Journal of Chromatography, 136 (1977) 85–93

CHROM. 9917

SOME 1,2-DIAMINOBENZENE DERIVATIVES AS REAGENTS FOR GAS CHROMATOGRAPHIC DETERMINATION OF SELENIUM WITH AN ELECTRON-CAPTURE DETECTOR

YASUAKI SHIMOISHI

Department of Chemistry, Faculty of Science, Okayama University, 3-1-1 Tsushima-naka, Okayama-shi 700 (Japan)

(First received December 3rd, 1976; revised manuscript received January 3rd, 1977)

SUMMARY

1,2-Diaminobenzene and its derivatives react with selenous acid in acidic solution to form the piazselenols, which can be extracted into toluene. Microgram amounts of selenium can be determined spectrophotometrically by measuring the absorbance of these piazselenols extracted into toluene. A more sensitive method, in which the piazselenols extracted into toluene are detected by electron-capture gas chromatography, has been developed. In order to find a more sensitive reagent, 13 piazselenols were synthesized. The retention behaviour and sensitivity in electron-capture detection gas chromatography and the distribution ratios between aqueous solution and toluene were studied for each piazselenol extracted into toluene. Of these piazselenols, 4,6-dibromopiazselenol, formed by the reaction of 1,2-diamino-3,5-dibromobenzene with selenous acid, was found to be best as regards sensitivity and distribution ratio. Under the optimal conditions for the formation and the extraction of the piazselenol, the practical detection limit was 1 ng. Selenium(VI) and total selenium in NBS Bovine Liver, SRM 1577, were determined successfully.

INTRODUCTION

Since Hoste[1] and Hoste and Gillis[2] proposed 3,3′-diaminobenzidine as a sensitive reagent for selenium, it has been applied to determination of selenium in various samples. Ariyoshi *et al.*[3] proposed 1,2-diaminobenzene as a more sensitive and stable reagent. Tanaka and Kawashima[4] described a critical study of some 4-substituted 1,2-diaminobenzenes as spectrophotometric reagents for selenium. 2,3- and 1,8-diaminonaphthalene were proposed by Parker and Harvey[5] and Murakami and Ishii[6], respectively. As the reaction products of 3,3′-diaminobenzidine and 2,3-diaminonaphthalene with selenium(IV) are strongly fluorescent, these reagents have been also used in fluorimetry. This method has been employed for the determination of trace amounts of selenium, because its sensitivity was several times greater than that of spectrophotometry. Nakashima and Tôei[7] proposed a very sensitive procedure

for selenium in which a gas chromatograph equipped with an electron-capture detector (ECD) is used with 1,2-diamino-4-chlorobenzene. Shimoishi and co-workers[8–13] have found that the sensitivity of 1,2-diamino-4-nitrobenzene for selenium is superior to that of 1,2-diamino-4-chlorobenzene, and it has been used for the determination of trace amounts of selenium in highly pure metals and organic materials.

In this work, the gas chromatographic (GC) properties of 13 piazselenols were studied in order to obtain a more sensitive reagent. The selenium(VI) and total selenium in NBS Bovine Liver, SRM 1577, were determined by using the best reagent, 1,2-diamino-3,5-dibromobenzene.

EXPERIMENTAL

Apparatus

A Shimadzu Model GC-5A gas chromatograph, equipped with a ^{63}Ni ECD, was used for the study of piazselenol derivatives. A glass column (1 m × 3 mm I.D.) was packed with 15% SE-30 on 60–80-mesh Chromosorb W. The detector temperature was maintained at 280° and the column temperature and nitrogen flow-rate were varied. A Shimadzu Model 101 recorder was used.

A Shimadzu Model GC-3AE gas chromatograph, equipped with a ^{3}H ECD, and connected with a Model EM-5 electrometer, was used for the determination of selenium. A glass column (1 m × 4 mm I.D.) was packed with 15% SE-30 on 60–80-mesh Chromosorb W. The column and detector temperatures were maintained at 200° and the nitrogen flow-rate was 34 ml·min^{-1}. A Shimadzu Model 250A recorder was used at a chart speed of 5 mm·min^{-1}.

