

Chromatographic analysis of the reaction between thiosalicylic acid and selenious acid in methanol

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ABSTRACT

A high-performance liquid chromatographic method using a monophenylated reversed-phase column for the determination of thiosalicylic acid, thiosalicylic acid disulphide and thiosalicylic acid selenotrisulphide was developed and applied to studies of the reaction between thiosalicylic acid and selenious acid in methanol. It was confirmed that the molar ratio of selenious acid to thiosalicylic acid is 1:4 at the equivalence point and that equimolar amounts of thiosalicylic acid selenotrisulphide and thiosalicylic acid disulphide are formed in this reaction. These results are consistent with previous information on this kind of reaction in an acidic aqueous solution.

INTRODUCTION

Compounds with mercapto groups are known to react with selenious acid. Busev [1] reported that some compounds reacted with selenious acid in a 4:1 molar ratio and some other compounds in a 2:1 ratio. Thiosalicylic acid is one of these mercapto compounds and has been used as a reagent for the determination of trace elements such as selenium and tellurium [2-4]. The purpose of these previous investigations was to determine selenium by various methods such as gravimetry or spectrophotometry or to study the equilibria of the reaction under acidic conditions by spectrophotometry.

Under highly acidic conditions (4-8 M hydrochloric acid), thiosalicylic acid is reported to react with selenious acid in a 4:1 molar ratio [3]. Al-Daher et al. [4] also showed that thiosalicylic acid reacts with selenious acid in a 4:1 molar ratio using potentiometric titration. However, Cresser and West [2] reported that the reaction

molar ratio of thiosalicylic acid to selenious acid was 3:1 in the pH range 0.5-2.5. These discrepancies in the reaction between thiosalicylic acid and selenious acid need to be investigated further and resolved.

We have already shown that a mixed precipitate of thiosalicylic acid selenotrisulphide and thiosalicylic acid disulphide can be obtained by the reaction of thiosalicylic acid and selenious acid in methanol and succeeded in the purification of the selenotrisulphide [5]. In this study, the reaction of selenotrisulphide formation in methanol was investigated quantitatively by high-performance liquid chromatography (HPLC) and the stoichiometry of the reaction between thiosalicylic acid and selenious acid was determined.

EXPERIMENTAL

Chemicals

Thiosalicylic acid, thiosalicylic acid disulphide and selenious acid were purchased from Nacalai Tesque (Kyoto, Japan). Thiosalicylic acid selenotrisulphide standard was prepared in our lab-

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oratory [5]. Methanol for sample preparation was of analytical-reagent grade and methanol of HPLC grade was used for the preparation of the mobile phase. All other chemicals were of analytical-reagent grade.

Sample preparation

Thiosalicylic acid (100 mg) was suspended in 1 ml of methanol and dissolved by heating on a water-bath (40°C), and various amounts of selenious acid (4.18-41.8 mg) were dissolved in 0.1 ml of methanol. Thiosalicylic acid solution was slowly added to the selenious acid solution while stirring and kept at constant temperature in a water-bath (40°C). Immediately after the addition of thiosalicylic acid, a yellowish white precipitate was formed. A small aliquot of the suspension was taken and diluted with the mobile phase for HPLC analysis as described below. A 5- μ l volume of sample was applied to the HPLC column.

High-performance liquid chromatography

A Develosil-ph5 column (250 mm \times 4.6 mm I.D.) (Nomura Chemical, Aichi, Japan) was attached to an HPLC system composed of a PU-980 pump, a UV-970 detector and an 807-IT integrator (JASCO, Tokyo, Japan). The composition of the mobile phase was methanol-0.05 M phosphate buffer (pH 7.0)-triethylamine (30:70:0.02, v/v/v). The flow-rate was 0.8 ml/min and the detection wavelength was 250 nm. Analysis was performed at room temperature. For the determination of molar absorption coefficients at 250 nm of thiosalicylic acid, thiosalicylic acid disulphide and thiosalicylic acid selenotrisulphide, the compounds were dissolved in the mobile phase at concentrations of 50, 25 and 25 μ M, respectively, and the absorption was measured with a spectrophotometer.

