Luminescence of some Piazselenols

A New Fluorimetric Reagent for Selenium*

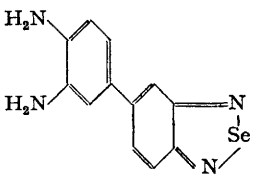
BY C. A. PARKER AND L. G. HARVEY

(*Admiralty Materials Laboratory, Holton Heath, Poole, Dorset*)

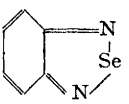
The reaction of selenous acid with three aromatic *ortho*-diamines has been investigated. Selenous acid reacts with 2,3-diaminonaphthalene in acid solution to form the strongly fluorescent 4,5-benzopiazselenol. This compound can be extracted from the acid aqueous phase by organic solvents and used for determining down to 0·002 μg of selenium. As a reagent for the fluorimetric determination of selenium, 2,3-diaminonaphthalene is considerably more sensitive and convenient than the 3,3′-diaminobenzidine previously recommended.

The 3,4-benzopiazselenol formed by reaction with 1,2-diaminonaphthalene is only weakly fluorescent, but, in carefully de-aerated solutions, also emits a phosphorescence band. A similar phosphorescence band is emitted by de-aerated solutions of the unsubstituted piazselenol.

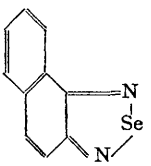
SELENOUS acid reacts with aromatic *ortho*-diamines to produce compounds containing the selenodiazol five-membered ring system. The reaction with an excess of 3,3′-diaminobenzidine to form 3′,4′-diaminophenylpiazselenol (**I**) has been adapted for the absorptiometric[1,2,3] and fluorimetric[4,5] determination of small amounts of selenium. For fluorimetric work of high sensitivity, this reagent suffers from several disadvantages. First, the fluorescence sensitivity of the compound is comparatively low. It has a fluorescence efficiency of only 5·5 per cent. in aerated solution, and the extinction coefficient at the exciting wavelength is also comparatively low, so that the fluorescence sensitivity index is only 0·003 at 2·29 μ^{-1} (436 mμ). As noted previously,[5] the search for an alternative fluorimetric reagent for selenium giving a higher fluorescence efficiency and extinction coefficient could be well worth-while if



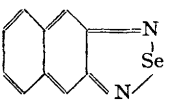
(I)
3′,4′-Diaminophenylpiazselenol



(II)
3,4-Benzo-1,2,5-selenodiazol (piazselenol)



(III)
3,4-Benzopiazselenol



(IV)
4,5-Benzopiazselenol

exceedingly low concentrations of selenium have to be determined. The second disadvantage of 3,3′-diaminobenzidine as a reagent is that it contains two pairs of *ortho*-diamine groups, only one of which reacts with selenium under the conditions of test, so that the resulting piazselenol is still comparatively strongly basic and can only be extracted from aqueous solution into organic solvents by increasing the pH value of the solution. This complicates the analytical procedure in presence of metals whose hydroxides are precipitated in neutral or alkaline solution and necessitates their removal or the addition of suitable complexing

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agents, which have to be purified to a high degree. It would clearly be desirable to use a reagent reacting with selenous acid in acid solution to produce a selenodiazol derivative extractable from acid solution. *ortho*-Diamines containing only two amino-groups produce either neutral or very weakly basic selenodiazols and would therefore be expected to fit these requirements. The luminescence of three such compounds was therefore investigated, namely, those derived from *o*-phenylenediamine, and 1,2- and 2,3-diaminonaphthalene (**II**, **III** and **IV**, respectively). The first of these compounds was used by Ariyoshi, Kiniwa and Toei[6] for the absorptiometric determination of selenium, and the second was prepared by Hinsberg,[7] but the third does not appear to have been reported previously.

Experimental

Apparatus—

For measurement of the luminescence emission spectra, a sensitive recording spectrophosphorimeter[8,9] fitted with an E.M.I. 9558QB red-sensitive photomultiplier was used. This instrument was capable of distinguishing between short-lived fluorescence emission and phosphorescence emission of lifetime longer than about 0·25 milli-second. For measurement of the spectra shown in Fig. 5 (p. 564), the chopper was removed from the beam of exciting light, so that the phosphorescence bands were recorded at their true intensities relative to the fluorescence bands.