Reagents

All reagents were of analytical-reagent grade unless stated otherwise.

1,2-Diamino-3,5-dibromobenzene dihydrochloride, saturated solution in concentrated hydrochloric acid. 1,2-Diamino-3,5-dibromobenzene was synthesized from 2-nitroaniline by simple bromination with bromine, followed by reduction with tin or zinc in hydrochloric acid. The pure dihydrochloride was prepared; the reagent obtained was dissolved in about 1 *M* hydrochloric acid on a water-bath, activated charcoal was added and the solution filtered. The salt was precipitated by adding an equal volume of concentrated hydrochloric acid to the filtrate. The reagent (0.2 g) was dissolved in 100 ml of concentrated hydrochloric acid. Although the reagent was dissolved immediately in concentrated hydrochloric acid, a small amount of the reagent might be precipitated after standing for 1 day. This solution can be used for at least 1 week.

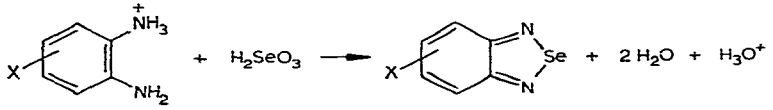
Selenium(IV) stock solution. Selenium dioxide (704 mg) was dissolved in 500 ml of distilled water and the selenium concentration was determined gravimetrically[14] to be 0.957 mg·ml^{-1}. Working solutions were prepared by dilution of the stock solution, which was stable for at least 6 months.

Solutions of piazselenol and its analogues. A constant weight of piazselenol or its analogue was dissolved in toluene to prepare a $2 \cdot 10^{-4}$ *M* solution. Working solutions were prepared by dilution.

RESULTS AND DISCUSSION

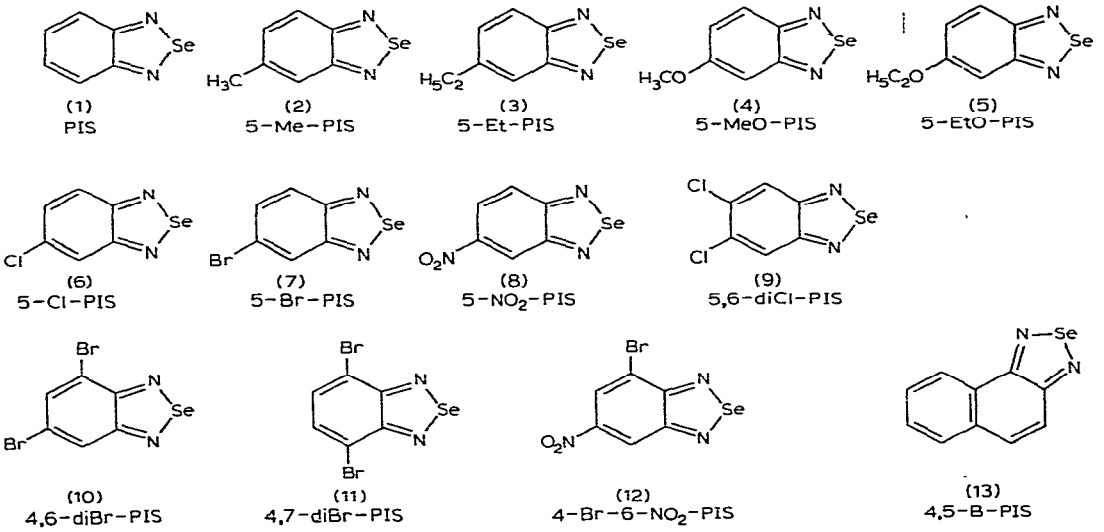
Synthesis of piazselenol and its analogues

The kinetics and mechanism of the reaction between aromatic *o*-diamines, such as 3,3′-diaminobenzidine[15], 2,3-diaminonaphthalene[16] and 1,2-diaminobenzene[17] and selenous acid (H_2SeO_3) were investigated. The results indicate that the reacting species are the monoprotonated diamine and the undissociated selenous acid. The reaction scheme is as follows:



Therefore, the reaction must be carried out at the pH between $pK_{a\,R_1}$ and $pK_{a\,Se}$, where $K_{a\,R_1}$ is the acid dissociation constant of the diprotonated diamine and $K_{a\,Se}$ is the first acid dissociation constant of selenous acid. As this reaction is very selective and specific, it is scarcely effected by most ions.

