THE DETERMINATION OF SELENIUM AND CHROMIUM IN NATURAL WATERS.

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by David James Harper.

July 1983.

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Methods are described for the determination of all dissolved forms of selenium at low and high levels in river, estuarine and sea waters. In the methods investigated, substituted piazselenols are formed from selenite and substituted 1,2,-diamino-benzenes and extracted into solvents ready for analysis by fluorimetry (Whitelaw, 1975) or gas chromatography using electron capture detection (Shimoishi and Tôei, 1978, Measures and Burton, 1980a). The use of 1,2-diamino-3,5-dibromo-benzene (D.A.D.B.B.) and 4-nitro-1,2-diamino-benzene (D.A.N.B.) with G.C./E.C.D. analysis gave detection limits of $1ngSe^{4+}l^{-1}$ and $6ngSe^{4+}l^{-1}$ and coefficients of variation of 0.5 and 1.0 at $180ngSe^{4+}l^{-1}$ respectively. The use of diamino-naphthalene as a reagent for fluorimetric analysis of selenite was also investigated. A detection limit of $50ngSe^{4+}l^{-1}$ was achieved in distilled water. However, severe interference from Fe (III), NO_3^- and NO_2^- limited the usefulness of the method to concentration of above $1ugSe^{4+}l^{-1}$.

Other forms of selenium were determined by conversion to selenite followed by the D.A.D.B.B./G.C. analysis, concentrations being estimated by subtraction. Selenium (0) and (-II) were oxidised by bromine and total selenium was converted to selenite by reduction with hydrobromic acid and oxidation with bromine. These procedures did not affect the limit of detection or reproducibility of the D.A.D.B.B./G.C. method. All organo-selenium compounds were decomposed by ultra violet irradiation.

Methods are also described for the determination of low levels of dissolved chromium (both Cr^{3+} and total Cr) and particulate chromium in river, estuarine and coastal waters. The methods of Cranston and Murray (1980) have been investigated. In that for total chromium the sample was treated with hydrous iron (II) oxide suspension which reduces Cr^{6+} to Cr^{3+}. This was subsequently adsorbed by hydrous iron (III) oxide, formed by aerial oxidation. The precipitate was recovered by filtration, dissolved in hydrochloric acid, and chromium was determined by electrothermal atomic absorption spectrophotometry. Chromium(III) was determined by carrying out the same procedure using hydrous (III) oxide suspension in place of that of iron (II). The slightly modified method was found to give a within batch standard deviation of $18ng\ l^{-1}$ for total chromium at a level of $250ng\ l^{-1}$ in tap water and a limit of detection of $32ng\ l^{-1}$. Organo-chromium compounds can be decomposed by using ultra violet irradiation as a pretreatment stage.

The methods were applied to a survey of the Loughor estuary in South Wales.

Particulate chromium was determined by prolonged digestion of the membrane filter bearing the particulates with a mixture of nitric and hydrofluoric acids followed by flame of electrothermal A.A.S.. The method gave within batch standard deviations of $1.7\mu gCr\ g^{-1}$ for a Mersey mud ($90\mu gCr\ g^{-1}$) and $10\mu gCr\ g^{-1}$ for Mersey suspended matter ($163\mu gCr\ g^{-1}$) when the filtration stage was included.

ACKNOWLEDGMENTS.

I would like to thank all those members of university staff and students who have helped me in the course of this work. In particular, Jim Murphy and Bob Tennant for their invaluable technical assistance, Mrs Wainwright and Mrs Smith for handling the administration and John Towner, Kevin Murphy and Gordon Sanders for advice, encouragement and putting up with me.

Grants from the Department of the Environment and the Water Research Centre were gratefully received.

Finally, I would like to thank Prof. J.P. Riley for the consistent support, advice and encouragement I received whilst under his supervision.

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CHAPTER 1.

1. Introduction.

1.1. Description of Selenium.

Selenium was discovered in 1817 by Berzelius. As its general chemistry resembled that of tellurium (ex-Greek Tellus-Earth) it was named selenium (ex-Greek Selens-Moon). In nature, it is one of the more widely distributed elements and is one of the rarest of the essential elements (Crustal concentration 0.05$\mu g\ g^{-1}$, Taylor,1964).

The recent discovery of its importance as both an essential nutrient at low levels and a toxic material at higher levels has stimulated considerable interest in its environmental and geochemical cycles.

1.2. Geochemistry of Selenium.

1.2.1. Behaviour in Crustal Material.

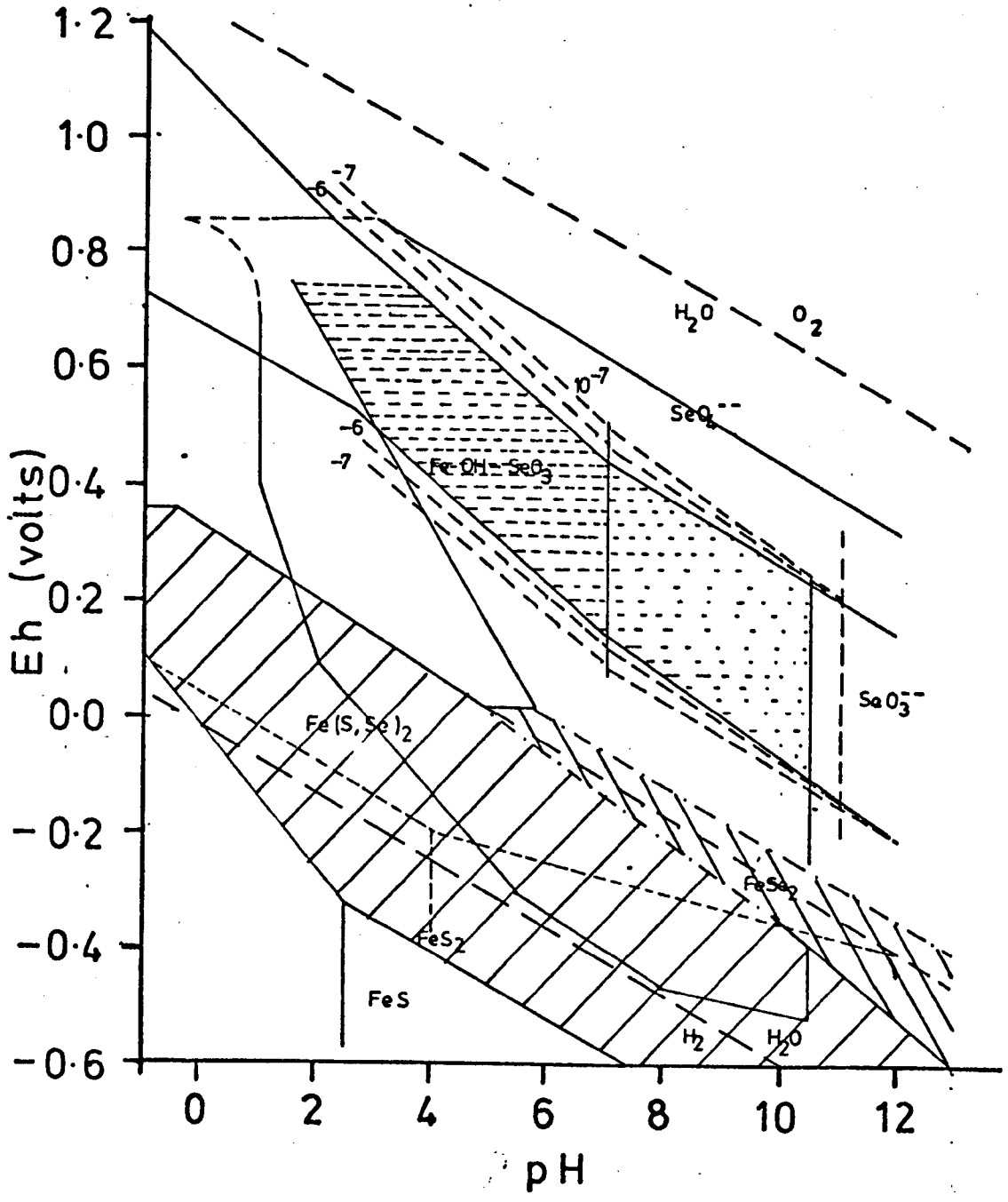
During the crystallisation of magma, selenium exhibits a behaviour roughly analagous to that of sulphur. It separates from the main body of the cooling magma along with sulphides of iron, cobalt, lead, uranium and some other metals, which escape as a supercritical solution in water or is given off as gaseous hydrogen selenide (Goldschmidt, 1954).

The supercritical aqueous solutions then flow through the surrounding rock and, on cooling, start to deposit minerals. If the solution contains sufficient sulphides for them to precipitate at high temperatures in reducing conditions, selenides will be associated with them. However, at lower temperatures and higher redox potentials there is an increasing

Caption for Fig. 1.

Eh-pH diagram of the predominant Se species in earth-surface aqueous environments. $FeSe_2$ (Ferroselite) field calculated for ΔG^o of -23.2h cal^{-1} $mole^{-1}$. Long dash-short dash line within Ferroselite field is $FeSe_2/Fe_2O_3$-Se^o boundry Dash lines parallel to Se (0)-Se (IV) and Se (IV) -Se(VI) boundries show displacement of these couples by adsorption on ferric hydroxide; vertical dashed lines bound a "transitional" zone of decreasing adscrption with increasing alkalinity. Short dash line within pyrite field represents Se^o-H_2Se and Se^o-HSe^- couples. Fe^{2+} is 10^{-3}M; ΣS, 10^{-1}M; and Σ Se, 10^{-5}M. (Howard, 1977).

Figure 1.



Eh/pH Diagram of Predominant Se Species in Water.

tendancy for selenide minerals to form before the sulphide minerals. In these cases selenides are often to be found associated with calcite, haematite and barite (D'yachkova et al, 1968).

The subsequent behaviour of selenium is determined by a combination of redox potential and the pH of the local aqueous environment. The sulphide body must be oxidised before selenium can be released. At low potentials, ferroselites and elemental selenium will be formed (Howard, 1969). However, at higher potentials it will be associated with hydrous ferric oxides as adsorbed selenite. Increasing alkalinity (greater than pH8) will lead to increasing desorption of selenite, which becomes quantitative at pH values over 11 (Howard, 1977) (vis Fig. 1.).

The latter effects have been well recorded in connection with "roll-type" uranium deposits (Howard, 1977, Lakin and Trites, 1958). Uranium and ferric selenides undergo repeated oxidation and reduction as they are mobilised resulting in the concentration of selenite minerals up to a level of up to 1000$\mu g\ g^{-1}$ in the surrounding strata.

Howard (1977) has presented a comprehensive thermodynamic description of this section of the geochemistry of selenium.

1.2.2. Behaviour in Fresh Water.

The leaching of selenium by ground water is dependant upon the iron content and the alkalinity of the soil in the drainage area.

Soil profiles by Slater et al (1937) have shown that

alkaline conditions in soils promote the oxidation of selenite (SeO_3^{2-}) to the more soluble selenate (SeO_4^{2-}) and, as selenate is in lower demand by plants than selenite, it will be leached far more easily by rain water. If, however, the soil is more acidic, the selenite is trapped in the soil/plant cycle due to its comparative lack of solubility and greater biotic demand.

The geochemistry of selenium, once in solution, is controlled by the local pH and Eh conditions and by the iron content. Hingston et al (1968) showed that selenite is specifically adsorbed by synthetic geothite, a hydrated ferric oxide mineral. The extent of the adsorption follows the equation:-

$$\%Se_{ads} = \frac{T.S.W}{V.Co} \times 100$$

where:- T = Adsorbed selenite, in moles m^{-2} surface.

S = Specific area of adsorbing solid, in $m^2\ g^{-1}$.

W = Weight of solid, in g.

V = Volume of Solution, in litres.

Co= Initial selenite concentration, in moles l^{-1}.

This equation applies over a pH range of 1-8,above which the adsorption capacity drops away sharply so that at pH 9.5 the capability drops by 50%.

Howard (1971) carried out a comparative study of adsorption of selenium by hydrated ferric oxides, kaolinite, mont-

morillonite and particulate organic material in fresh water. The ferric oxides were shown to scavenge one or two orders of magnitude more selenium than the clays. Particulate organic material was found to adsorb no dissolved selenium.

Measures and Burton (1978) made a comparative study of the behaviour of selenium in rivers of varying acidity and iron content. The River Beaulieu, an organic rich, acidic ferruginous river, was shown to have a low selenium content (about 80ngSe l^{-1}). This isprobably due to the acidic, iron rich nature of the drainage area. It was also found that the selenite content was below the detection limit of the analytical method used (about 2ngSe l^{-1})(Measures and Burton, 1980a).

This indicates that any selenite being introduced is being scavenged by hydrous iron oxides. However, the River Test and others flowing from chalky, more alkaline areas were shown to have a far higher total selenium content (about 370ngSe l^{-1}) with selenite accounting for about 7% of the total.

These results, together with those obtained by Kharkar et al (1968) for a number of major rivers, suggest an average total selenium content of about 200ng l^{-1} with a range of 75 - 400ng l^{-1}. Results for various Italian rivers have been given as considerably lower than these values (Dall' Aglio et al, 1978). However, as no record of the period or method of sample storage is given it is difficult to comment on them.

The highest recorded concentration of selenium in fresh water was recorded in the Animas River, Colorado where the level has reached 400µg l^{-1} (Scott and Voegli, 1961).

1.2.3. Behaviour in Estuaries.

Concentrations of total selenium in the estuarine zones of the River Beaulieu and the River Test (vis Sect. 1.2.2.) were also recorded by Measures and Burton (1978). The River Test, with a low loading of particulate material, showed a conservative decrease in dissolved selenium with increasing salinity. It can, therefore, be assumed that the only process controlling the concentration of selenium in this river is dilution.

The River Beaulieu, however, presented a more complex situation. Unfortunately, no data were collected between salinities of $0^{o}/_{oo}$ and $13^{o}/_{oo}$. At $13^{o}/_{oo}$ the concentration was higher than that of either end member after which it fell conservatively to sea water concentrations. It is likley that the behaviour between 0 and 13 $^{o}/_{oo}$ is similar to that of arsenic in rivers with a high particulate loading. A maximum is reached at about $5^{o}/_{oo}$ as adsorbed oxyanions are released and then conservative mixing takes place (Waslenchuk, 1979).

