

## NOTE

**Isolation and Characterisation of Cysteine Selenotrisulfide**

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Cysteine selenotrisulfide formed by the reaction of selenite with L-cysteine in acid solution has been isolated and characterised by thin layer chromatographic experiments, FAB-MS spectra and IR spectra. Thermal experiments revealed that the compound is stable up to 190°C. Abrasive stripping voltammetric results have shown that this compound undergoes electrochemical reduction in two steps.

Selenium is an essential micronutrient which exhibits both beneficial and toxic effects depending upon the concentration. Selenium deficiency has been associated with several diseases<sup>1</sup> and the beneficial effects of selenium include reduction of toxicity of heavy metals<sup>2</sup> and nephrotoxicity of the antitumour drug cisplatin without reducing its antitumour activity<sup>3</sup>. It is generally presumed that selenite is incorporated into the biological system through an intaction with thiols such as glutathione and cysteine. Ganther<sup>4</sup> has shown that selenite intake by the body is through a reductive metabolism in which the selenotrisulfide of glutathione is an intermediate. In view of the importance of selenotrisulfide in the metabolism of selenite, studies have been initiated on selenotrisulfides formed by selenite with biologically important thiols. However, selenotrisulfides are too labile to be characterised except a few. Selenotrisulfide formed from penicillamine was found to be exceptionally stable and could be isolated. This compound has been characterised by FAB-MS and NMR studies<sup>5</sup>. Selenotrisulfide formed from glutathione has been isolated by HPLC methods and a procedure has been developed for the determination of selenotrisulfide of glutathione using dual mercury/gold electrode<sup>6</sup>. The present paper reports the preparation of cysteine selenotrisulfide and its properties.

**Reagents:** L-cysteine hydrochloride and sodium selenite used were of analytical grade. Ultra pure water (Milli-Q, USA) was used for preparing solutions.

**Cysteine selenotrisulfide:** This compound was prepared by mixing 4 mL of cysteine hydrochloride (0.1 M) with 1.2 mL of sodium selenite (0.1 M) in 0.1 M HCl. A yellow solution was obtained and the mixture was kept at 5°C for 1 day. A light yellow colour precipitate was obtained and it was filtered, washed with cold water and cold ethanol, dried in vacuum over P<sub>2</sub>O<sub>5</sub> at room temperature. The

yield was *ca.* 70%. The purity of the compound was checked by TLC method by using 1-butanol : acetic acid : water (2 : 1 : 1) as the solvent. Cysteine and selenium on TLC plates were identified by spraying with 1% ninhydrin and 0.1 M sodium hydroxide respectively.

*Instruments for measurements:* The elemental analysis of the compound was carried out using Perkin-Elmer (Model 540) C, H, N Analyser. The IR spectrum was recorded using Perkin-Elmer (Model 983G) spectrometer. The FAB-Mass spectrum was obtained by using JEO SX 102/Da 600 Mass Spectrometer (HEOL, Tokyo, Japan) Data System. A Perkin-Elmer (PC-7 series) was used for TGA measurements. The cyclic voltammetric experiments have been carried out with EG&G PAR (Model 362) scanning potentiostat/galvanostat with X-Y recorder.

Cysteine(CSH) reacts with selenite similar to other thiols in acid medium, when taken in the ratio of 4:1 to give cysteine selenotrisulfide (CSSeSC) and cysteine (CSSC). The selenotrisulfide was isolated and the melting point of the compound was found to be 196°C. The compound decomposes to red colour liquid above the m.p. A single spot with  $R_f$  value 0.32 was obtained in TLC experiments. This single spot confirmed the presence of only one compound in the precipitate.

The analytical results are given below with the calculated values in brackets. C 22.57% (22.57%); H 3.70% (3.79%); and N 8.70% (8.77%). The molecular formula of the compound  $C_6H_{12}N_2O_4S_2Se$  corresponds to two cysteine molecules bonded to Se through sulphur.