RESULTS AND DISCUSSION

Fig. 1 shows representative chromatograms for the reaction mixture of thiosalicylic acid-selenious acid in which the molar ratios were (a) 1:8, (b) 1:4 and (c) 1:2. As shown in Fig. 1a, three peaks corresponding to thiosalicylic acid (peak 1), thiosalicylic acid disulphide (peak 2) and

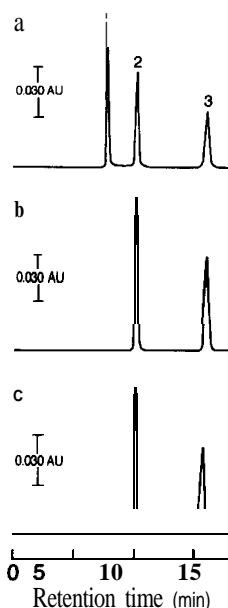


Fig. 1. Chromatograms of the reaction mixtures of thiosalicylic acid and selenious acid. The selenious acid:thiosalicylic acid molar ratios were (a) 1:8, (b) 1:4 and (c) 1:2. Peaks 1, 2 and 3 correspond to thiosalicylic acid (7.6 min), thiosalicylic acid disulphide (10.1 min) and thiosalicylic acid selenotrisulphide (15.8 min), respectively.

thiosalicylic acid selenotrisulphide (peak 3) could be resolved under the conditions used. The retention times of these compounds were 7.6, 10.1 and 15.8 min, respectively. These peaks were identified by comparison with authentic samples. Furthermore, these compounds were found to be stable during their passage through the column, as each standard sample gave a single, sharp peak.

When an ODS column was used [5], the retention times of thiosalicylic acid and its disulphide were too close to evaluate them separately under the conditions described. As neutrality was necessary to dissolve these compounds, 0.05 M phosphate buffer (pH 7.0) was used. Although the selenotrisulphides are usually unstable in neutral solutions [1,6,7], thiosalicylic acid selenotrisulphide was stable enough during the analysis. Triethylamine in the mobile phase improved the peak shape of thiosalicylic acid, which otherwise showed tailing.

From the peak area data obtained in HPLC

analysis of the reaction mixture, the contents of thiosalicylic acid, thiosalicylic acid disulphide and thiosalicylic acid selenotrisulphide were calculated as described below. The peak areas (%) of these compounds are given in Table I. The thiosalicylic acid: thiosalicylic acid disulphide: thiosalicylic acid selenotrisulphide molar ratio was calculated by dividing each peak area (%) by the molar absorption coefficients, which were 750, 1600 and 1600 m^2/mol , respectively. This molar ratio was corrected using a thio-salicylic acid disulphide or selenotrisulphide structure as a unit, so the values for thiosalicylic acid are represented as half of the usual molar number. The percentage contents of thiosalicylic acid and its products were then calculated so that their sum equalled 100 (Table I). These values were plotted against the reaction molar ratio of selenious acid to thiosalicylic acid (Fig. 2).

The thiosalicylic acid content decreased with increase in the molar ratio and reached zero at a molar ratio of 1:4. This result means that the reaction involving the thiol group is completed

TABLE I

PEAK AREA AND CALCULATED CONTENT OF (A) THIOSALICYLIC ACID, (B) THIOSALICYLIC ACID DISULPHIDE AND (C) THIOSALICYLIC ACID SELENOTRISULPHIDE OBSERVED FOR REACTION MIXTURES IN HPLC ANALYSIS

The content values (%) were calculated as follows: first, the A:B:C molar ratio was calculated by the division of the peak area (%) values by the molar absorption coefficients at the detection wavelength (250 nm), which were 750, 1600 and 1600 m^2/mol for A, B and C, respectively; second, the resulting values were represented as the corrected molar ratio using thiosalicylic acid disulphide or selenotrisulphide structure as a unit; finally, this corrected molar ratio was represented as content (%) after making their sum total 100.