Absorption spectra were measured with a Beckman DK2 recording spectrophotometer.

Preparation of selenodiazols—

3,4-*Benzo*-1,2,5-*selenodiazol* (*piazselenol*)—A 0·75-g portion of selenium (as selenous acid) was dissolved in 50 ml of water, and the solution was added to a solution of 1 g of *o*-phenyldiamine in 250 ml of water. The pale buff-coloured precipitate formed was separated by filtration, dried by suction and recrystallised once from water at 70° C. It was then sublimed three times to give colourless needle-shaped crystals melting at 75° C (corrected); Hinsberg[10] recorded a melting-point of 76° C.

Naphtho-1′,2′,3,4-[1,2,5-*selenodiazol*] (3,4-*benzopiazselenol*)—Commercial 1,2-diaminonaphthalene was recrystallised twice from aqueous solution to give silvery leaf-shaped crystals melting at 96° C. A 0·5-g portion of selenium (as selenous acid) was dissolved in 50 ml of hot water, and the solution was added to a solution of 1 g of the recrystallised amine in 250 ml of hot water. The precipitate was separated from the hot solution by filtration, washed with hot water, dried by suction and recrystallised twice from ethanol, yielding pale buff-coloured needles melting at 128° C (corrected); Hinsberg[7] recorded a melting-point of 128° to 129° C.

Naphtho-2′,3′,3,4-[1,2,5-*selenodiazol*] (4,5-*benzopiazselenol*)—Commercial 2,3-diaminonaphthalene was recrystallised from water to give buff-coloured leaf-shaped crystals melting at 190° C (corrected). A 1-g portion of the recrystallised amine was dissolved in 250 ml of 0·1 N hydrochloric acid, and 0·5 g of selenium (as selenous acid) dissolved in 100 ml of 0·1 N hydrochloric acid was added; a bright-red precipitate appeared almost immediately. After 10 minutes, the precipitate was separated by filtration, dried over silica gel and recrystallised from light petroleum (boiling range 40° to 60° C) to give large red needles melting, with decomposition, at about 290° C. Analysis showed the compound to contain 50·3 per cent. of carbon, 2·7 per cent. of hydrogen, 11·8 per cent. of nitrogen and 32·7 per cent. of selenium; the theoretical contents for $C_{10}H_6N_2Se$ are, respectively, 51·5, 2·6, 12·0 and 33·9 per cent. (The selenium content was determined by combustion in oxygen as described previously,[5] but with use of a 500-ml conical flask). Dilute solutions of the compound in cyclohexane or dekalin were stable, but concentrated solutions in toluene deposited a white insoluble substance. This was separated, dried, and analysed; it was found to contain 50·9 per cent. of carbon, 2·7 per cent. of hydrogen, 11·9 per cent. of nitrogen and 32·6 per cent. of selenium. It was concluded that the white compound was a dimer or polymer of the 4,5-benzopiazselenol.

Purification of solvents—

Cyclohexane—The grade sold as "special for spectroscopy" was used without further purification.

Dekalin—Commercial dekalin (boiling range 186° to 194° C) is a mixture of *cis* (b.p. 194° C) and *trans* (b.p. 186° C) isomers and contains appreciable amounts of tetralin (b.p.

207° C) and naphthalene (b.p. 218° C). The boiling-point of tetralin is sufficiently near to that of the *cis* isomer to make purification by fractionation difficult. *cis*-Dekalin can be converted into the *trans* isomer by means of aluminium chloride,[11] and this treatment was adopted before subjecting the liquid to fractionation.

A 500-ml portion of commercial dekalin was stirred in a closed flask with 125 g of anhydrous aluminium chloride for 7 days. The solvent was then decanted and treated for a further 7 days with a fresh 125 g of aluminium chloride; the dekalin was then fractionated in a heated column. About 300 ml of solvent boiling over the range 186° to 188° C were collected. This contained only a trace of tetralin and gave negligible fluorescence when irradiated with light of wavenumber 2·73 μ^{-1} (366 mμ).