Sugimura et al, (1976) traced the total selenium content in an estuary in which the concentration in the river water was less than that in the sea water. The nature of the river is not reported but as the selenium concentration is so low it is reasonable to assume that it is of similar character

to the River Beaulieu. No change in concentration was found between salinities of $0^{o}/_{oo}$ and $20^{o}/_{oo}$ after which a sharp increase to sea water concentrations occurs.

The reason for this behaviour is uncertain but may be partially attributable to the analytical technique used (vis Sects. 2.2.2. and 2.3.1.).

Before the geochemistry of selenium in estuaries can be understood much more data are required and information about the form of the element and the extent to which it is adsorbed by the colloidal fraction in relation to the pH, Eh and ionic strength are required.

1.2.4. Behaviour of Selenium in Sea Water.

Sillen (1961) has suggested that the speciation of selenium in sea water is dependent upon the local redox conditions and that it might serve as a redox indicator. This theory was based on the oxygen-water couple:-

$$\tfrac{1}{2}O_2\ (aq.) + 2H^+ + 2e^- \rightleftharpoons H_2O \qquad \text{Log } K = 41.55 \ (25^{o}C)$$

At a pH of 8.1 the pE, in oxygen saturated water is 12.5. Under these conditions selenate should be the dominant species.

However, when analytical methods, apparently capable of determining the speciation of selenium, were developed (Chau and Riley, 1965, Shimoishi, 1973.) only selenite was found. Chau and Riley (1965) explained this as the result of kinetic, as opposed to thermodynamic, effects.

More recent methods (Sects. 2.3.3., 2.3.4.,.2.3.5. and 2.3.6.) have been developed that are capable of evaluating both selenite and selenate. The few results which have been obtained for open ocean waters have proved selenium to have a unique redox chemistry.

Results obtained by Sugimura et al (1976), Measures and Burton (1980 b) and Measures et al (1980) for North West Pacific, North East Atlantic and East Pacific respectively. Beneath a minimum in the total selenium in the photic zone there is a sharp rise to a maximum at the oxygen minimum in the Pacific, and then a gentle rise into deep water. In the Atlantic the upper layers were found to be almost devoid of selenite, whereas it comprised a major part of the total in the Pacific. This is consistent with different nutrient chemistry in the different areas. Selenate contributed considerably more to the maximum at the oxygen minimum than did selenite. The steady rise to deep water concentrations is mainly due to selenate. Any variation from this general pattern can be explained by the presence of different water masses.

In general a good correlation has been found between selenite and silicon profiles and selenate and silicon/phosphorous profiles. This implies two processes are taking place.

Silicon profiles represent the slow, non-oxidative dissolution of the refractory phases showing that the major downward flux of selenite is associated with the tests of the

biota (Measures and Burton, 1980 b). However, Fowler and Benayoun (1976) have shown that selenite is preferentially taken up by tissue and is reduced to selenide (Se -II), replacing the -SH in amino acids, such as methionine. Upon decay this will be oxidised up to both selenite and selenate. Any scavenging of selenium by hydrous iron oxides will also remove selenite.

It must be assumed, therefore, that reduction of selenate occurs in deep water or that selenite, being formed in the photic zone, is immediately incorporated into the refractory phases (Measures et al, 1980).

The data, so far presented, suggests that the element does not act in a manner consistent with its thermodynamic properties, partly because of kinetic effects, but mainly as a result of its involvement in the biosphere.

1.2.5. Behaviour of Selenium in Marine Sediments.

Information on the behaviour of selenium in marine sediments is very limited, mainly because of the problems associated with storage. Unless the sample is highly oxygenated all the selenium can be lost in as little as 24 hours unless it is stored in 1M nitric acid.

An extensive survey of the Black Sea sediment has, however, been carried out (Volkov and Sokolova, 1977). The concentration of selenium in the total sediment varied from an average of 0.25$\mu g\ g^{-1}$ in sands and coquinae to 2.4$\mu g\ g^{-1}$ in limy-clay muds. In the non-carbonate fraction the concentrations were 0.4 and 6.0$\mu g\ g^{-1}$ respectively. These data confirm the findings of Rankama and Sahama (1950) and Vorobjew (1969) that

selenium is enriched in clay sediments.

Volkov and Sakolova (1977) found that selenium was enriched only to a minimal extent in oxidised sediments and in iron-manganese concretions (1-1.3$\mu g\ g^{-1}$) in which it appears to be in the form of selenate weakly bound to the iron hydroxides. The same authors have explained this as an effect of an increase in acidity.

In anaerobic sediments there was a strong correlation between selenium maxima and those of organic carbon, pyritic sulphur and total reduced sulphide, though it seems most likely that its behaviour is closest to that of pyritic sulphur, especially iron and copper pyrites (Leutwein and Stark, 1957).

1.3. Anthropogenic Sources.

The greatest man-made sources of selenium to the environment are the manufacture of concrete and the combustion of fossil fuels (coal contains up to 8$\mu g\ g^{-1}$ and oil up to 0.4$\mu g\ g^{-1}$) which Bertine and Goldberg (1971) have estimated together to introduce 1.1×10^{9}gSe yr^{-1}. Although of little global significance there are some point sources that have local effects, the most important of which are the smelting of sulphide ores (vis Sect. 1.2.1.), the manufacture of some sulphur compounds and the dumping of sulphuric acid. There are probably also inputs from those industries using selenium compounds. These include the manufacture of selenium oxychloride, selenium rubbers, photo-electric cells and glass. However, no global estimation of these inputs have been made.

1.4. Total Cycle in Sea Water.

Three major sources of the element to the sea have been identified. Kharkar et al (1968) and Measures and Burton (1978) agree that about 7.2×10^{-9}gSe yr^{-1} is introduced by rivers on the basis of the average concentration of total dissolved selenium in river water. A further 1.1×10^{9}gSe yr^{-1} is introduced from anthropogenic sources (Sect. 1.3.) and about 1.2×10^{9}gSe yr^{-1} from volcanic activity (Rankama and Sahama, 1950). These give a total input of 9.5×10^{9}gSe yr^{-1}.

Measures et al (1980) calculated that some 3×10^{9}gSe yr^{-1} is removed from the upper layer of the oceans by the biota. A steady state, therefore, requires the additional removal of 6.5×10^{9}gSe yr^{-1} by adsorption onto clays and iron and manganese oxides.

1.5. Role of Selenium in the Environment.

1.5.1. Selenium as a Nutrient.

Plants will take up any form of selenium although selenite is preferred (Johnson et al, 1967). Once incorporated it is associated with the amino acid fraction, mainly as selenomethionine, and with various enzymes including glutathione peroxidase (Shamberger, 1981). Certain plants, such as Astragalus, can accumulate high levels of the element (up to 1000μgSe g^{-1}) and can constitute a severe hazard to grazing animals (Harvath, 1976). It has also been shown that an inverse relationship exists between selenium intake and the occurrence of some forms of cancer (Shamberger, 1981). Certain forms of heart disease appear to be discouraged by a seleniferous diet, especially hypertension caused by cadmium at

sub-lethal levels (Perry and Erlanger, 1977).

Animals require selenium for both the formation of various amino acids and enzymes and as an anti-oxidant. For this reason the amount of selenium required in a diet depends on the form of the element and the composition of the rest of the diet. The recommended intake for man is between 100 and 200$\mu g\ dy^{-1}$.

In animals, the lack of selenium can lead to various symptoms depending on the species, thus lambs suffer a form of muscular dystrophy (white muscle disease) and hens suffer retarded growth (Harvath, 1976).

1.5.2. Selenium and Mercury in Nature.

Parizek and Oslodolova (1967) discovered the first indications that selenite is antagonistic to both inorganic and organic forms of mercury. Since then it has been found that as the mercury content, of both mammals and fish, increases so does the retention of selenium from the diet. The normal Hg:Se ratio is 0.002-0.02 but at high mercury concentrations this ratio increases to 0.5-1.6 (Bernhard, 1981). The reduction in mercury toxicity has been attributed to the formation of a 1:1 mercury/selenium complex (Beijer and Jernelöv, 1978). It appears to be especially effective against the effect of methyl mercury.

1.5.3. Toxicity of Selenium.

The majority of the reported cases of selenium poisoning arise in naturally seleniferous areas. The earliest known occasion was reported in 1290ad when Marco Polo wrote that

his pack animals were sloughing their hooves in Turkestan. Contemporary cases of this in Colorado and western Australia have been well reported (Shamberger, 1981).

Cattle have been found to lose hair, slough hooves and suffer cirrhotic livers and sheep mortality has been reported in areas where the grazing contained 16$\mu g\ g^{-1}$ selenium (dry weight). The toxicity varies widely with species, the form of selenium and according to other elements of the diet. However, in general, the toxic level in food and grazing can be taken to be between 5 and 15 $\mu g\ g^{-1}$ and in drinking water as 10$\mu g\ l^{-1}$ (U.S. Dept. of Health and Education and Welfare, 1962)

The physiological effects of excess selenium in man include lassitude, depression, dermatitis, gastric disorders and bad teeth (Harvath, 1976).

The reasons for the toxicity are unknown but it is possible that the removal of sulphydryl groups by the selenium may lead to morbidity by preventing oxidative processes.

CHAPTER 2.

2. Methods for the Analysis of Selenium in Natural Waters.

2.1. Preconcentration.

A considerable number of methods have been described for the separation of selenium, several of which could be used for the concentration of the element from waters. Such methods include; co-precipitation, distillation of the bromide, evolution of gaseous hydrogen selenide, retention of complexes on macroreticular resin and solvent extraction of organic derivatives.

2.1.1. Precipitation in the Elemental Form.

Vogel (1951) has described a gravimetric method for the determination of selenium as dissolved selenite and selenate. In this the oxyanions are reduced to the element in 8M hydrochloric acid by the addition of sulphur dioxide. On heating, the amorphous, red allotrope formed is converted to the grey, consolidated form which is filtered and weighed. Although not sensitive enough for use at environmental concentrations the method was found to be a convenient method of determining standards (Sect. 3.1.2.).

2.1.2. Co-precipitation.

2.1.2.1. Iron Hydroxide.

In natural systems iron hydroxides are responsible for the scavenging of a large proportion of dissolved selenite. A number of workers have used this principle to preconcentrate selenite from natural waters (Ishibashi et al, 1967, Chau and Riley, 1965, Whitelaw, 1975.).

Iron (III) chloride is added to the sample and the iron is precipitated as the hydroxide by adjusting the pH of the solution to between 4 and 6. Although the iron can, subsequently, be largely removed by ion exchange (Chau and Riley, 1965, Whitelaw, 1975) or by separation of the selenium as the bromide by distillation of the bromide (Johnson, 1958), residual quantities can interfere seriously with some methods of determination.

2.1.2.2. Copper/A.P.D.C. Complex.

See Section 6.4..

2.1.3. Evolution of Hydrogen Selenide.

Selenite can be reduced in the presence of nascent hydrogen to give hydrogen selenide. In practise the reduction is usually carried out by the addition of sodium borohydride to the sample, (made 4M with respect to hydrochloric acid.). The hydrogen selenide is then stripped from the solution by the excess hydrogen formed and can be trapped either in a liquid nitrogen cold trap (Cutter, 1978), or by bubbling through an I_3^- solution (Maher, 1980.). Subsequent determination can be carried out by atomic absorption spectrophotometry (Goulden and Brooksbank, 1974, Cutter, 1978, Maher, 1980, Cheam and Agemien, 1980.), or inductively coupled plasma emission spectrometry (Pahlavanpour et al, 1980.).

This method of preconcentration was tested in this work and the results are recorded in Section 6.1..

2.1.4. Distillation of Selenium Tetrabromide.

Selenium tetrabromide can be distilled by boiling the sample with sulphuric acid, potassium bromide and sodium

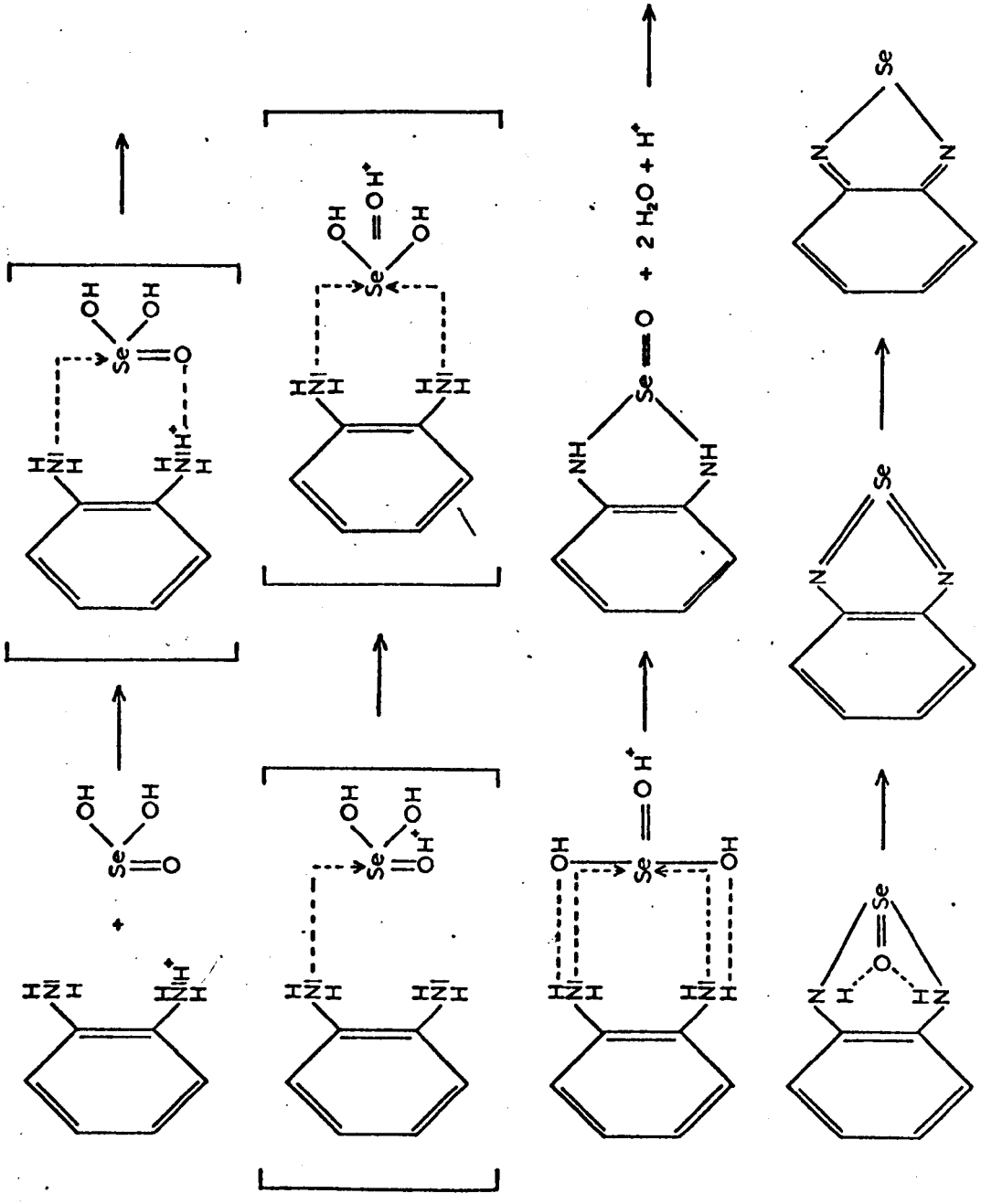


Fig. 2. Reaction scheme for formation of piazselenols.

hypochlorite. The bromide is collected by condensation in a cold trap of sulphuric acid (Johnson, 1958).