$\text{H}_2\text{SeO}_3:\text{RSH}$	Peak area (%)			Content (%)		
	A	B	C	A	B	C
0	88	12	0	89	11	0
1:20	67	22	11	68	21	11
1:8	37	35	28	39	34	27
1:4	0	52	48	0	52	48
1:3	0	51	49	0	51	49
1:2	0	53	47	0	53	47

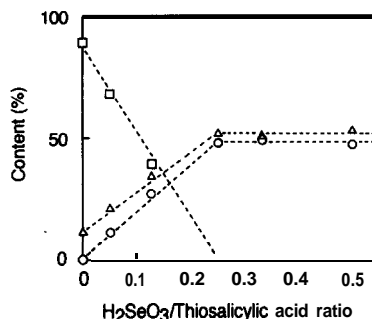


Fig. 2. Contents of thiosalicylic acid, thiosalicylic acid disulphide and thiosalicylic acid selenotrisulphide in the reaction mixture with various selenious acid:thiosalicylic acid molar ratios. The values are given in Table I. \square = Thiosalicylic acid; Δ = thiosalicylic acid disulphide; \circ = thiosalicylic acid selenotrisulphide.

when the molar ratio is 1:4. Both the thiosalicylic acid disulphide and selenotrisulphide contents increased almost linearly with increase in the molar ratio and reached the maximum (ca. 50%) when the molar ratio was 1:4, and these values remained constant where the molar ratio exceeded 1:4. These results indicate that equimolar amounts of thiosalicylic acid disulphide and selenotrisulphide were formed at least at molar ratios $>1:4$. Analysis of thiosalicylic acid in the absence of selenious acid showed that ca. 10% was present as thiosalicylic acid disulphide, which was probably formed by air oxidation after dissolution and/or during the analysis. Although the content of disulphide was slightly higher than that of selenotrisulphide, this difference decreased with increase in the selenious acid: thiosalicylic acid molar ratio ($=0.29$, which suggests that the amount of thiosalicylic acid disulphide formed by air oxidation is proportional to the residual amount of thiosalicylic acid).

The results described above indicate that the reaction between thiosalicylic acid and selenious acid in methanol proceeds via the following equation:



That is, thiosalicylic acid reacts with selenious acid at a molar ratio of 4:1 in methanol, and equimolar thiosalicylic acid disulphide and thiosalicylic acid selenotrisulphide are formed by

this reaction. It was further demonstrated that **Se(IV)** was reduced to **Se(II)** by the reaction with thiosalicylic acid.

These results differ from those of Al-Daher et al. [4] except for the reaction molar ratio. They analysed the composition of the product obtained at the equivalence point in the **potentiometric** titration and found the molar ratio of selenium and thiosalicylic acid in the product to be 1:4. They did not give any information on the oxidation state of selenium in the reaction. However, as shown in this study, the product should have been an equimolar mixture of thiosalicylic acid disulphide and thiosalicylic acid selenotrisulphide containing **Se(II)**, as the reaction conditions they used seem essentially the same as ours.

Regarding the reaction molar ratio of thiosalicylic acid to selenious acid, our result obtained with a methanol solution is consistent with previous reports in which it was determined in an acidic solution by spectrophotometry [3] or in 95% ethanol and **acetate-HCl (pH 2.3)** by potentiometric titration [4], although different molar ratios were described by Cresser and West [2].

In this study, the reaction between thiosalicylic acid and selenious acid in methanol was **investi-**

gated using HPLC. This is the first direct demonstration that thiosalicylic acid reacts with **selenious acid** at a molar ratio of **4:1** in methanol, that equimolar disulphide and selenotrisulphide are formed and that **Se(IV)** is reduced to **Se(II)** in this reaction. These results were in good agreement with those observed in the acidic aqueous solution, and it is considered that the same reaction can occur both in acidic aqueous and in methanol solutions in the case of thiosalicylic acid and selenious acid. This HPLC method will be useful to investigate further the reaction involving thiosalicylic acid **selenotrisulphide**.

REFERENCES

- 1 A.I. Busev, *Talanta*, 11 (1964) 485.
- 2 M.S. Cresser and T.S. West, *Analyst*, 93 (1968) 595.
- 3 B.W. Budesinsky and J. Svec, *J. Inorg. Nucl. Chem.*, 33 (1971) 3795.
- 4 I.M. Al-Daher, F.A. Fattah and K.A. Najm, *Analyst*, 115 (1990) 645.
- 5 Y. Saito and M. Chikuma, *Fresenius' J. Anal. Chem.*, submitted for publication.
- 6 H.E. Ganther, *Biochemistry*, 7 (1968) 2898.
- 7 P.D. Whanger, S.C. Vendeland, J.T. Deagen and J.A. Butler, in B. Momčilović (Editor), *Trace Elements in Man and Animals 7*, IMI Institute for Medical Research and Occupational Health, University of Zagreb, Zagreb, 1991, Ch. 17-11.