Choice of conditions for fluorimetry—

Preliminary tests, in which 100-μg amounts of selenium (as selenous acid) were allowed to react with 100-mg portions of 2,3-diaminonaphthalene in 500-ml volumes of solutions of various acidities, showed that the rate of reaction decreased with increase of acidity and that, for concentrations of hydrochloric acid greater than 0·1 N, reaction was not complete, even after 2½ hours at room temperature.

Further tests were carried out in 1·0, 0·1 and 0·01 N hydrochloric acid at 50° C, the concentration of selenium being decreased to 5 μg per 500 ml. Portions (50 ml) of solution were periodically removed, cooled to 20° C and extracted with 5-ml amounts of cyclohexane; the amount of 4,5-benzopiazselenol was then determined fluorimetrically. With 0·1 N hydrochloric acid, reaction was complete after 15 minutes at this temperature, although still incomplete after 1 hour in N acid solution. It was found that, by increasing the time of reaction to 20 minutes at 50° C in 0·1 N hydrochloric acid, the concentration of reagent could be decreased by one half, reaction of 0·5 μg of selenium per 50 ml of solution still being complete. These conditions were therefore chosen for the fluorimetric determination.

Purification of 2,3-diaminonaphthalene—

It was found that recrystallised 2,3-diaminonaphthalene gave rise to a comparatively high blank fluorescence when treated as described in absence of selenous acid. This fluorescence did not arise from selenium, but from traces of fluorescent impurity present in the reagent and extracted by the organic solvent from solution in 0·1 N hydrochloric acid. To minimise this blank fluorescence, the reagent was freshly prepared each day as described below.

> Recrystallised 2,3-diaminonaphthalene (0·05 g) was dissolved in 50 ml of 0·1 N hydrochloric acid, and the solution was heated at 50° C for 20 minutes. It was then cooled to 20° C, extracted twice with 10-ml portions of dekalin to remove the fluorescent impurities, and spun in a centrifuge. This procedure, and that described below for determining selenium, was carried out in a room lit only by yellow "safe" lights.

Fluorimetric determination of selenium

Volumes of standard solutions of selenous acid in 0·1 N hydrochloric acid, containing between 0·005 and 0·025 μg of selenium, were transferred to separate 60-ml beakers, and each solution was diluted to 45 ml with 0·1 N hydrochloric acid. The beakers were covered with watch-glasses and placed in a water bath maintained at 50° C. After 5 minutes, 5 ml of 2,3-diaminonaphthalene reagent solution were added to the contents of each beaker, and the solutions were mixed and left in the bath for 20 minutes. Each was then cooled to 20° C, transferred to a separating funnel and vigorously shaken with 5 ml of purified dekalin for 1 minute. Each dekalin extract was shaken with two successive 25-ml portions of 0·1 N hydrochloric acid to remove the last traces of reagent, spun in a centrifuge for 2 minutes and transferred to a 1-cm spectrofluorimeter cuvette for measurement. The intensity of the fluorescence excited by light of wavenumber 2·73 μ^{-1} was found to be proportional to the concentration of selenium up to at least 0·5 μg, although the normal range for measurement was 0·002 to 0·03 μg of selenium per 5 ml of dekalin. The reagent blank reading at the wavenumber corresponding to the uncorrected peak in the fluorescence emission spectrum of the selenium compound was equivalent to 0·002 μg of selenium in 5 ml of dekalin. Comparison of the spectra with those obtained from known concentrations of 4,5-benzopiazselenol in dekalin showed that the over-all recovery of selenium was 95 per cent. (see Fig. 1).