2.1.5. Retention of Complexes on a Macroreticular Resin.

The macroreticular resin, Amberlite X.A.D. 2 has been found to retain the complex formed between selenite and sodium diethyl-dithiocarbamate (D.D.T.C.) (Sugimura and Suzuki, 1977.). The selenium can then be eluted with a mixture of 2M nitric acid and concentrated perchloric acid (19:1) and, after evaporation and dissolution in a suitable medium, is ready for analysis. A study of this method is recorded in Section 6.2..

2.1.6. Solvent Extraction of Organic Derivatives.

The most useful organo-selenium compounds, for the aspect of analysis, are the piazselenols, which are formed when the selenite ion reacts with a 1,2-diamino-aromatic compound. The reaction is, to all extents and purposes, unique to selenite. Piazselenols also tend to be fairly insoluble in water, but soluble in solvents such as toluene or cyclohexane. These factors make piazselenols ideal for the separation and preconcentration of dissolved selenium. As most substituted piazselenols can be formed easily a reagent can be tailor-made to suit a subsequent method of determination.

As these compounds were used extensively in the present work it is useful to discuss their formatiom in some detail.

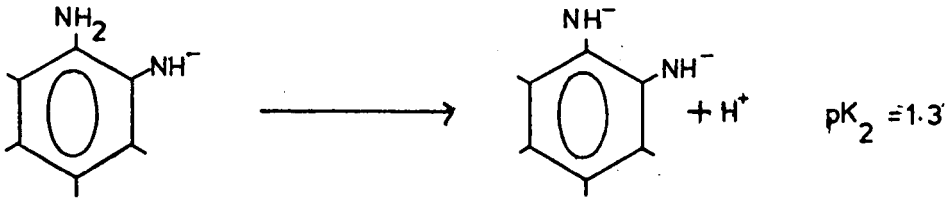
2.1.6.1. The Formation of Piazselenols.

Hinsberg (1889) noted that, in mildly acidic media, a pH dependent reaction took place between 1,2-diamino-benzene and selenite, this lead to the formation of piazselenol. The conditions for the reaction were investigated by Ariyoshi et al

(1960), who found that, for maximum efficiency, the pH conditions must be such that the selenious acid is predominantly undissociated;

$$H_2SeO_3 \text{ ---------- } H^+ + HSeO_3^- \qquad pK_1 = 2.6.$$

and the 1,2-diamino-benzene is in its monoprotonated state.



It is clear, therefore, that, for an efficient reaction between selenious acid and 1,2-diamino-benzene, the pH of the solution must be between 1.3 and 2.6. The precise mechanism of the reaction was subsequently studied by Bareza (1964), who showed that it proceeds as is shown in Figure 2.

Substitution of different groups on the diamino compound may lead to considerable changes in the pK values. It is, therefore, necessary to investigate the optimum reaction conditions for every compound used.

2.2. Analytical Techniques.

The methods used for selenium analysis fall into two groups: those relying on an intrinsic property of selenium itself, such as atomic absorption, electrochemical techniques or neutron activation, or those relying on a property of a selenium compound such as, colorimetry, fluorimetry or gas chromatography.

Table 1.

Detection Limits and References of Various 1,2-Diamino-Benzene Reagents for the Colorimetric Determination of Selenium.

Reagent.	Detection Limit.	Reference.
1,2-diamino-benzene	0.25mgSe l^{-1}.	Ariyoshi *et al*, 1960.
4-dimethylylamine-1,2-diamino-benzene.	1.0mgSe l^{-1}	Demeyere and Hoste, 1962.
4-methyl-1,2-diamino benzene.	0.01mgSe l^{-1}	Tanaka and Kavashima, 1965.
4-chloro-1,2-diamino benzene	0.01mgSe l^{-1}	"
4-nitro-1,2-diamino benzene	0.01mgSe l^{-1}	"

2.2.1. Colorimetric Determination of Piazselenols.

The strong absoption of visible and U.V. light by piazselenols has been taken advantage of by Hoste (1948) and Hoste and Gillis (1955) for the determination of selenite. In their method, for which the detection limit of 0.25mgSe l^{-1} was claimed, the selenite was reacted with diamino-benzidine and the absorbance was measured at 348nm. This procedure was adapted by Chau and Riley (1965) for the analysis of selenium in sea water after co-precipitation on ferric hydroxide, thereby reducing the detection limit to 0.01µgSe l^{-1}. A number of diamino derivatives have also been used to improve on the detection limit, (vis Table 1). However all these methods are subject to considerable interference from iron(III), vanadium (II), chromium(III) and tin(II) but, in some instances, this can be masked by chelating agents such as E.D.T.A. (Tanaka and Kavashima, 1965.).

2.2.2. Fluorimetric Techniques.

The development of the colorimetric determination of selenium using piazselenols led to investigations into the fluorescing properties of a number of these compounds. Parker and Harvey (1962) found that 4,5-benzo-piazselenol, formed from 1,2-diaminonaphthalene, showed the strongest fluorescence of the compounds tested and this derivative has been used, in conjunction with various methods of preconcentration, for the determination of selenium in waters (Lott et al, 1963, Cukor et al, 1964, Whitelaw, 1975, Sugimura and Suzuki, 1977.).

The fluorometric methods are subject to considerable

interference from a number of cations and anions. At low selenium levels these can only be masked by adding abundant complications to the method, thus increasing the chances of contamination. At higher levels (greater than 1μgSe l^{-1}), however, these become much less significant.

A more complete discussion of the fluorimetric methods is given in Chapters 5 and 6.

2.2.3. Gas Chromatographic Techniques.

Substituted piazselenols are volatile and can, thus, be isolated using a gas chromatograph. The electron capture detector, E.C.D., is extremely sensitive to electrophilic groups and so, by substituting halogen or nitro groups onto the benzene ring of diamino-benzene, a very sensitive method of selenium analysis can be developed.

Nakashima and Tôei (1968) achieved a sub-microgram detection limit for selenium in waters using 1,2-diamino-4-chlorobenzene as the reagent. Shimoishi and Tôei (1970) found that the 4-nitro derivative was equally sensitive and it was adopted for the analysis of selenium in natural waters (Shimoishi, 1973), marine sediments (Gosink and Reynolds, 1975) and the biota (Poole et al, 1977). In an attempt to improve the sensitivity of the gas chromatography a survey was carried out by Shimoishi (1977) into the E.C.D. response to a number of substituted piazselenols. It was found that, of those compounds tested, 3,5-dibromo-piazselenol gave the greatest response, some three times that of the 4-nitro derivative. The same author went on to adopt 1,2-diamino-3,5-dibromo-benzene as a

reagent in the determination of selenium in natural samples, obtaining a detection limit of about 1ngSe l^{-1} (Shimoishi and Tôei, 1978.). Measures and Burton (1980a) later obtained a similar sensitivity using 4-nitro-1,2-diamino-benzene. A study of various gas chromatographic reagents was made in the present work which are recorded in Chapters 3 and 4.

2.2.4. Atomic Absorption Techniques.

Kirkbright and Wilson (1974) have discussed a flame/atomic absorption method for the determination of selenium in waters using the 196.0nm absorption line. Unfortunately, interferences so numerous and severe were found that the method was useless for the analysis of environmental samples (Smith, 1975.). Thompson and Thomerson (1974) found that the introduction of hydrogen selenide, generated by the reduction of selenite, (vis Sect. 2.3.1.), into a heated quartz glass tube in the light path of a spectrophotometer could give a detection limit of about 1.8μgSe l^{-1}. Goulden and Brooksbank (1974), who also automated the method, improved on this by burning the gas inside the tube in a hydrogen/air flame, gaining a detection limit of about 0.1μgSe l^{-1}. The interferences associated with this determination are, however, severe, and although some of them can be masked by the formation of chloro complexes, by working at high concentrations of hydrochloric acid, they render the method almost useless in the analysis of environmental samples, (Vijan and Wood, 1976.).

Attempts have also been made, therefore, to separate selenium from the interferences. This was achieved by trapping

the hydrogen selenide in a liquid nitrogen trap. By releasing the trapped gas in a single pulse a detection limit of 5ngSe l^{-1} can be achieved (McDaniel et al, 1976, Cutter, 1978.). Maher (1980) increased the sensitivity to give a detection limit of 0.5ngSe l^{-1} by trapping the gas in an I_3^- solution followed by direct injection into a carbon furnace atomiser.

Unfortunately, the evolution of hydrogen selenide is severely hampered by the presence of dissolved chlorine, precluding the use of the above methods in the analysis of drinking water and some polluted waters (Cutter, 1978.).

2.2.5. Electrochemical Methods.

Lingane and Niedrach (1948 and 1949) investigated the polarographic behaviour of the various oxidation states of dissolved selenium. Christian (1965) used this to develop a polarographic method of selenium analysis achieving a limit of detection of 0.2μgSe(IV) in a sample by using a 1,2-diaminobenzene preconcentration stage (vis Sect. 2.1.6.). Agasyan (1967) performed some preliminary work on the sensitivity of various solid metal electrodes for the voltametric stripping method of determination. Vadja (1970) achieved a detection limit of 8μgSe l^{-1} using cathodic stripping of a mercury electrode. This was superceded by the anodic stripping of a gold plated, rotating, glassy carbon electrode, which was found to be capable of measuring selenite concentrations down to 0.04μgSe l^{-1} (Andrews and Johnson, 1975, Posey and Andrews, 1981.).

2.2.6. Neutron Activation Analysis.

Kharkar et al (1968) have described a neutron activation

procedure for the determination of selenium in sea and fresh waters which involves the freeze drying of the sample followed by irradiation with thermal neutrons according to the equation $^{74}Se(n,\gamma)^{75}Se$. The selenium-75 is then isolated by an elaborate, time consuming radiochemical separation, involving some eight stages and, finally, counting. However, although the electron capture cross section of ^{74}Se is high, (50±7 barns), its natural abundance is low, 0.87%, and the long half-life of the ^{75}Se produced, 121 days, makes it difficult to achieve adequate sensitivity.

2.3. Methods for the Identification and Analysis of the Various Species of Selenium.

The majority of methods for the determination of dissolved selenium are specific to the selenite (SeO_3^{2-}) ion. As the element commonly occurs in waters as the selenate (SeO_4^{2-}) ion, it is necessary to convert the 6+ oxidation state to the 4+ oxidation state prior to analysis of the total selenium concentration. The majority of published analytical methods, therefore, consider that either the 6+ or the reduced (2- and 0) states are negligible, depending on the redox conditions of the sample and estimate the form assumed present by the difference between total selenium and selenite. A number of methods for the determination of total selenium which appeared to be applicable to the analysis of waters will be reviewed below.

2.3.1. Co-precipitation of Total Selenium with Tellurium.

One approach to the determination of total selenium is to add tellurium (IV) to the sample and to reduce the latter to the elemental state with hydrazine sulphate and, in doing so

to co-precipitate selenium, all forms of which are simultaneously reduced to the elemental state as well. After filtration, the selenium is re-oxidised to the 4+ state by dissolving the precipitate in nitric acid and boiling with 6M hydrochloric acid. After analysis, the previously determined selenium 4+ concentration is subtracted from the total concentration to determine that of the selenium 6+ (Sugimura and Suzuki, 1977.).

2.3.2. Reduction with Titanium Trichloride and Oxidation with Bromine/Bromide Redox Buffer.

All oxidised forms of selenium are reduced to the element by heating the sample with titanium trichloride and "selenium-free" sulphuric acid. The selenium is then selectively oxidised to the 4+ state by warming with excess bromine/bromide redox buffer (Shimoishi and Tôei, 1978.). Once again no differentiation can be made between the 2-, the 0 and the 6+ states. The characteristics of this method have been investigated in the present work and the evaluation of the technique can be found in Section 7.7..

2.3.3. Reduction/Oxidation of Selenium by Hydrobromic Acid and Bromine.

The efficiency of a number of oxidising agents for the conversion of selenium 2- and 0 to selenium 4+ has been investigated by Uchida et al. (1980). Bromine, which was found to be the most efficient for this purpose, has the additional advantage that any excess reagent can be removed by the simple expedient of adding hydrazine hydrochloride prior to analysis.