Piazselenols were prepared as follows. 1,2-Diaminobenzene or its analogue was dissolved in 0.01–1 *M* hydrochloric acid and selenous acid solution was added to this solution. The corresponding piazselenol was immediately precipitated. The resulting crystals of the piazselenol derivative were filtered, washed with a small amount of water and then recrystallized twice from ethanol or toluene. If the purified material is stored in a calcium chloride desiccator, it is stable for at least 2 years. The piazselenols synthesized and used are shown below (1–13).



Piazselenols 1, 2, 6, 8 and 13 were synthesized by using commercially available aromatic 1,2-diaminobenzene derivatives (Tokyo Chemical Industry, Tokyo, Japan). Compounds 3–5 had been synthesized earlier[18]. Compounds 7 and 9–12 were obtained from the corresponding 1,2-diaminobenzene derivatives. The analytical results

for these new compounds are as follows. 5-Bromopiazselenol (7): $C_6H_3N_2BrSe$ requires C 27.51, H 1.15, N 10.69%; found, C 26.96, H 1.08, N 10.27%. 5,6-Dichloropiazselenol (9): $C_6H_2N_2Cl_2Se$ requires C 28.77, H 0.80, N 11.09%; found, C 29.23, H 0.86, N 11.62%. 4,6-Dibromopiazselenol (10): $C_6H_2N_2Br_2Se$ requires C 21.14, H 0.59, N 8.22%; found, C 21.27, H 0.57, N 8.34%. 4,7-Dibromopiazselenol (11): $C_6H_2N_2Br_2Se$ requires C 21.14, H 0.59, N 8.22%; found, C 21.31, H 0.57, N 8.18%. 4-Bromo-6-nitropiazselenol (12): $C_6H_2N_3O_2BrSe$ requires C 23.48, H 0.66, N 13.69%; found, C 23.52, H 0.68, N 13.68%.

Gas chromatographic properties of piazselenol derivatives

Piazselenol and its derivatives have the characteristics of organometallic compounds rather than metal complexes, and have clear melting points (Table I). Moreover, as these compounds can sublime at temperatures lower than the melting points without decomposition, it implies that these compounds can be used for GC analysis.

TABLE I

PROPERTIES OF PIAZSELENOL DERIVATIVES

Piazselenol	*M.p. (°C)*		*Retention index (180°)*	*Relative sensitivity*	*Distribution ratio*	
	Found	*Literature*[18]			*Found (pH = 0)* *	*Literature (pH = 1)*[4]
PIS	73–74	74	1307	1.0	172	154
5-Me-PIS	72–73	72–73.5	1421	1.4	379	657
5-Et-PIS	73–73.5	72–73.5	1515	1.2	4010	
5-MeO-PIS	109–110	108–111	1541	1.7	297	
5-EtO-PIS	102	99.5–102.5	1611	1.6	1661	
5-Cl-PIS	121	119–119.5	1454	17	1405	2580
5-Br-PIS	135		1547	30	2576	
5-NO_2-PIS	222–223	220–221	1653	128	250	367
5,6-diCl-PIS	163–164		1632	102	N.B.	
4,6-diBr-PIS	217–218		1812	363	N.B.	
4,7-diBr-PIS	273		1864	172	N.B.	
4-Br-6-NO_2-PIS	221–222		1908	255	946	
5,6-B-PIS	129	127.5–128.5	1800	25	N.B.	

* N.B. = Not back-extracted.