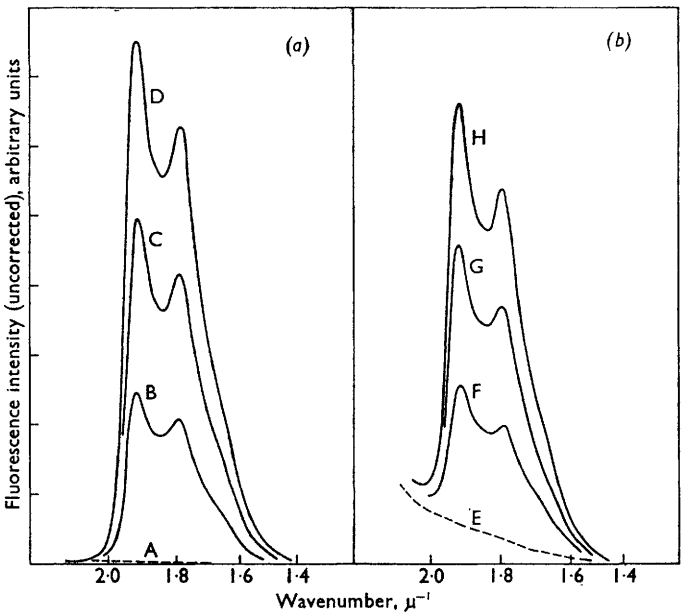

Fig. 1. Fluorescence emission spectra of 4,5-benzopiazselenol in aerated dekalin. (*a*) Pure compound at concentration of: curve A, 0·0 μg per ml; curve B, 0·0053 μg per ml; curve C, 0·0106 μg per ml; curve D, 0·0159 μg per ml. (*b*) Solutions derived from selenous acid by the prescribed procedure and containing selenium equivalent to: curve E, 0·0 μg per ml; curve F, 0·0014 μg per ml; curve G, 0·0028 μg per ml; curve H, 0·0042 μg per ml.

Excitation with 2·73-μ^{-1} (366 mμ) light; half-band width 0·025 μ^{-1} at 2·5 μ^{-1}

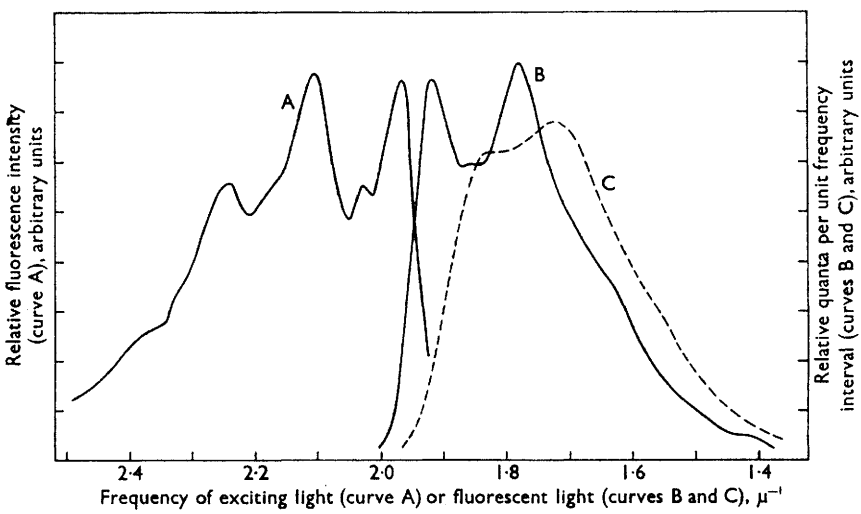

Fig. 2. Corrected fluorescence excitation and emission spectra of 4,5-benzopiazselenol at concentration of 0·7 μg per ml: curve A, excitation spectrum in cyclohexane; curve B, emission spectrum in cyclohexane; curve C, emission spectrum in toluene containing 10 per cent. v/v of cyclohexane.