Selenium 6+ can be quantitatively reduced and selenium 2- and 0 partially oxidised to selenium 4+ by boiling the sample with hydrobromic acid. The oxidation can be completed by the addition of a little bromine (Uchida et al, 1980.). This method was investigated in the present work and the results obtained are given in Section 7.2..

2.3.4. Reduction of Selenium 6+ by U.V. Irradiation.

Armstrong et al (1966), and Shuali et al (1969), have reported that , in mildly alkaline conditions, at about pH 10, nitrate is partially reduced to nitrite by U.V. irradiation. Measures and Burton (1980a) have adapted this principle to the reduction of selenium 6+ to selenium 4+, finding a maximum, reliable reduction of about 86% at a pH of 8.1. An investigation of this technique is reported in Section 7.3..

2.3.5. Reduction of Selenium 6+ by Boiling with Hydrochloric Acid.

It has long been known that selenium 6+ is reduced to selenium 4+ when boiled in 4M hydrochloric acid. However, if boiling is protracted the reduction will continue beyond this stage, reducing the selenium down to the elemental form. This procedure also has the disadvantage that the final acidity precludes the use of the majority of methods of subsequent analysis.

2.4. Determination of Dimthyl Selenide and Dimethyl Diselenide.

Chau et al (1975) and Cutter (1978) outlined a GC/AA method for the analysis of dimethyl selenide and dimethyl diselenide in environmental samples with a detection limit of C.1ngSe.

CHAPTER 3.

3. Development of Gas Chromatographic Methods for the Analysis of Selenium 4+ in Waters.

3.1. Reagents Used.

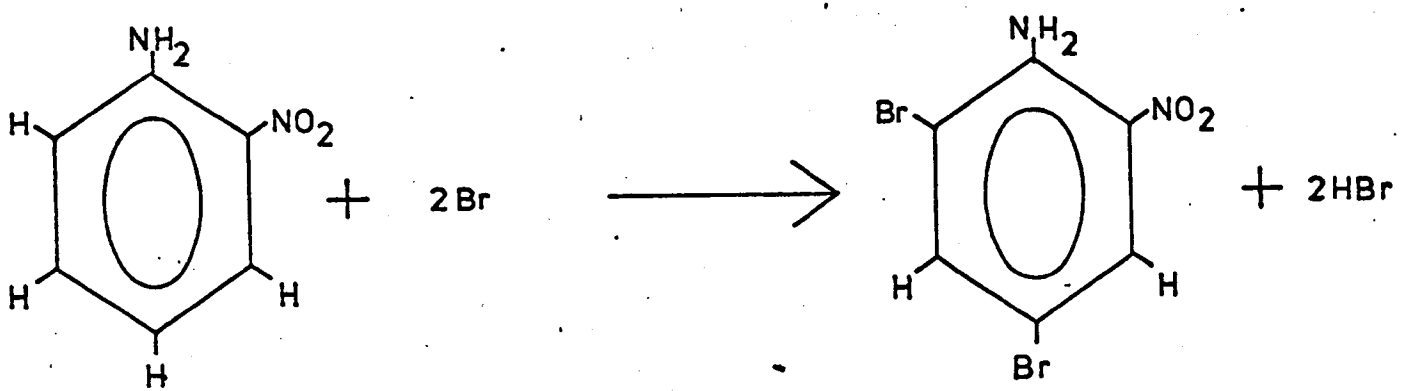
After a study of the literature (Sect. 2.2.3.) , it was decided to study three reagents; 1,2-diamino-3,5-dibromo-benzene, (D.A.D.B.B.), 1,2-diamino-4-bromo-benzene, (D.A.B.B.), and 1.2-diamino-4-nitro-benzene, (D.A.N.B.). D.A.N.B. is commercially available (B.D.H.), D.A.B.B. was supplied by Dr. E.J. Newman, of B.D.H. Chemicals and D.A.D.B.B. was synthesised from 2-nitroaniline.

3.1.1. Synthesis of 1,2-diamino-3,5-dibromo-benzene.

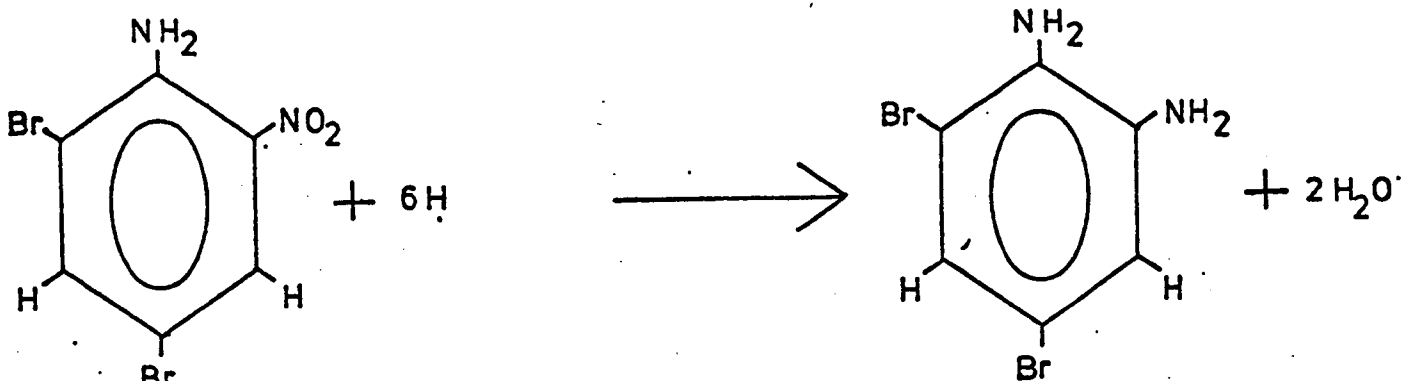
As this is not commercially available, it was necessary to synthesise this compound. 2-nitroaniline was brominated in the 4 and 6 positions and then the nitro group was reduced (vis Fig. 3.). No difficulty was experienced in the bromination stage, however, considerable problems were encountered in the reduction stage.

Initially, attempts were made to reduce the brominated nitroaniline using granulated tin and hydrochloric acid as suggested by Shimoishi and Tôei (1978), without details. Only about 3% of theoretical was recovered as the free base, using steam distillation for isolation (Jackson and Russe, 1906.). A number of other reduction methods were attempted. Reduction by zinc dust and sodium hydroxide (Hirsberg, 1889), iron turnings and acetic acid (Neilson et al, 1962), hydrazine hydrate and raney nickel, stannous chloride and hydrochloric acid, hydrogen

Figure 3. Synthesis of D.A.D.B.B..



Bromination



Reduction

sulphide in aqueous, alcoholic ammonia, sodium borohydride with palladium on charcoal and hydrazine hydrate with palladium on charcoal all gave very little or no yeild of the amine. It is now believed that the reduction, in most cases, succeeded but the free base oxidised rapidly on extraction.

On the advice of Dr. R.A.W. Johnstone of the Organic Chemistry Department of the University of Liverpool, attention was directed to the use of phosphinic acid with a palladium on charcoal catalyst (Entwistle et al, 1977.). After some initial difficulty it was found that a good yield could be obtained by extracting the final product as the dihydrochloride. The identity of the compound was confirmed by elemental analysis (C.H.N.) and mass spectroscopy (vis Sect. 3.1.1.3.).

3.1.1.1. Bromination of 2-nitroaniline.

2-nitroaniline, (13.8g; 0.1 moles.), was dissolved in 120ml of glacial acetic acid and a solution of 10ml (0.4moles) of bromine in 15ml of glacial acetic acid was stirred in. After the rapid discharge of the red colour of the bromine the mixture was poured into 800ml of deionised water. The yellow precipitate which formed was filtered off under suction and then washed with water. The compound was then recrystallised from aqueous ethanol (Jackson and Russe, 1906.). The recovery of the purified 3-dibromo-5-nitro-6-amino-benzene (D.B.N.A.B.) was about 27g (about 90% of theoretical.).

3.1.1.2. Reduction of D.B.N.A.B. with Phosphinic Acid and Palladium on Charcoal.

D.B.N.A.B. (5.9g; 0.02 moles) was dissolved in the minimum

volume of methanol and 12ml of phosphinic acid was added followed by 2g of 5% palladium on charcoal. After stirring, the initial effervescence was allowed to die down and then the mixture was refluxed at about 60°C for about 30mins until colourless, more phosphinic acid being added if necessary.

The solution was filtered immediately, using suction, care being taken that the filtrate remained acidic (pH 2). The filtrate was evaporated down to a small volume using a rotary evaporator. Excess sodium methoxide in methanol was added to the resultant brown liquid which was then stirred, under nitrogen, for an hour. A considerable excess of concentrated hydrochloric acid was then stirred into the mixture for five minutes. The precipitated sodium hydroxide was filtered off and the filtrate was rotary evaporated at 35°C. The resultant light mauve solid was recrystallised from 1M hydrochloric acid (yield, 50-60% of theoretical).

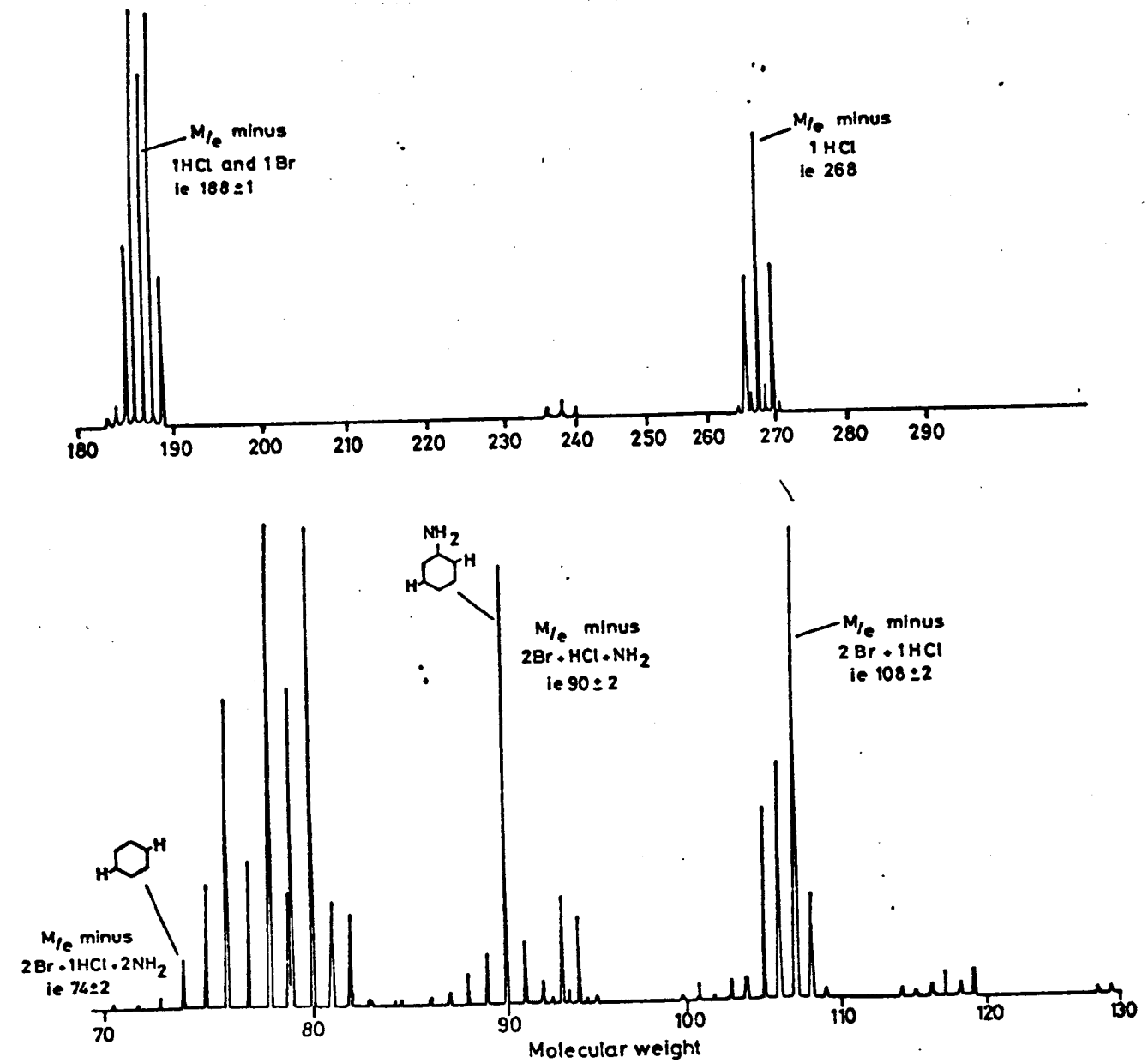
This scheme of synthesis was used for much of the work on determining the optimum conditions for the formation of the piazselenol. This work also involved the use of selenium-75 tracer (Sect. 3.2.1.); however, when the method was transferred to the G.C., the product proved to contain unacceptable levels of electron capturing substances. It was decided, therefore, to develop a different scheme. A private communication with Dr. Y. Shimoishi, (Okayama University), led to the following technique, which was used for all subsequent work carried out on the gas chromatograph.

Table 2.

Typical Results of an Elemental Analysis of D.A.D.B.B..

Element.	% by Weight of $C_6H_7N_2Br_2Cl$	% by Weight Found.		Mean.
		1	2	
N	9.26	9.20	9.28	9.24
C	23.80	23.85	23.81	23.83
H	2.31	2.05	2.51	2.28

Figure 4. Mass Spectrometer Response to D.A.D.B.B.:HCl.



3.1.1.3. Reduction of D.B.N.A.B. with Tin and Hydrochloric Acid.

The bromination of 2-nitroaniline was carried out as described above. The nitro compound (6g) was then dampened with ethanol in a round bottomed flask (250ml) and then refluxed at about 60°C with granulated tin (15g) and hydrochloric acid (50ml, concentrated) until the solution and the solid lost all hint of the orange colour of the D.B.N.A.B.. The mixture was allowed to cool, the solid was then filtered off and dissolved in a minimum of boiling 1M hydrochloric acid, decolourising charcoal was added and the solution was filtered into a similar quantity of concentrated hydrochloric acid. The pink or white solid was filtered off and recrystallised at least twice more in a similar fashion. The D.A.D.B.B. formed and purified in this manner was dried and stored in a vacuum desiccator(yield, 60-70% of theoretical.).