In GC, the retention value is influenced by the interaction between the solute and the liquid stationary phase, and is mainly affected by the substituents in the homologues. The retention values, expressed as Kováts retention indices[19], of the piazselenol derivatives and of standard *n*-alkanes were calculated from retention time measured at 180° (Table I).

Fig. 1, a plot of retention index against the molecular weight of piazselenol derivatives, shows three straight lines, each of which starts at the point representing piazselenol. The top line contains 5-methyl-, 5-ethyl-, 5-methoxy-, 5-ethoxy- and 5-nitropiazselenol, the second line 5-chloro, 5,6-dichloro- and 4-bromo-6-nitropiazselenol and the third line 5-bromo-, 4,6-dibromo- and 4,7-dibromopiazselenol. The differences in the slopes of these lines depend upon the affinity between the solute and the liquid stationary phase. As the stationary phase (SE-30) is non-polar, the

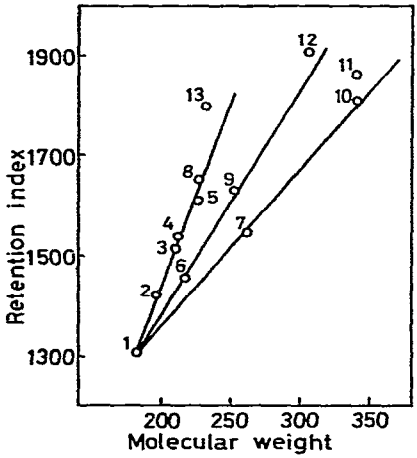


Fig. 1. The plots of retention index *versus* molecular weight. 1 = PIS; 2 = 5-Me-PIS; 3 = 5-Et-PIS; 4 = 5-MeO-PIS; 5 = 5-EtO-PIS; 6 = 5-Cl-PIS; 7 = 5-Br-PIS; 8 = 5-NO_2-PIS; 9 = 5,6-diCl-PIS; 10 = 4,6-diBr-PIS; 11 = 4,7-diBr-PIS; 12 = 4-Br-6-NO_2-PIS; 13 = 4,5-B-PIS.

interaction of chloro and bromo derivatives with SE-30 would be less than that of alkyl derivatives. Therefore, the slopes of the lines for chloro and bromo derivatives are smaller than that for the alkyl derivatives, but the polar nitro compound falls on the top line for an unknown reason. If a new mono- or di-substituted compound is prepared, the retention index could be predicted from Fig. 1.

Sensitivity of piazselenol derivatives for electron-capture detection

The ECD is very sensitive to electron-withdrawing groups in organic substances. The first GC–ECD determination of selenium was applied to 5-chloropiazselenol, which was formed from selenium(IV) and 1,2-diamino-4-chlorobenzene[7]. Of the commercially available 1,2-diaminobenzene derivatives, 1,2-diamino-4-nitrobenzene was found to be best and four times more sensitive than 1,2-diamino-4-chlorobenzene. Microgram amounts of selenium in pure sulphuric acid[8], pure tellurium[9], sea water[10], plant materials[11], copper[12] and milk products[13] were determined successfully with 1,2-diamino-4-nitrobenzene by GC–ECD.

Young and Christian[20] determined selenium in human blood and urine by GC–ECD using 2,3-diaminonaphthalene. They stated that the sensitivity was almost the same as that for 4,5-dichloropiazselenol. The selenium–2,3-diaminonaphthalene complex could not be obtained in the pure state, because the complex was unstable. Stijve and Cardinale[21] determined selenium in various substrates by GC–ECD using 1,2-diamino-4,5-dichlorobenzene.