Excitation with 2·73-μ^{-1} (366 mμ) light for curves B and C; half-band width 0·025 μ^{-1} at 2·5 μ^{-1}

Absorption and fluorescence spectra

Preliminary examination showed that solutions of 4,5-benzopiazselenol were far more fluorescent than those of the other selenium compounds. Tests were first made in toluene, as this solvent had proved satisfactory for extracting the piazselenol derivative previously investigated.[5] In toluene, or toluene containing a small amount of cyclohexane, the 4,5-benzopiazselenol exhibited yellow fluorescence (see Fig. 2, curve C), but the solutions were not stable and deposited a white amorphous solid that appeared to be a polymer (see p. 559). It seemed that some kind of association effect occurred in the toluene solutions, even before appreciable decomposition had taken place, since the absorption and fluorescence spectra in this solvent were much more diffuse and shifted to lower wavenumbers compared with the spectra of solutions in cyclohexane. In the latter solvent, the compound exhibits green fluorescence (see Fig. 2, curve B), the fluorescence emission spectrum showing fine structure similar to the rather weak low-frequency absorption band occurring in the blue region of the spectrum in this solvent (see Fig. 3, curve C). A much more intense absorption band

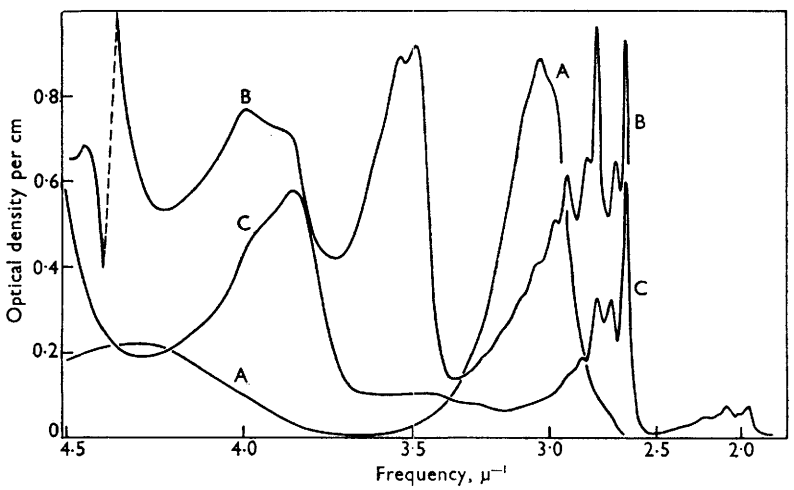

Fig. 3. Absorption spectra of piazselenols in cyclohexane: curve A, 9·5 μg of piazselenol per ml; curve B, 14·9 μg of 3,4-benzopiazselenol per ml; curve C, 4·4 μg of 4,5-benzopiazselenol per ml

appears in the near-ultraviolet region and yet a third band in the middle-ultraviolet region (see Fig. 3, curve C). Excitation in all three absorption bands gives rise to fluorescence, and the complete excitation spectrum (Fig. 4) is similar in shape to the absorption spectrum.

In contrast, the fluorescence from the other two compounds (**II** and **III**) was weak. **3,4**-Benzopiazselenol gave rise to a recognisable emission spectrum when excited with light of wavenumber 2·73 μ^{-1} (366 mμ). Piazselenol did not absorb appreciably at 2·73 μ^{-1} and, when excited with light of wavenumber 3·19 μ^{-1} (313 mμ), gave only very weak fluorescence that may have arisen from traces of impurity.

The fluorescence efficiencies of the three compounds, determined by comparision with a solution of quinine bisulphate in 0·1 N sulphuric acid, are compared in Table I with that of the piazselenol derivative formed from 3,3′-diaminobenzidine, which was previously used in determining selenium. Values of the fluorescence sensitivity index[12] are also shown in Table I; the sensitivity attained for 4,5-benzopiazselenol with excitation at 2·73 μ^{-1} was some twenty times greater than that attained for 3′,4′-diaminophenylpiazselenol.

Owing to its high volatility, cyclohexane is not an ideal solvent for use in a method involving extraction, particularly if experiments including de-aeration by passage of nitrogen are contemplated. Dekalin is much less volatile, and absorption and fluorescence spectra in this solvent are similar to those in cyclohexane. This solvent was therefore chosen for the fluorimetric method ultimately adopted. The fluorescence efficiency of 4,5-benzopiazselenol in dekalin is shown in Table I. Dekalin has the further advantage over cyclohexane that the quenching of fluorescence by air is less.