A mass spectrometer was used to check the compound. This showed the presence of hydrochloride, bromide and amino groups in the expected proportions (vis Fig. 4.). These results were confirmed by elemental analysis (Table 2.).

3.1.2. Reagents.

i) D.A.D.B.B. Solution.

A 0.12% solution of D.A.N.B. was prepared in toluene-washed hydrochloric acid. This was washed three times with toluene and could be stored, under refrigeration, 4°C, for at least a month.

ii) D.A.N.B. Solution.

Solid D.A.N.B. was recrystallised from 1M hydrochloric

acid. The amber crystals were dried and stored in a vacuum desicator. A 1% w/v solution in 10% hydrochloric acid was prepared. This was washed five times with toluene and stored under toluene. Under refrigeration, about 4°C, this solution will remain stable for at least two weeks.

iii) D.A.B.B. Solution.

A 0.24% w/v solution of D.A.B.B. was prepared by dissolving 0.24g of the compound in 100ml of 50% hydrochloric acid. This was washed three times with toluene and stored under refrigeration at 4°C.

iv) Hydrochloric Acid.

The cleanest hydrochloric acid, with respect to selenium was found to be M. & B. Laboratory Grade chemical. Prior to use this was washed with toluene.

v) Toluene.

Analytical Grade toluene was fractionated at 111-112°C, the middle third being retained for use.

vi) Perchloric Acid.

40% perchloric acid was prepared by a 1:2 dilution of 60% perchloric acid (AnalaR) with deionised water.

vii) Standard Selenite Solution.

Sodium selenite (0.219g) was dissolved in 1M nitric acid and diluted to 100ml with the same acid. This solution, which contained 1mgSe l^{-1} was found to be stable indefinitely.

As the exact purity of the selenite was uncertain the solution was standardised gravimetrically. Hydrochloric acid and hydrazine sulphate, (Erdy, 1965) and sulphur dioxide in

hydrochloric acid (Vogel, 1951) gravimetric methods were first tested against standards made from "Specpure" selenium shot dissolved in nitric acid. The latter method was found to be more satisfactory, giving a recovery of 98%, and was adopted for the standardisation.

A 25ml aliquot of the standard was diluted to 100ml with deionised water and treated with 150ml of concentrated hydrochloric acid and 50ml of sulphur dioxide saturated concentrated hydrochloric acid. After 2hrs the volume was reduced to 100ml by gentle boiling, which converted the red, amorphous selenium to the consolidated, grey selenium. The volume of the mixture was increased to 200ml with deionised water and the selenium was filtered off using a pre-weighed glass sinter (4). After washing with ethanol, the selenium was dried to constant weight at 100°C.

The purity of the standards has been allowed for in all results.

3.2. Determination of the Optimum Reaction Conditions for the Formation of the Piazselenols.

The most favourable reaction conditions were determined using the radioactive isotope, selenium-75, obtained, without carrier, from the radiochemical centre at Amersham. Counting was carried out using a Pye scintillation counter fitted with a NaI (Tl) well crystal connected to a Panax Equipment Limited power unit, P7102A scaler unit and P7202 timer. All results showed a standard deviation of ± 3%.

It must be noted that, due to the difference in media, the readings for samples are 10% high relative to aqueous

Table 3.

The Effect of Hydrochloric Acid Concentration on the Recovery of Selenium Using the D.A.D.B.B. Reagent.

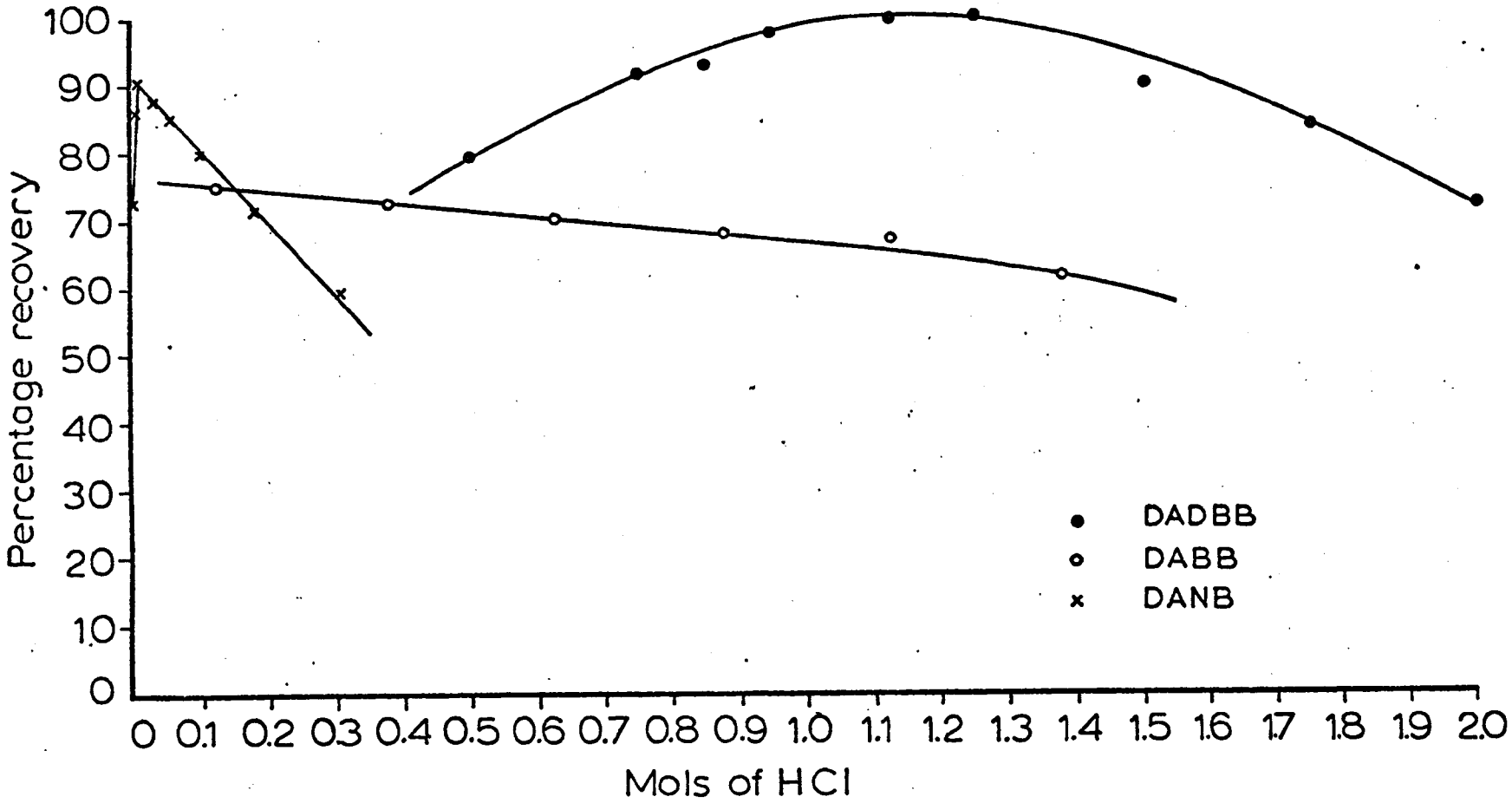
HCl (M) After Reagent Addition.	Percentage ^{75}Se Recovered.
0.50	79
0.75	92
0.85	93
0.95	98
1.15	100
1.25	101
1.50	90
1.75	84
2.00	72

Table 4.

The Effect of Hydrochloric Acid Concentration on the Recovery of Selenium Using the D.A.B.B. Reagent.

HCl (M) After Reagent Addition.	Percentage ^{75}Se Recovered.
0.125	80
0.375	79
0.625	77
0.875	74
1.125	73
1.375	67

Figure 5. Effect of hydrochloric acid molarity on formation of piazselenols.



standards. This effect has been corrected for in all the results given below.

3.2.1. Concentration of Selenium-75 Used.

The selenium-75 provided was diluted by a factor of 10,000 to a level of about 0.02μCi ml^{-1}, giving a reading of appoximately 60,000 counts per minute per millilitre. 1ml of the solution was used in all experiments and 1ml aliquots of 2ml toluene extractions were counted to determine the recovery.

3.2.2.1. Hydrochloric Acid Concentration Required for Maximum Recovery Using D.A.D.B.B..

1ml of the selenium-75 solution was added to 250ml of toluene saturated deionised water, various quantities of concentrated hydrochloric acid were added so as to give concentrations of between 0.5 and 2.0M after the addition of 10ml of the D.A.D.B.B. reagent. After 2½hrs the solution was shaken vigerously with a 2ml aliquot of toluene, by hand, for 2mins. A 1ml aliquot of this extract was then counted. Since this volume contains only half of the selenium-75 added the corrected counts were compared with that of 1ml of a two times dilution of the aqueous standard. The percentage extraction was then determined (vis Table 3.).

The results, (Fig.5), showed that quantitative extraction is achieved at hydrochloric acid concentrations between 1.15 and 1.25M.

3.2.2.2. Hydrochloric Acid Concentration Required for Maximum Recovery Using D.A.B.B..

A 1ml aliquot of selenium-75 solution and 5ml of the

Table 5.

The Effect of Hydrochloric Acid Concentration on the Recovery of Selenium Using the D.A.N.B. Reagent.

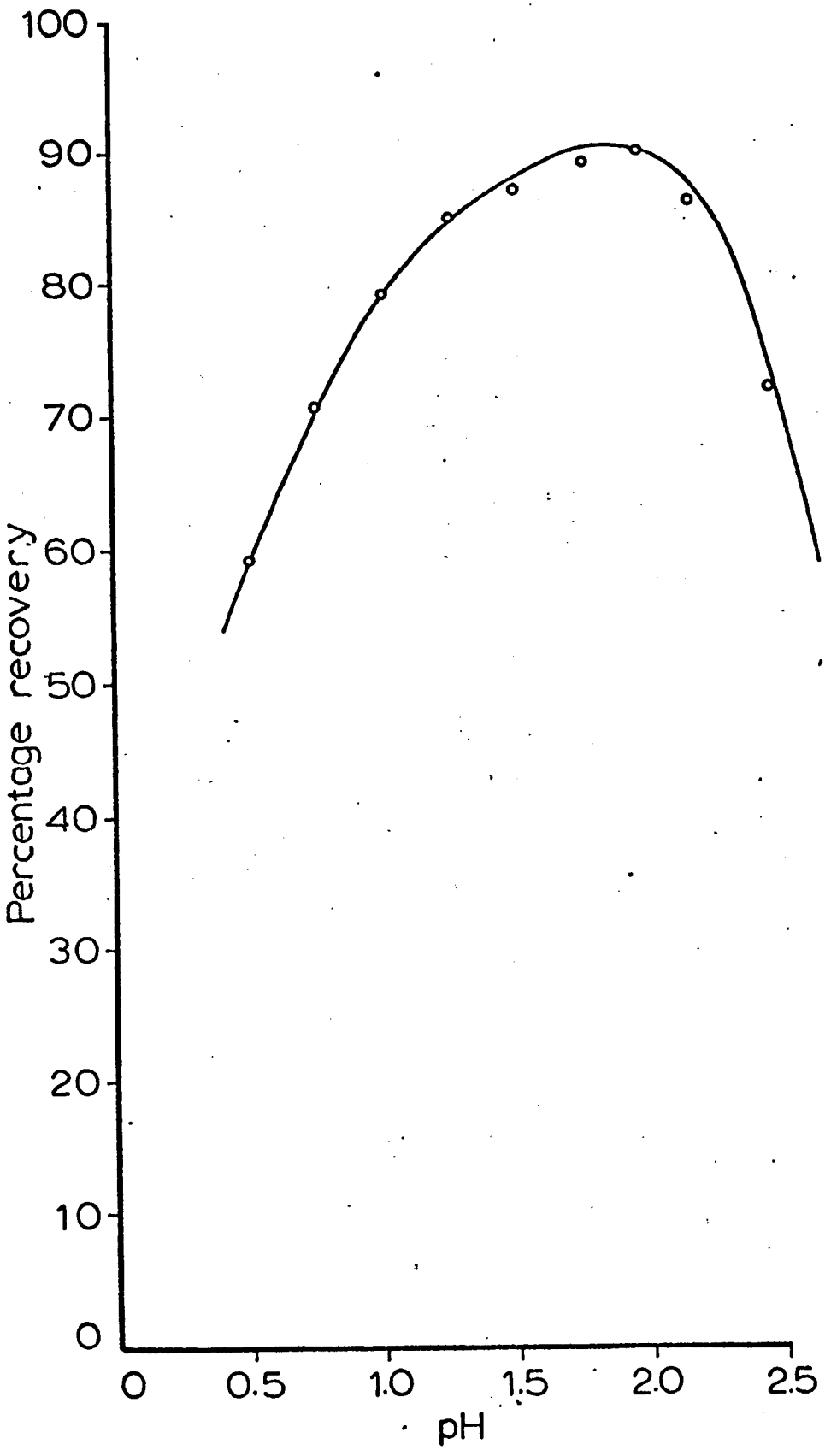
pH	HCl (M) After Reagent Addition.	Percentage ^{75}Se Recovered.
0.50	0.31	59
0.75	0.18	71
1.00	0.10	79
1.25	0.06	85
1.50	0.031	87
1.75	0.017	89
1.94	0.011	90
2.14	0.007	86
2.45	0.003	72

Table 6.

Reaction Time Required for the Maximum Recovery of Selenium Using the D.A.D.B.B. Reagent.

Time (mins.).	% ^{75}Se Recovered.
24	62
38	79
57	84
73	90
100	99
120	100

Figure 6. Effect of pH on formation of nitro-piazselenol.