In order to find a more sensitive reagent for selenium, the 13 piazselenol derivatives were tested for sensitivity in GC–ECD. The detector temperature was kept at 280° and the carrier gas (nitrogen) flow-rate was kept constant at 29 ml·min^{-1}. Each piazselenol derivative at three different concentrations in toluene was injected into the gas chromatograph at a column temperature of 120–210° and the retention time, peak height and peak area were measured. At a constant column temperature, a linear relationship was found between the solute concentration and the peak height

or area; the shorter the retention time, the larger the peak height or area becomes. The retention time was then measured at various temperatures, and the peak height and area were extrapolated to a constant retention time (3 min). The relative sensitivity was calculated from the ratio of the peak height or area of the derivative to that of piazselenol at the retention time of 3 min. There were no differences between the relative sensitivities as calculated from the peak height and the peak area. The results are given in Table I.

The order of sensitivity of the substituents is $H < Cl < Br < NO_2$, and of the compounds studied 4,6-dibromopiazselenol had the highest sensitivity, being three times greater than that of 5-nitropiazselenol. 4-Bromo-6-nitropiazselenol did not have as high a sensitivity as was predicted from the mono-substituted compound.

Distribution ratio of piazselenol derivatives

Piazselenol and its derivatives can be extracted into toluene over the whole pH range. When the pH of the aqueous phase is higher than $pK_{a\,R_2}$, where $K_{a\,R_2}$ is acid dissociation constant of the monoprotonated diamine, the excess of reagent would be extracted into toluene together with the piazselenol. Consequently, the piazselenol derivative should be extracted at a pH lower than $pK_{a\,R_2}$.

The distribution ratio was determined gas chromatographically. Toluene (1.0 or 0.5 ml) containing a known amount of the piazselenol derivative was equilibrated with 200 ml of 1 *M* hydrochloric acid saturated with toluene. The distribution ratio was calculated from the decrease in the peak height in the gas chromatogram. The results are shown in Table I.

The distribution ratios of the derivatives are generally large; in particular, dichloro and dibromo derivatives could not be back-extracted into 200 ml of 1 *M* hydrochloric acid. The results indicate that the extraction of the piazselenols is quantitative. 4,6-Dibromopiazselenol has not only the highest sensitivity for the electron-capture detection but also the highest distribution ratio. Hence, 1,2-diamino-3,5-dibromobenzene would be the most sensitive reagent for selenium.

Determination of selenium with 1,2-diamino-3,5-dibromobenzene

The recommended procedure for the determination of selenium(IV) with 1,2-diamino-3,5-dibromobenzene is as follows. A 2-ml volume of a saturated solution of the reagent in concentrated hydrochloric acid is added to 1 *M* hydrochloric acid containing selenium(IV); the final hydrochloric acid concentration is about 2 *M*. After allowing the mixture to stand for 2 h at room temperature, the 4,6-dibromopiazselenol formed is extracted with 1 ml of toluene by mechanical shaking for 5 min. The toluene extract is washed with 2 ml of 9 *M* hydrochloric acid by shaking for 1 min, 2 μl of the toluene extract is injected into the gas chromatograph and the peak height is measured.

Fig. 2 shows the effect of the amount of reagent on the determination of 28.7 ng of selenium(IV). When the reagent is present in more than a 12,000 molar excess over selenium(IV), it reacts quantitatively with the selenium(IV) to form 4,6-dibromopiazselenol. On the other hand, 1,2-diamino-4-nitrobenzene needs more than a 50,000-fold molar excess for the complete formation of 5-nitropiazselenol[8]. Therefore, 2 ml of a saturated solution (about 0.2%) of the reagent in concentrated

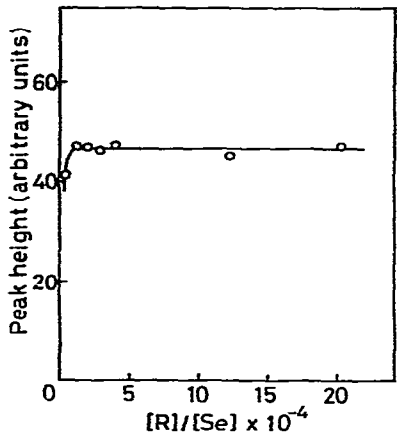


Fig. 2. Effect of reagent concentration. Sensitivity setting, 64 × 0.01 V. Amount of selenium, 28.7 ng.

hydrochloric acid was used in the following experiment; the complete formation of the piazselenol requires more than 1 h.