The IR spectrum of the compound is shown in Fig. 1. The important absorption frequencies observed are  $3000\text{ cm}^{-1}$  (N—H absorption);  $613\text{ cm}^{-1}$  (C—S absorption). The absorption at  $520\text{ cm}^{-1}$  is attributed to the presence of S—Se group. The absence of absorption frequencies for SH and S—S indicates that no free SH or S—S group is present in the compound.

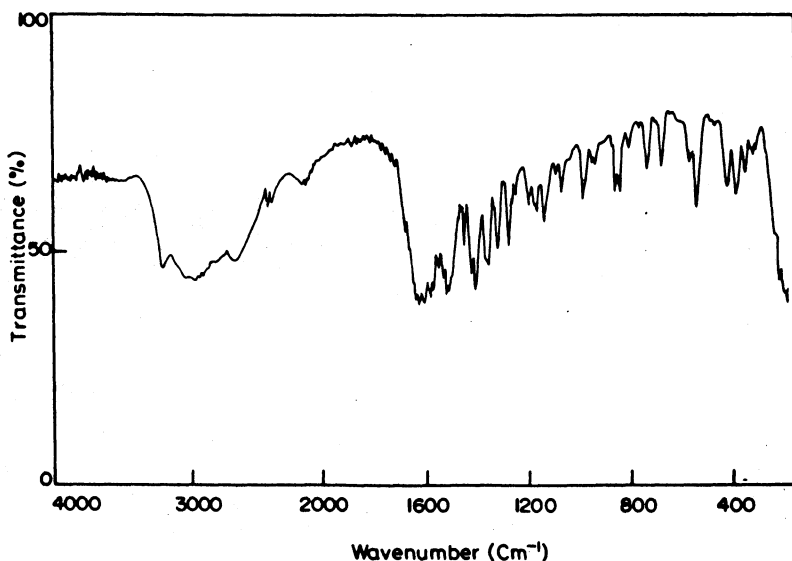


Fig. 1. IR spectrum of cysteine selenotrisulfide

In FAB-MS spectra, the molecular ion peak  $(M+1)^+$  was observed at  $m/z$  321 for cysteine selenotrisulfide, which confirmed the molecular weight 320, consistent with the molecular formula  $C_6H_{12}N_2O_4S_2Se$  deduced from elemental analysis.

The thermogravimetric behaviour of the compound is shown in Fig. 2. There was no marked change in the weight of the compound when the temperature is

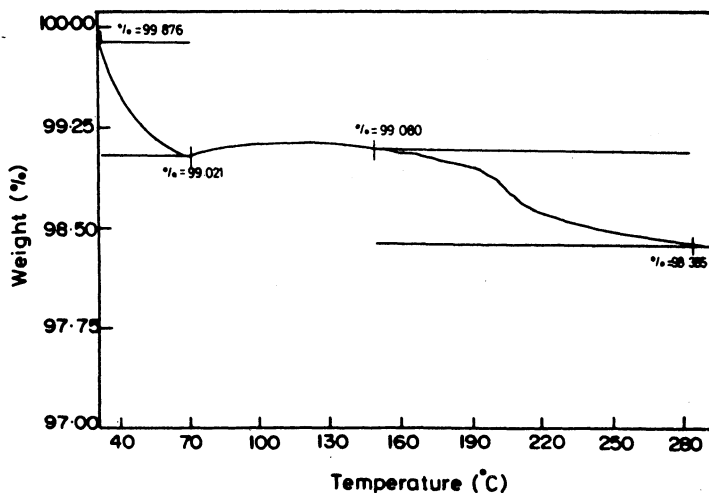
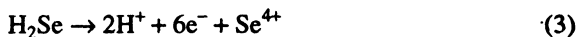
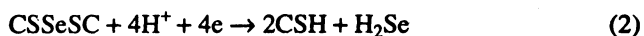
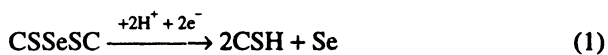


Fig. 2. TGA curve of cysteine selenotrisulfide

increased up to 155°C. The initial reduction in the weight of the compound (up to 1%) when the temperature is increased to 70°C, may be due to the removal of moisture absorbed by the compound. Though a small change in the weight of the compound from 155° to 180°C was observed, the compound did not decompose appreciably liberating any volatile products in the temperature range studied.