D.A.B.B. solution were added to 250ml of toluene saturated, deionised water. Varying quantities of concentrated hydrochloric acid were then mixed in. After 2½hrs the solution was extracted with 2ml of toluene and the recovery was determined by counting a 1ml aliquot (vis Table 4.). The results (Fig.5) of these experiments showed that complete extraction could not be achieved. The maximum recovery of 80% being at a hydrochloric acid concentration of 0.125M, corresponding to the addition of the reagent alone.

3.2.2.3. Hydrochloric Acid Concentration Required for Maximum Recovery Using D.A.N.B..

A 1ml aliquot of selenium-75 solution and 5ml of the D.A.N.B. solution were added to 250ml of deionised water, saturated with toluene. The pH was then adjusted to various values between 0.5 and2.45 with 0.5M hydrochloric acid. After 2½hrs the solution was extracted with 2ml of toluene and the recovery was determined by counting a 1ml aliquot (Table 5.).

The results (Fig. 5 and 6) of the experiments showed that complete extraction could not be achieved, the maximum recovery being 90% at a pH between 1.7 and 2.0.

3.2.3. Time Required for Maximum Extraction.

The speed with which the reaction takes place depends on two factors. An increase in the quantity of reagent added will increase the speed of reaction, however, it will also increase the value of the blanks. Therefore, the quantity of reagent added was limited to that which would be consistent with a negligible blank. An increase in temperature will also

Table 7.

Reaction Time Required for the Maximum Recovery of Selenium Using the D.A.B.B. Reagent.

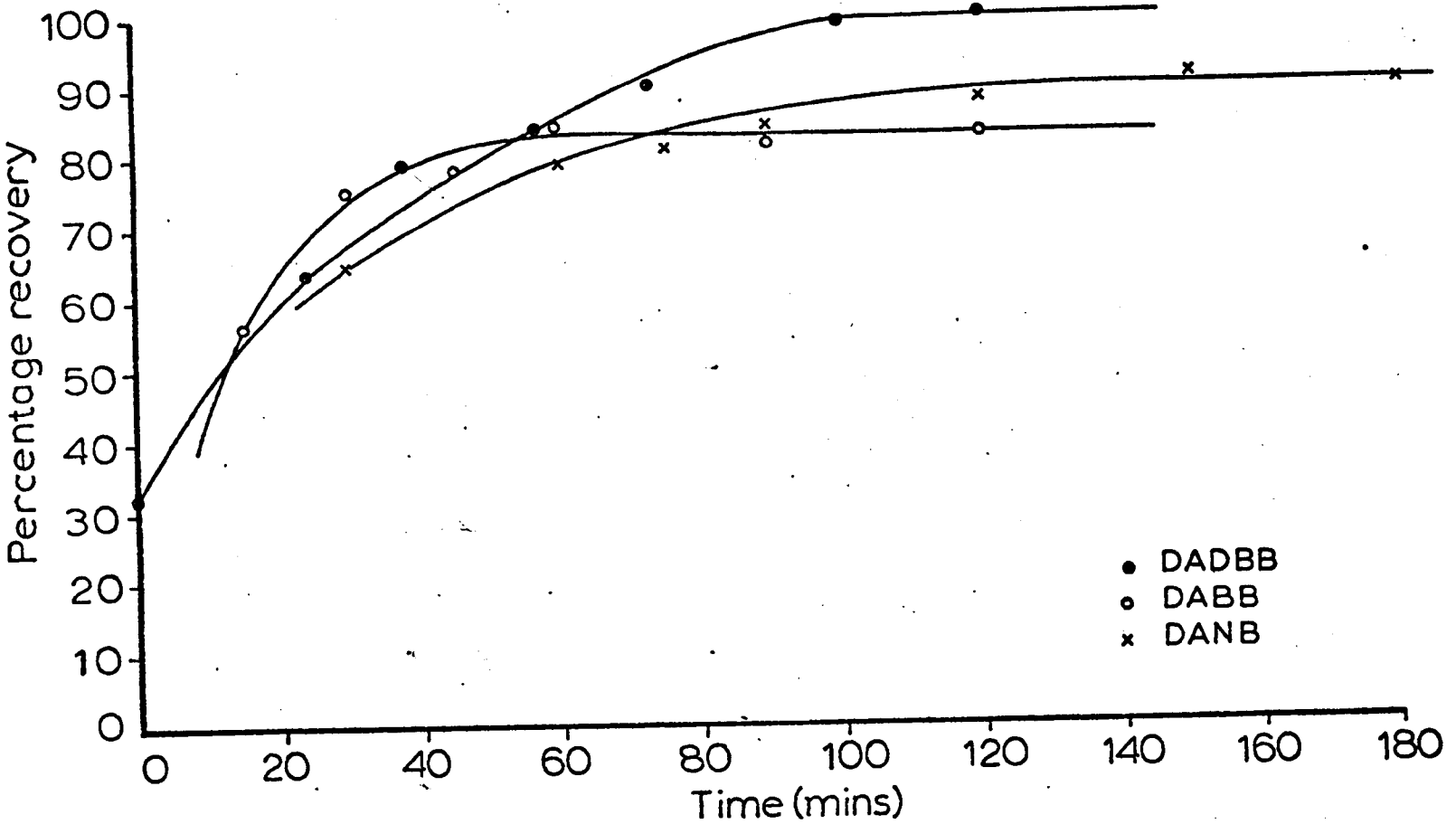
Time (mins.).	% ^{75}Se Recovered.
15	56
30	75
45	78
60	84
90	81
120	82

Table 8.

Reaction Time Required for the Maximum Recovery of Selenium Using the D.A.N.B. Reagent.

Time (mins.).	% ^{75}Se Recovered.
30	65
60	79
75	81
90	84
120	88
150	90
180	90

Figure 7. Rates of formation of piazselenol under optimized conditions.



increase the rate of reaction. Unfortunately as 1,2-diamino-benzenes are fairly unstable, this will increase the interference from decomposition products. All the following investigations were carried out at room temperature. The disparity between the observed results for D.A.N.B. and those obtained by Measures and Burton (1980a), who obtained a maximum extraction after 75mins, is probably due to differences in temperature, since the present data were obtained in winter when the laboratory and the water were at a temperature well below standard (about 14°C).

3.2.3.1. Time Required for Maximum Extraction Using the D.A.D.B.B. Reagent.

The procedure used in Section 3.2.2.1. was repeated using a hydrochloric acid concentration of 1.2M. Extractions were then carried out at intervals between 24 and 120mins after addition of the reagent. The results of these experiments (Table 6, Fig. 7) indicated that, to ensure maximum extraction of selenium the reaction must be allowed to proceed for at least two hours.

3.2.3.2. Time Required for Maximum Extraction Using the D.A.B.B. Reagent.

The procedure used in Section 3.2.2.2. was repeated at an acid concentration of 0.125M, extractions being carried out at intervals of between 15 and 120mins.. It was found that a maximum extraction could be achieved after 75mins. (Table 7, Fig. 7).

Table 9.

Volume of Reagent Solution Required for the Maximum Recovery of Selenium Using the D.A.D.B.B. Reagent.

ml of D.A.D.B.B. Reagent Added.	% of ^{75}Se Recovered.
10.0	99
8.3	98
6.7	89
5.0	85
3.4	69
1.7	49
0.3	19

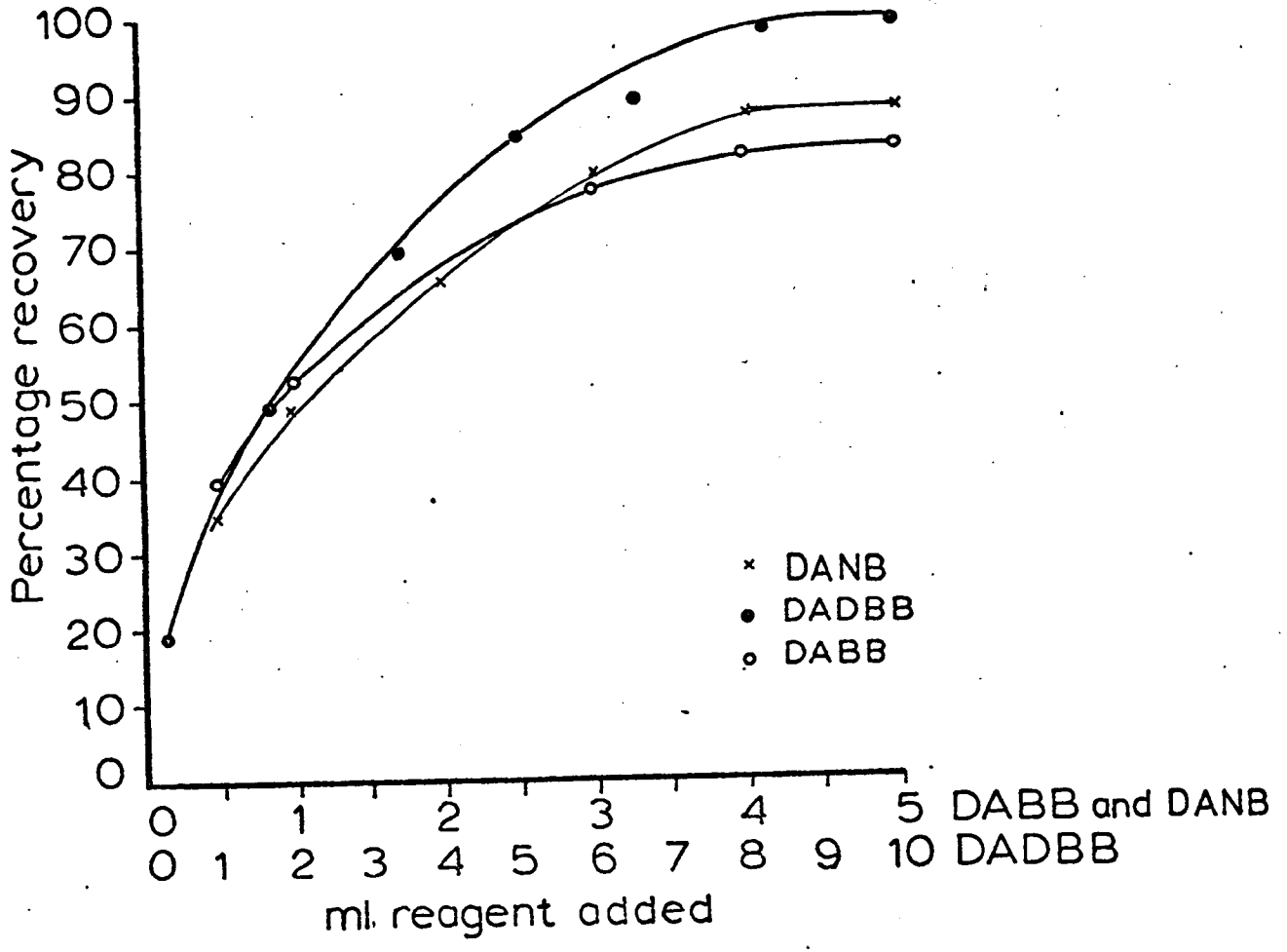
Table 10.

Volume of Reagent Solution Required for the Maximum Recovery of Selenium Using the D.A.B.B. Reagent.

ml of D.A.B.B. Reagent Added.	% of ^{75}Se Recovered.
5.0	83
4.0	82
3.0	77
2.0	62
1.0	52
0.5	39

Figure 8.

Effect of variation of amounts of diamino-compound on piazselenol formation



3.2.3.3. Time Required for Maximum Extraction Using the D.A.N.B. Reagent.

The procedure described in Section 3.2.2.3. was repeated using a final pH of 1.85±0.05, extractions being carried out at intervals between 30 and 180mins.. It was found that a reaction time of 150 mins. was necessary to achieve maximum extraction (Table 8, Fig. 7.).

3.2.4. Reagent Concentration Required.

In order to obtain a maximum recovery a very large excess of reagent is required in all three cases, in the order of $x10^6$. As the quantity of reagent is so large, in comparison to the reagent used up by selenite, the concentration of the selenium anion, within reason, does not affect the percentage recovered.

3.2.4.1. Reagent Excess Required for Maximum Extraction Using the D.A.D.B.B. Reagent.

Using conditions otherwise identical to those described in Sections 3.2.2.1. and 3.2.3.1. the effect of variation of the amount of reagent solution added was investigated. It was found that, for complete extraction of the selenium it was necessary to use 10ml of the D.A.D.B.B. reagent solution (Table 9, Fig. 8). This volume was used in all subsequent work.

3.2.3.2. Reagent Concentration Required for Maximum Extraction of Selenium Using the D.A.B.B. Reagent.

Using conditions otherwise identical to those described in Sections 3.2.2.2. and 3.2.3.2. the effect of variation of the amount of reagent solution added was investigated. It was

Table 11.

Volume of Reagent Solution Required for the Maximum Recovery of Selenium Using the D.A.N.B. Reagent.

ml of D.A.N.B. Reagent Added.	% of ^{75}Se Recovered.
5.0	89
4.0	88
3.0	80
2.0	66
1.0	49
0.5	35

found that 5ml of the D.A.B.B. reagent solution was necessary to ensure a maximum recovery of selenium (Table 10, Fig. 8). This volume was used in all subsequent work.

3.2.4.3. Reagent Excess Required for Maximum Extraction Using the D.A.N.B. Reagent.

Using conditions otherwise identical to those described in Sections 3.2.2.3. and 3.2.3.3., the effect of variation of the amount of D.A.N.B. reagent solution added was investigated. It was found that 5ml of the solution was required to obtain a maximum recovery of selenium (Table 11, Fig.8). This volume was used in all subsequent work.

3.3. Final Reaction Conditions Required to Obtain Maximum Extraction of Selenium.

3.3.1. Sample Preparation.

Prior to analysis samples were filtered through either a glass fibre filter (GF/C) or a membrane filter (0.45um). The glass fibre filters were cleaned by heating to 250°C in a muffle oven for 2hrs and then washed through with deionised water. Membrane filters were thoroughly washed through with deionised water before use.

The 250ml samples were then shaken with 25ml of toluene to saturate the water with the solvent and to remove any toluene-solu ble material.