The effect of the hydrochloric acid concentration was examined between 1 and 6 *M*. The reaction proceeds completely even at 6 *M* hydrochloric acid. When the toluene extracted from hydrochloric acid of concentration 2 *M* or less was injected directly into the gas chromatograph, the peaks of the reagent itself and unknown substances overlapped with the peak of 4,5-dibromopiazselenol. In more concentrated hydrochloric acid, the overlapping peaks became smaller. The peaks could be eliminated effectively by washing the toluene extract with 9 *M* hydrochloric acid and the blank minimized as shown in Fig. 3.

Calibration graphs were constructed by using known amounts of selenous acid (Fig. 4). The sensitivity is 2.5 times higher than that with 5-nitropiazselenol, and 1 ng of selenium in 1 ml of toluene can be determined quantitatively.

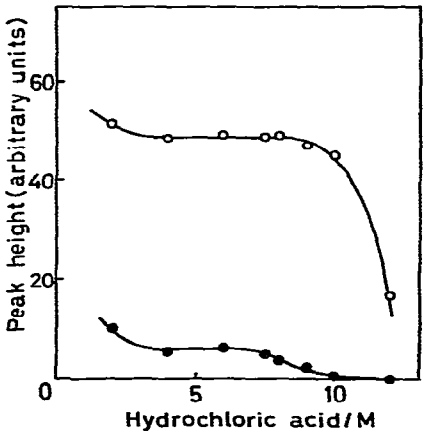


Fig. 3. Effect of the HCl concentration for washing of toluene extract. Sensitivity setting, 64 × 0.01 V. ○, Selenium (28.7 ng) + blank; ●, blank.

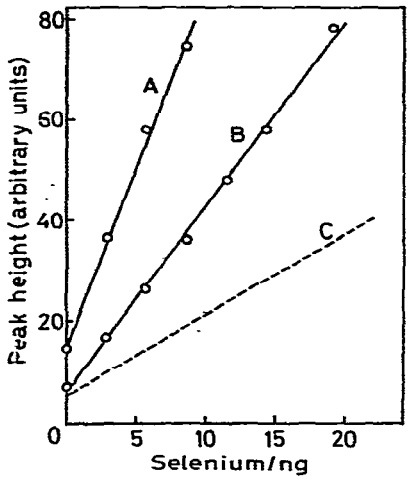


Fig. 4. Calibration graphs for selenium. Sensitivity settings: A, 16 × 0.01 V; B and C, 32 × 0.01 V. ○, 1,2-Diamino-3,5-dibromobenzene. Broken line, 1,2-Diamino-4-nitrobenzene.

Determination of selenium in NBS Bovine Liver, SRM 1577

Trace amounts of total selenium and selenium(VI) in NBS Bovine Liver were determined with 1,2-diamino-3,5-dibromobenzene. The digestion method used was as described earlier[13]. To determine the total selenium, the sample (50 or 100 mg) was digested with 10 ml of concentrated nitric acid until the volume was reduced to about 0.3–0.5 ml and the oxides of nitrogen were decomposed with 3 ml of 1 *M* urea solution. Then, in order to reduce selenium(VI) to selenium(IV), 5 ml of concentrated hydrochloric acid were added and the mixture was kept at 100° for 10 min. To determine the selenium, excluding the sexivalent state, the digestion method was the same as above, except for the treatment by concentrated hydrochloric acid. One tenth of the digestion solution was used for the determination of selenium.

