Effect of pH on color intensity.

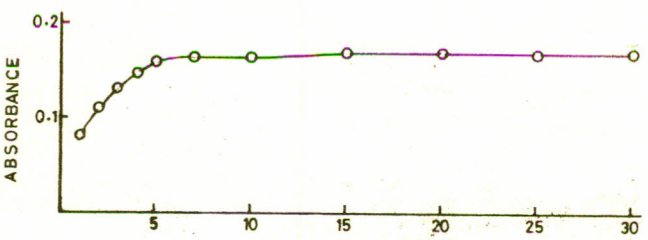


Fig. 2. Effect of time on color intensity.

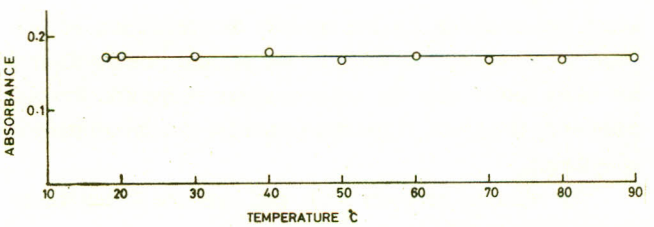


Fig. 3. Effect of temperature on color intensity.

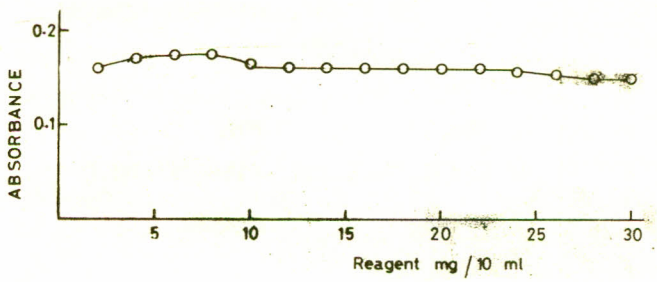


Fig. 4. Effect of reagent concentration on color intensity.

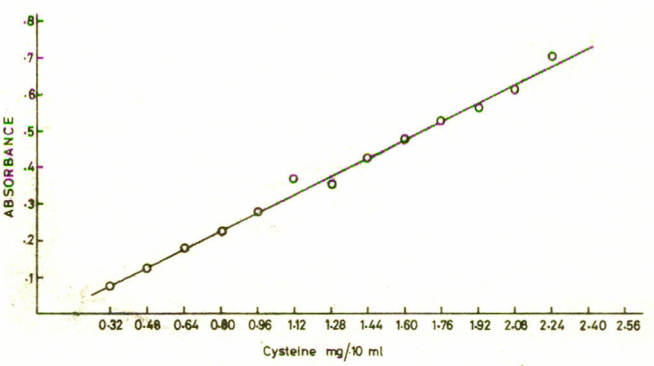


Fig. 5. Calibration graph

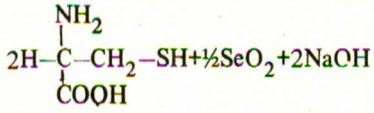
Table 1. Determination of cysteine.

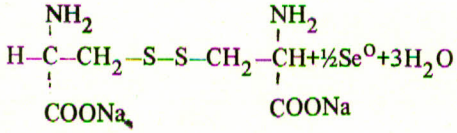
Amount taken (mg)	Amount found (mg)	Error (%)
0.70	0.70	0.00
0.56	0.56	0.00
0.70	0.71	+ 1.42
0.45	0.44	– 2.22
0.48	0.49	+ 2.08
0.40	0.42	+ 0.05
0.68	0.68	0.00
0.72	0.71	– 1.38
0.60	0.60	0.00
1.52	1.55	+ 1.97
1.82	1.76	– 2.19
2.03	2.04	+ 0.49
2.28	2.30	+ 0.87

Average of 5 determination.

which are available for the specific determination of cysteine in presence of cystine. In the present investigation it has been found that the color reaction is specific for cysteine and, therefore, it has been used for the determination of cysteine.

The reaction between SeO_2 and cysteine in NaOH can be described as below.





The orange color is due to very fine suspension of selenium metal which appears as a result of reduction of SeO_2 by cysteine in alkaline medium. This suspension was stabilized by the addition of PVA.

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