3.3.2.1. Final Reaction Conditions Used with the D.A.D.B.B. Reagent.

The washed sample was transferred to a 500ml separating funnel and 15ml of cleaned, concentrated hydrochloric acid

and 10ml of D.A.D.B.B. reagent were added. After mixing thoroughly, the mixture was allowed to stand, in subdued light, for two hours. 1ml of fractionated toluene was added and the funnel was shaken vigorously for two minutes. The organic layer was allowed to settle out and then isolated.

3.3.2.2. Final Reaction Conditions Used with the D.A.B.B. Reagent.

The washed sample was transferred to a 500ml separating funnel and 5ml of the D.A.B.B. reagent was added. After 100mins the solution was extracted into 1ml of fractionated toluene, as above.

3.3.2.3. Final Reaction Conditions Used with the D.A.N.B. Reagent.

The washed sample was transferred to a 500ml separating funnel and 5ml of the D.A.N.B. reagent was added. The pH was checked and if it did not lie between 1.7 and 2.0 it was adjusted to 1.85 ± 0.10 with either 0.5M hydrochloric acid or 0.5M sodium hydroxide, as appropriate. After 150mins the solution was extracted with fractionated toluene, as above.

CHAPTER 4.

4. Gas Chromatography of Piazselenols.

Once optimum conditions for the formation of the piazselenols chosen, and their method of extraction had been established, the conditions required for the best gas chromatographic separation and electron capture detection were investigated.

4.1. Equipment.

The gas chromatograph used was a Pye Unicam, 104 series instrument, fitted with a 10mCi ^{63}Ni electron capture detector.

Initially, as recommended by Shimoishi (1977), a 1mx4mm (i.d.) glass column, packed with a stationary phase consisting of 15% SE 30 on Chromosorb W (60-80 mesh), A.W., D.M.C.S. was used for the separation. Argon, purified and dried by passage through a column of molecular sieve, was used as the carrier gas at a flow rate of 28ml min^{-1}. Argon/methane, 90%:10%, cleaned by passage through a column of molecular sieve, at a flow rate of 12ml min^{-1}, was used as the purge gas for the electron capture detector. The column oven was maintained at a temperature of 200°C and the detector oven was maintained at a temperature of 250°C.

4.2. Gas Chromatography of Substituted Piazselenols.

The piazselenols formed from selenite and D.A.D.B.B., D.A.B.B. and D.A.N.B. were synthesised by dissolving the appropriate reagent in 1M hydrochloric acid and then adding a solution of sodium selenite. The precipitate formed was filtered off and recrys.tallised from aqueous ethanol. After filtering and then drying in vacuo, standard solutions of

of the piazselenols were prepared in fractionated toluene. These were then diluted, with toluene, to give solutions containing about 10ngSe ml^{-1}. 2µl of these solutions were injected into the column of the gas chromatograph and the response of the electron capture detector to the dibromo-, momobromo- and nitro-piazselenols, expressed in terms of selenium were found to be 1.0:0.1:0.35 respectively. Those results are similar to those obtained by Shimoishi (1977).

It was therefore decided to continue the investigations of the D.A.D.B.B. reagent, because of its superior response, and the D.A.N.B. reagent, because of its commercial availability.

4.3. Gas Chromatography of the D.A.D.B.B. Extract.

The 3,5-dibromo-piazselenol was formed in deionised water spiked with 120ngSe l^{-1} and extracted, as is described in Section 3.3.2.1.. A 2µl aliquot of the extract was then injected into the column. A massive peak was found to precede and mask the piazselenol response. Washing twice with 3ml of 40% perchloric acid was found to reduce the extent of this peak considerably, but another peak, coincident with that of the piazselenol, remained. In an attempt to eliminate this peak a number of other washes were tried including hydrochloric acid (1M, 2M, 5M and concentrated), sodium carbonate, phosphoric acid (1M, 2M, 5M and concentrated) and water. The acid washes produced only a small improvement in comparison to the effect of the perchloric acid wash, even when followed by a water wash. Sodium cabonate and water washes had no effect.

The gas chromatographic conditions of gas flow and temperature were altered in an unsuccessful attempt to resolve the peak. The loading of the stationary phase was then decreased to 10%, 5% and, finally, 2.5%, the peak, however, could not be resolved in this way.

As SE 30 is a very non-polar stationary phase, it was decided to investigate a number of increasingly polar phases, mainly of the OV silicone oil type. OV 17 (3%), on a 1m column, redistributed the peaks that interfered but a considerable overlap of responses still existed which limited the detection limit to about 80ngSe l^{-1} ($4.6 \times \sigma$). OV 101 (3%) did not improve the limit of detection but it did further resolve the two interfering peaks. Still more polar stationary phases, diethyl glycol succinate, Silar 10C and OV 275 all showed a further improvement in resolution, OV 275 proving to be by far the best. Although the interfering peak was not entirely resolved from that of the piazselenol, the separation was sufficient to reduce the detection limit to about 20ngSe l^{-1}.

At this point in the work a new method of synthesis of D.A.D.B.B. was developed (<u>vis</u> Sect. 3.1.1.3.) which more than halved the interference, reducing the limit of detection to about 8ngSe l^{-1}. This was then reduced to about 1ngSe l^{-1} by moving the extraction apparatus to a laboratory free of sulphuric acid fumes, which contain a significant concentration of selenium.

Figure 9. GC record for chromatography of (a) 40ngSe^{-1} (b) a blank with OV275 column. Through whole procedure. (chart speed 1 cm/2.5 minutes).

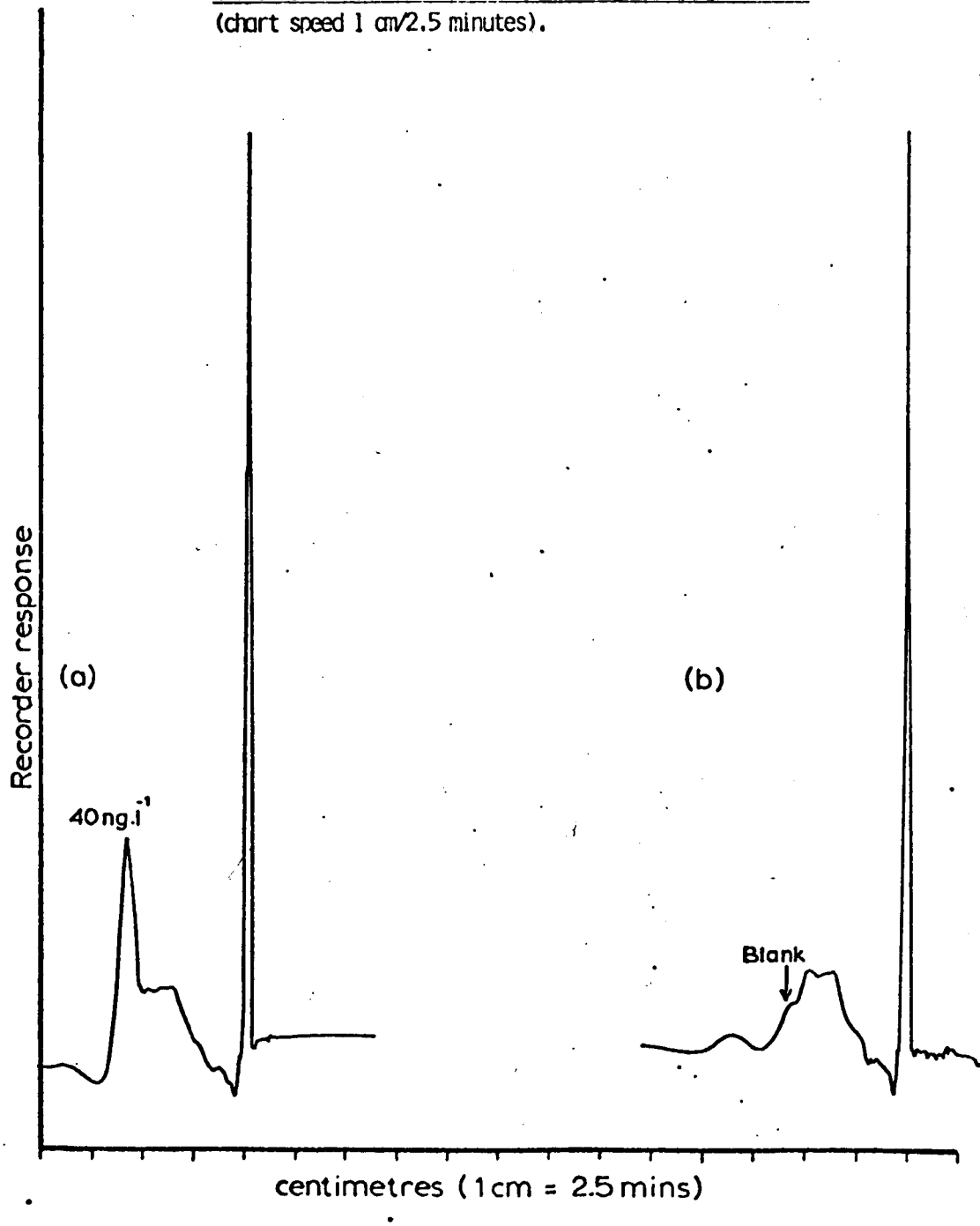
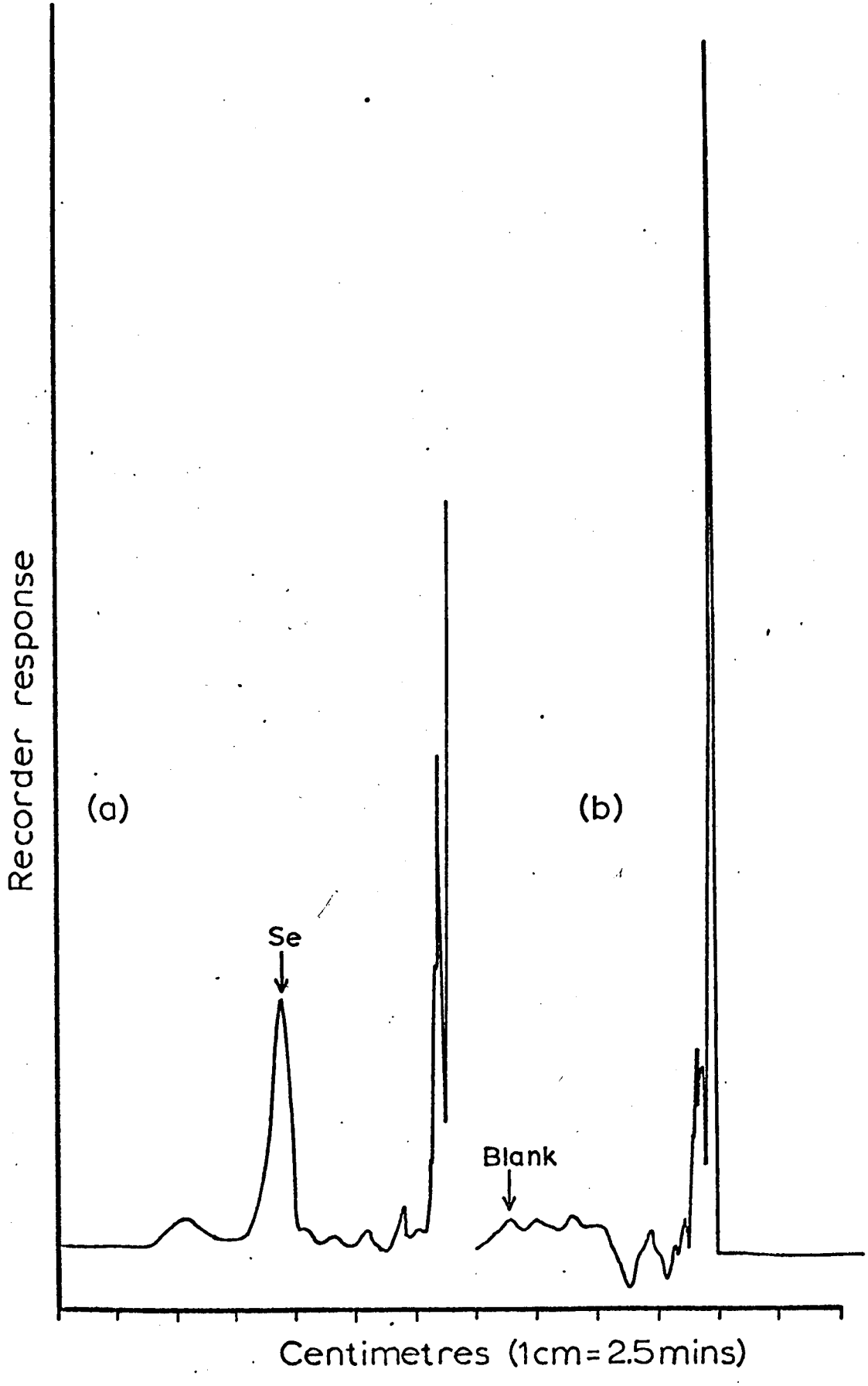


Fig. 10. GC record for chromatography of (a) 40ng $Se l^{-1}$
(b) a blank using OV330 column (chart speed 1cm/2.5 minutes



4.3.1. Gas Chromatographic Conditions for OV 275.

A glass column, 2m x 4mm (i.d.) was packed with 4% OV 275 on a support of Chromosorb W (60-80 mesh, A.W., D.M.C.S.). After purging for 12hrs at 200°C the column was used at 190°C with argon as the carrier gas at a flow rate of 55ml min^{-1}. The detector oven was maintained at 265°C. Fig. 9 shows a typical set of responses from the running of a blank and a standard solution containing 40ngSe l^{-1}.

These conditions were used for much of the subsequent work. However, when the yet more polar stationary phase OV 330 became available it was found to give a better separation than OV 275, especially at low concentrations of selenium.

4.3.2. Gas Chromatographic Conditions for OV 330.

A glass column, 2m x 4mm (i.d.) was packed with 3% OV 330 on a support of Chromosorb W (60-80 mesh, A.W., D.M.C.S.). After purging, at 205°C for 12hrs, the column was used at a temperature of 195°C. Argon/methane (90%/10%) was used as the carrier gas, at a flow rate of 65ml min^{-1}. The detector oven was maintained at a temperature of 265°C. Fig. 10 shows some typical charts obtained from the analysis of a blank and a spiked sea water solution containing 10ngSe l^{-1}.