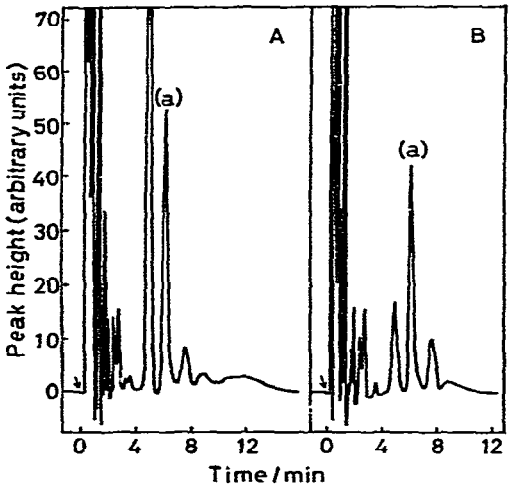


Fig. 5. Determination of selenium in 0.1 g of NBS Bovine Liver. A, Total selenium; B, organoselenium and selenite. (a) = 4,6-Dibromopiazselenol.

TABLE II
SELENIUM CONTENT OF NBS BOVINE LIVER, SRM 1577 *(μg/g)*
Results are the means of five determinations.

	NBS value	*Gas chromatographic method*	
		1,2-Diamino-4-nitrobenzene	*1,2-Diamino-3,5-dibromobenzene*
Selenium (VI)		0.30 ± 0.07	0.31 ± 0.11
Total Selenium	1.1 ± 0.1	1.24 ± 0.04	1.22 ± 0.04

The resultant piazselenol was extracted into 1 ml of toluene, which was washed with 4 ml of 1 *M* sodium hydroxide solution and then with 2 ml of 9 *M* hydrochloric acid. The gas chromatograms obtained are shown in Fig. 5, and the selenium content was calculated from the peak height. 1,2-Diamino-4-nitrobenzene was also used to determine the selenium in the same sample. The chromatograms in Fig. 5 are clearer than those obtained with 1,2-diamino-4-nitrobenzene, because in the latter instance a 10-fold amount of the sample was used without dilution because of its lower sensitivity. The results are shown in Table II. The precision is as good, but the results are higher than the NBS value.

ACKNOWLEDGEMENTS

The author thanks Professor Kyoji Tôei for his encouragement and helpful discussions throughout this work. He also thanks the Ministry of Education for financial support.

REFERENCES

1 J. Hoste, *Anal. Chim. Acta*, 2 (1948) 402.
2 J. Hoste and J. Gillis, *Anal. Chim. Acta*, 12 (1955) 158.
3 H. Ariyoshi, M. Kiniwa and K. Tôei, *Talanta*, 5 (1960) 112.
4 M. Tanaka and T. Kawashima, *Talanta*, 12 (1965) 211.
5 C. A. Parker and L. G. Harvey, *Analyst (London)*, 87 (1962) 558.
6 T. Murakami and E. Ishii, *Kogyo Kagaku Zasshi*, 66 (1963) 1652.
7 S. Nakashima and K. Tôei, *Talanta*, 15 (1968) 1475.
8 Y. Shimoishi and K. Tôei, *Talanta*, 17 (1970) 165.
9 Y. Shimoishi, *Bull. Chem. Soc. Jap.*, 44 (1971) 3370.
10 Y. Shimoishi, *Anal. Chim. Acta*, 64 (1973) 465.
11 Y. Shimoishi, *Bull. Chem. Soc. Jap.*, 47 (1974) 997.
12 Y. Shimoishi, *Bull. Chem. Soc. Jap.*, 48 (1975) 2797.
13 Y. Shimoishi, *Analyst (London)*, 101 (1976) 298.
14 L. Erdey, *Gravimetric Analysis, Part 2*, Pergamon Press, Oxford, New York, Toronto, Sydney, Braunschweig, 1965.
15 L. Barcza, *Mikrochim. Acta*, (1964) 967.
16 P. Cukor and P. F. Lott, *J. Phys. Chem.*, 69 (1965) 3232.
17 L. Barcza, *Mikrochim. Acta*, (1964) 136.
18 M. Gotô and K. Tôei, *Talanta*, 12 (1965) 124.
19 E. Kováts, *Helv. Chim. Acta*, 41 (1958) 1915.
20 J. W. Young and G. D. Christian, *Anal. Chim. Acta*, 65 (1973) 127.
21 T. Stijve and E. Cardinale, *J. Chromatogr.*, 109 (1975) 239.