Oxygen quenching of luminescence

De-aeration of solutions of 4,5-benzopiazselenol was found to increase the intensity of the fluorescence band by a factor of about 3 (in cyclohexane) or 2 (in dekalin), without otherwise changing its form. The weak fluorescence band of 3,4-benzopiazselenol was unaffected by de-aeration, but a new band, completely absent with aerated solutions, appeared

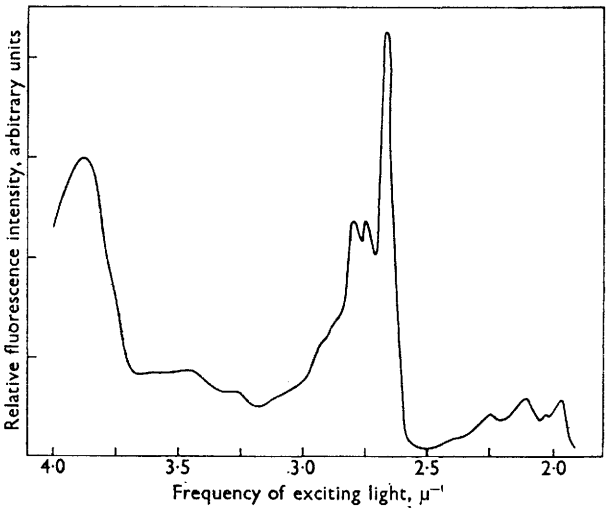

Fig. 4. Corrected fluorescence excitation spectrum of 4,5-benzopiazselenol in cyclohexane at concentration of 1μg per ml. Half-band width of excitation monochromator 0·02 μ^{-1} at 2·5 μ^{-1}

in the red region. A similar band appeared when solutions of piazselenol were completely de-aerated (see Fig. 5). The quenching effect of oxygen on the latter two bands was so great as to suggest that their origin was an excited state of extremely long lifetime. This was confirmed by taking measurements with the choppers of the spectrophosphorimeter out of phase. The red bands were found to correspond to phosphorescence having a lifetime of about 0·2 to 0·3 milli-second. The phosphorescence efficiencies were determined by comparison with solutions of quinine bisulphate and are shown in Table I.

Table I

Luminescence results for piazselenols

Fluorescence efficiency	Fluorescence sensitivity index $\left(\frac{\phi D}{H}\right)$	Phosphorescence efficiency	Phosphorescence sensitivity index $\left(\frac{\phi D}{H}\right)$	Air-quenching factor for fluorescence	Excitation frequency, μ^{-1} (mμ)	Fluorescence emission maximum, μ^{-1}	Phosphorescence emission maximum, μ^{-1}
Piazselenol in cyclohexane—							
Very small	Very small	0·0009	0·00012	—	3·19 (313)	—	1·51
3,4-*Benzopiazselenol in cyclohexane*—							
0·0014	0·00017	0·0052	0·00048	1·0	2·73 (366)	2·50	1·66
4,5-*Benzopiazselenol in cyclohexane*—							
0·52	0·12*	—	—	3·1	2·73 (366)	1·92, 1·78	—
4,5-*Benzopiazselenol in dekalin*—							
0·46	0·094*	—	—	2·1	2·73 (366)	1·92, 1·78	—
4,5-*Benzopiazselenol in toluene containing* 10 *per cent. of cyclohexane*—							
0·53	0·097	—	—	2·8	2·73 (366)	1·72	—
3′,4′-*Diaminophenylpiazselenol in toluene*—							
0·076	0·0045	—	—	1·4	2·29 (436)	1·65	—

* At 1·92 μ^{-1}.

Although the phosphorescence efficiencies are comparatively low, the use of these compounds for determining trace amounts of selenium might have some advantages, because, if a phosphorimeter were used to measure the phosphorescence, all scattered light and fluorescence from traces of impurities in the solution could be completely eliminated,[9] and the blank value arising from other fluorescent impurities would therefore be extremely low. This possibility was not further investigated, since it was found that sufficiently high efficiency and sensitivity could be attained by using the fluorescence of 4,5-benzopiazselenol.