Scholz *et al.*<sup>7</sup> have reported a new method for electrochemical characterization of solid compounds, in which the solid is transferred to the surface of paraffin wax impregnated graphite electrode (PIGE) by abrasion, and the electrode is used as working electrode. Cyclic voltammetric experiments with such an electrode provide some information about the solid. The voltammogram obtained for cysteine selenotrisulfide electrode is shown in Fig. 3. It shows two cathodic peaks and an anodic peak. The first cathodic peak corresponds to the reduction of cysteine selenotrisulfide to cysteine and elemental selenium and the second peak corresponds to the formation of hydrogen selenide. The anodic peak is due to the oxidation of hydrogen selenide to selenite. The mechanism can be tentatively given as



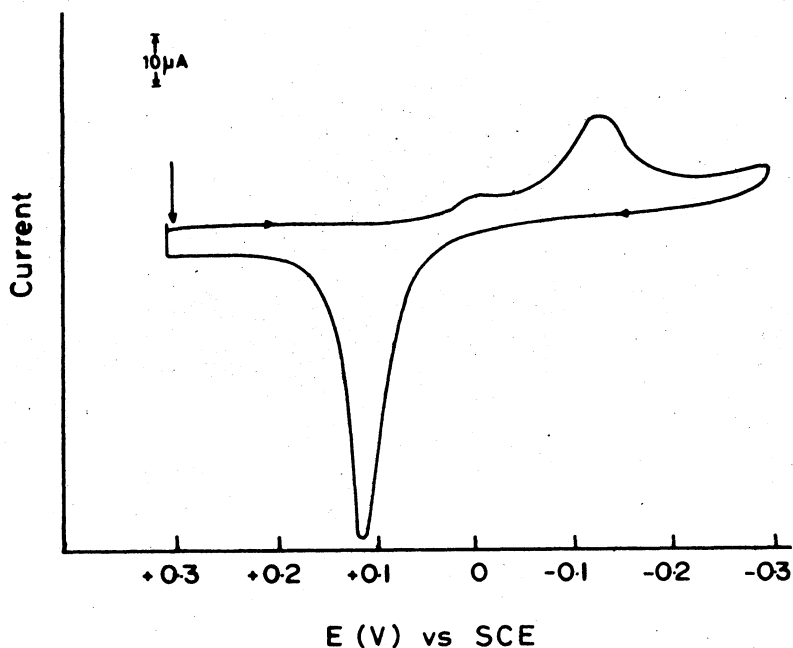


Fig. 3. Abrasive cyclic voltammogram of cysteine selenotrisulfide in 1 M HCl; scan rate 100 mV/s

The larger current obtained for the anodic process suggests that the oxidation may involve six electrons, leading to the formation of selenite which requires a more detailed investigation. The results presented here suggest that cysteine selenotrisulfide can be prepared in pure form and is quite stable

#### REFERENCES

1. J.T. Salonen and J.K. Huttunen, *Ann Clin. Res.*, **18**, 30 (1986).
2. R.C. Schnell, K.S. Park, M.H. Davies, B.A. Merrick and S.W. Weir, *Toxicol. Appl. Pharm.*, **95**, 1 (1988).
3. G.S. Baldew, C.J.A. Van den Hammer, G. Los, N.P.E. Vermeulen, J.J.M. de Goeij and J.G. McVie, *Cancer Res.*, **49**, 3020 (1989).
4. H.E. Ganther, *Biochem.*, **10**, 4089 (1971).
5. T. Nakagawa, Y. Hasegawa, Y. Yamaguchi, H. Tanaka, M. Chikuma, H. Saukurai and M. Nakayama, *Biochem. Biophys. Res. Commun.*, **135**, 183 (1986).
6. H.M.A. Killa and D.L. Rabenstein, *J. Chromatogr.*, **465**, 359 (1989).
7. F. Scholz and B. Lange, *Trends in Anal. Chem.*, **11**, 359 (1992).

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