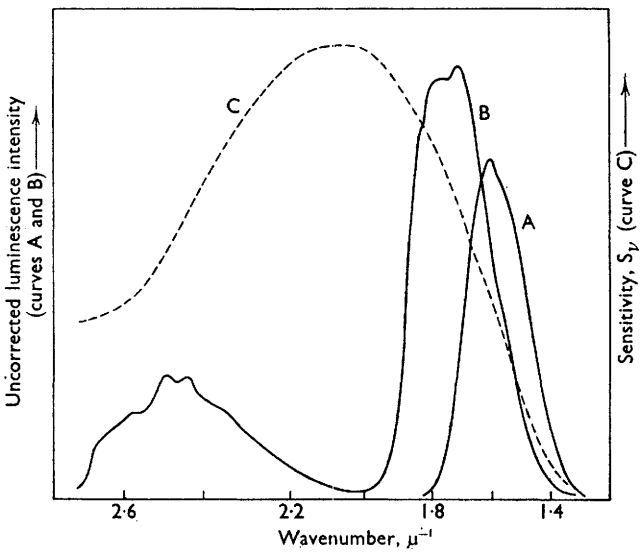

Fig. 5. Luminescence emission from piazselenol (4·4 μg per ml) and 3,4-benzopiazselenol (5·7 μg per ml) in cyclohexane at 20° C. Curve A, phosphorescence band of piazselenol: excitation with 3·19-μ^{-1} (313 mμ) light; half-band width 0·066 μ^{-1} at 2·5 μ^{-1}. Curve B, phosphorescence and fluorescence bands of 3,4-benzopiazselenol: excitation with 2·73-μ^{-1} (366 mμ) light; half-band width 0·018 μ^{-1} at 2·5 μ^{-1}. Curve C, sensitivity (S_ν) curve of quartz-prism spectrometer fitted with E.M.I. 9558QB photomultiplier

Determination of selenium with 2,3-diaminonaphthalene

2,3-Diaminonaphthalene will react with traces of selenium in both acid and neutral solution, but the rate of reaction decreases as the acidity is increased. Rates of reaction were determined at various pH values, and a concentration of 0·1 N hydrochloric acid was finally chosen for the method. At this concentration, the rate of reaction is sufficiently rapid; at the same time, the acidity is sufficient to keep most metals in solution. After reaction, the 4,5-benzopiazselenol can be extracted into a small volume of dekalin.

The factor limiting sensitivity was the blank fluorescence arising from traces of impurity in the 2,3-diaminonaphthalene reagent. This blank value was minimised by recrystallisation of the reagent in the dark and by pre-extraction with dekalin of a solution of the reagent in 0·1 N hydrochloric acid. To minimise subsequent decomposition, the entire procedure for determining selenium was carried out in a room illuminated by only yellow safe lights. The fluorescence spectra of solutions derived from the reaction of small amounts of selenium, together with that of a typical blank solution, are compared in Fig. 1 with the spectra of dilute solutions of pure 4,5-benzopiazselenol in dekalin. The residual blank fluorescence (Fig. 1, curve E) was due to traces of organic impurity, and, at the frequency of the peak in the fluorescence spectrum of the selenium compound, had a value corresponding to about 0·002 μg of selenium. This blank value was reproducible, and it was estimated that a weight of selenium equivalent to the blank value could reliably be detected.

The solutions yielding the spectra shown in Fig. 1 were not de-aerated. De-aeration increases the fluorescence of the selenium compound by a factor of 2, and it might therefore be expected that the relative magnitude of the blank fluorescence arising from impurities would be correspondingly decreased. Unfortunately, de-aeration produced some increase in the fluorescence of the impurity, and it was decided that the inconvenience of de-aeration was not justified by the relatively small over-all gain in sensitivity. The use of air-saturated solutions was therefore adopted in the final procedure.

Interfering elements

Preliminary tests have shown that, if the experiment is carried out in the presence of ethylenediaminetetra-acetic acid, relatively few elements interfere. A more detailed investigation of interfering elements is in progress, and it is hoped that the results will be reported later, with an account of the use of the method for determining traces of selenium in high-purity gallium arsenide.

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