4.4.1 Evaluation of the Method Using the D.A.D.B.B. Reagent.

The sensitivity of the method varied from day to day by up to 20% due to variations in the response of the detector, however, over a set of analyses, performed in one day, the difference in sensitivity was not significant. In addition, the value of the blank changed with different batches of reagent.

Table 12.

Coefficient of Variation of 6 Replicate Analyses of Selenium (IV) Using OV 275 and OV 330 Stationary Phases.

Conc. of Se^{4+} (ng l^{-1})	Attenuation of E.C.D. Signal.	OV 275 Planimeter Reading.	OV 275 Coeff. Variatn.	OV 330 Planimeter Reading.	OV 330 Coeff. Variatn.
0	$2x10^6$	23	4	15	2
20	$2x10^6$	45	2	35	1
40	$2x10^6$	66	2	53	1
120	$5x10^6$	77	1	60	0.5
180	$5x10^6$	91	1	75	0.5

Table 13.

Calibration Curves of the D.A.D.B.B. Reagent at Selenium (IV) Concentrations Between 0 and 480ng l^{-1}.

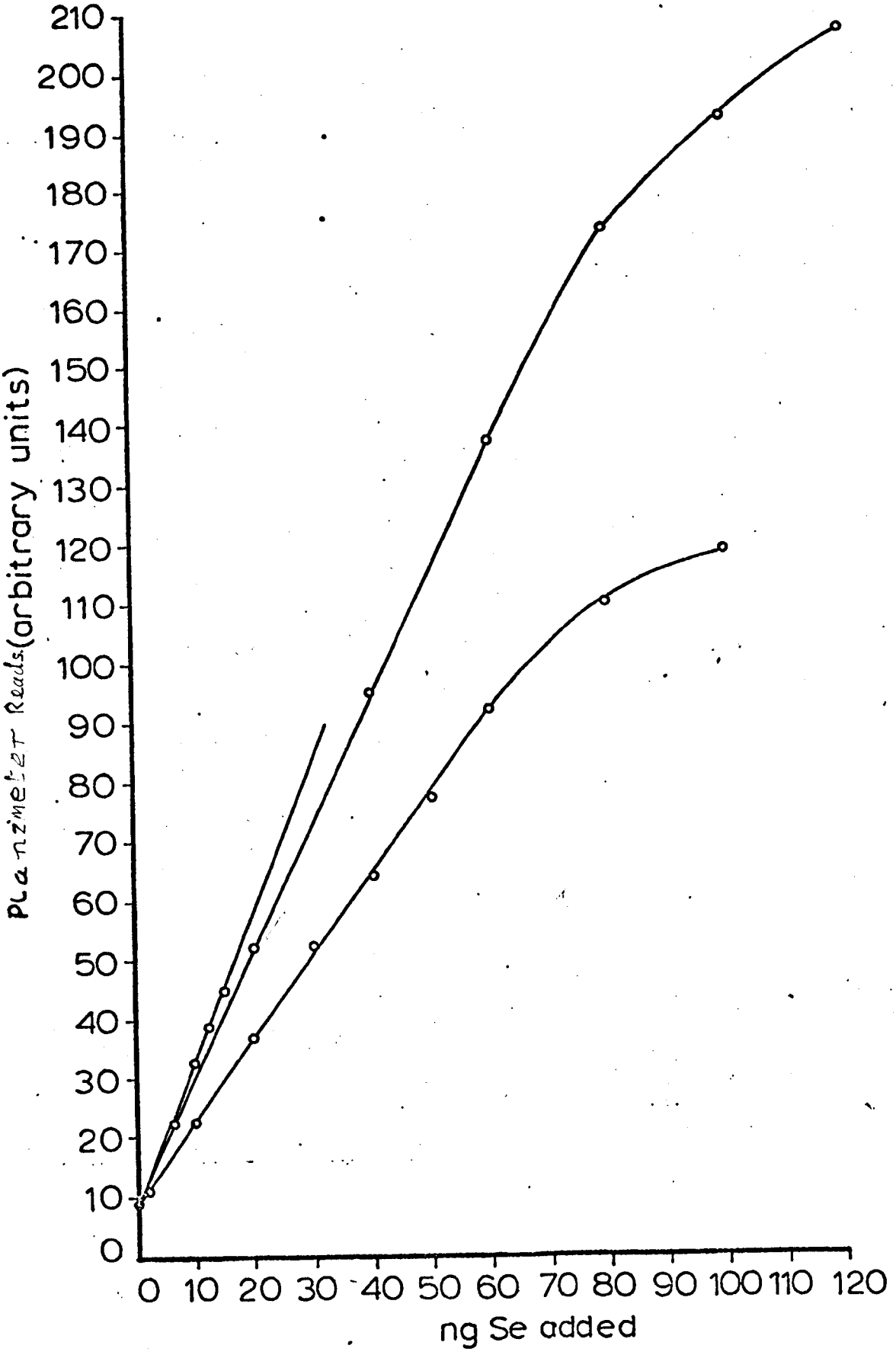
Concentration of Selenium (ng l^{-1}).	Planimeter Readings. Curve 1.	Curve 2.
0	9	8
40	-	22
80	52	36
120	-	52
160	95	64
200	-	77
240	137	92
320	173	110
400	192	118
480	207	-

Table 14.

Calibration Curve for the D.A.D.B.B. Reagent at Selenium(IV) Concentrations Between 0 and 60ng l^{-1}.

Concentration of Selenium (ng l^{-1}).	Planimeter Readings.
0	1.5
8	12.0
16	17.0
24	21.5
40	32.0
50	38.0
60	44.0

Figure 11. Calibration curves obtained with OV-330 column.



Calibration curves and standard deviations were obtained by spiking deionised water with known quantities of selenite to give a final concentration in a range of 0 to 480ngSe l^{-1}. The procedure described in Section 3.3.2.1. was then carried out. After washing the toluene extract twice with two 3ml volumes of 40% perchloric acid, 2µl of the solvent were injected into the chromatographic column under the conditions described in Sections 4.3.1. and 4.3.2.. Examination of the recorder charts showed that the most reproducible results were obtained by measurement of peak area, using a planimeter. Replicate analyses (6) were carried out at various levels of selenite using both the OV 275 and the OV 330 stationary phases (vis Table 12).

A proportion of the total error can be attributed to the reproducibility of the readings from the planimeter, which becomes increasingly significant at low concentrations. The use of an integrator would probably have improved the reproducibility to a noticable, but not dramatic, extent. However, the results recorded on Table 12 give detection limits of 2ng Se l^{-1} and 1ngSe l^{-1} using OV 275 and OV330 respectively.

4.4.2. Determination of Characteristics of Calibration Curves Using the D.A.D.B.B. Reagent.

Data for a series of calibration curves were collected by carrying out the analyses of a number of samples of deionised water spiked with various quantities of standard selenite solution. The stationary phase OV 330 was used for the gas chromatographic separation (vis Tables 13 and 14, Fig. 11). The difference in in gradient between the curves arises from

Table 15.

Examination of the Effect of Other Substances on the Determination of Selenium.

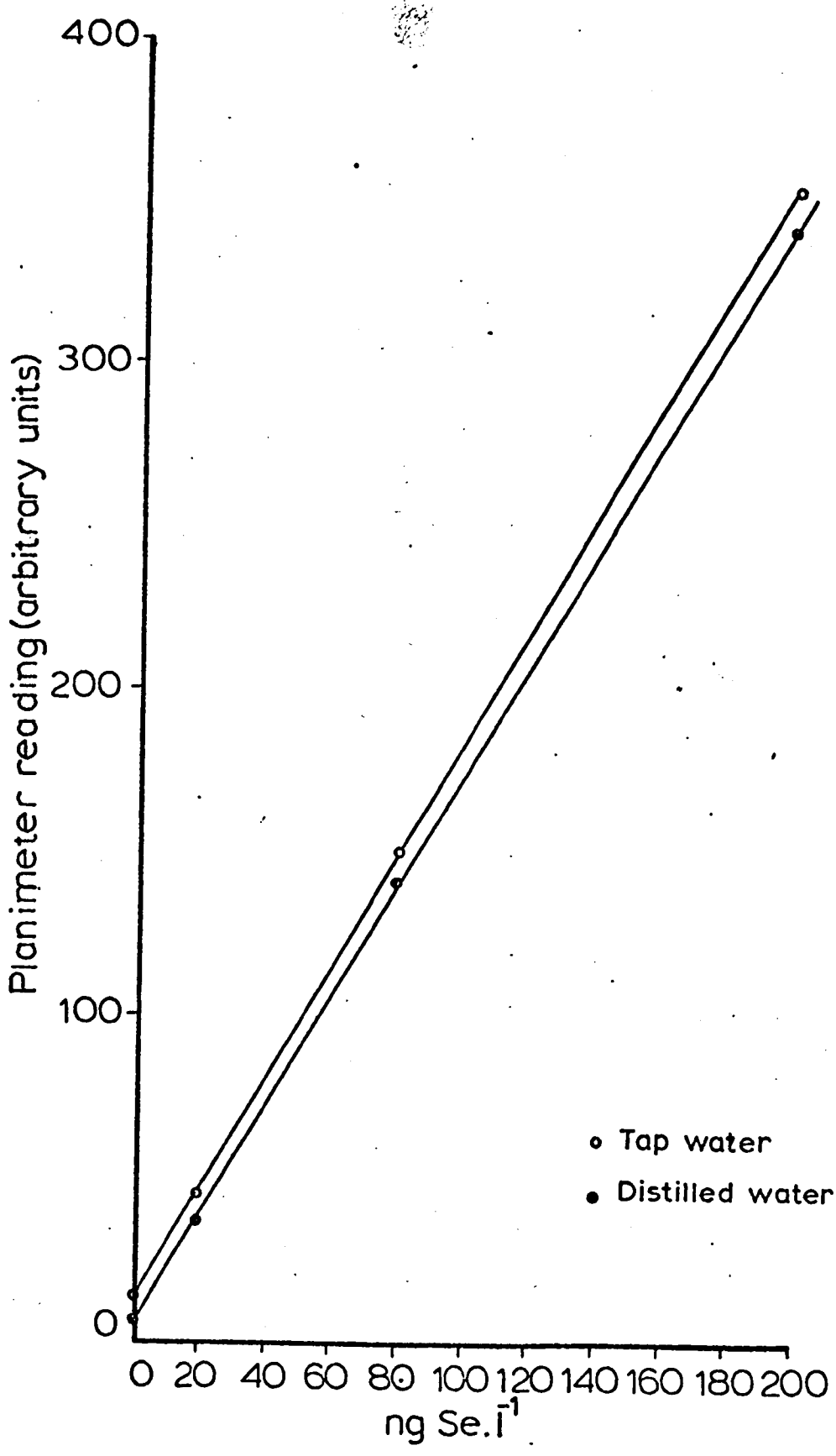
I	Conc. ($\mu g\ l^{-1}$).	% Recovered at 40ngSe l^{-1}.	% Recovered at 200ngSe l^{-1}.
Li	100	100	100
B	1000	102	101
Sb	10	102	100
Cu	5000	100	98
Fe	5000	102	99
Pb	5000	102	98
Ni	2000	100	98
Mn	1000	104	99
Al	1000	100	98
Co	20	98	100
Ba	3000	100	102
Cd	20	98	98
Cr	50	100	98
Ca	$3x10^5$	97	101
Na	$3x10^5$	100	101
Mg	$1x10^5$	100	100
Bi	200	102	100
K	$2x10^4$	105	98
Ag	50	99	100
Mo	500	97	100
Sr	3000	97	101
Br^-	1000	100	101
F^-	1000	98	100
Fulvic Acid.	$1x10^4$	98	99
Hg	1	100	100
As	50	102	100
PO_4^-	5000	100	103
Det.	5000	105	100
Sn	1000	98	102
Zn	100	102	98
CN^-	100	98	99
Phenol	1000	102	101
I^-	1000	102	99
SCN^-	1000	100	97
NTA	1000	98	100
EDTA	1000	100	97

Table 16.

Recovery of Selenium Spikes from North Sea Surface Water and Tap Water Using the D.A.D.B.B. Method.

Selenium IV Spike ng Se l^{-1}	Distilled Water PLANIMETER READING	North Sea Surface Water		Tap Water.	
		Planimeter Reading.	% age Recovered	Planimeter Reading	% age Recovered
0	1	10	-	20	-
20	35	43	97	52	95
120	141	150	100	162	101
200	343	355	101	362	100

Figure 11a. Calibration curve tapwater and distilled water.



variations in the sensitivity of the electron capture detector. The response can be seen to deviate from linearity at a selenium concentration of about 240ng l^{-1}.

4.4.3. Testing of Possible Interferences.

The effects of possible interfering substances on the determination were then investigated. The concentrations of the various substances tested for interference were chosen according to those recommended by the Water Research Centre, Medmenham Laboratory (S.C.A. paper No. 226). No significant interference was encountered at selenium levels of either 40ngSe l^{-1} or 200ngSe l^{-1} (Table 15).

4.4.4. Spiked Sea Water and Tap Water Tests.

The recovery of selenite from samples of water collected in the North Sea and ordinary tap water was tested by spiking them with known quantities of selenite and comparing the results with those obtained with spiked deionised water (vis Table 16). The results show that total recovery of selenium was achieved from both tap and sea water at selenite concentrations of 20, 120 and 200ngSe l^{-1} (Fig. 11a).

4.4.5. Conclusions on the D.A.D.B.B./G.C. Method for the Determination of Selenite.

It was concluded that the D.A.D.B.B./G.C. method for the analysis of selenite in waters which has been reported above, and which is described in detail below, has a detection limit of 1-2ngSe l^{-1}, using the Pye Unicam 104 series gas chromatograph available. No interference is produced by either minor elements, at high concentrations or by major elements at their natural sea water concentrations.

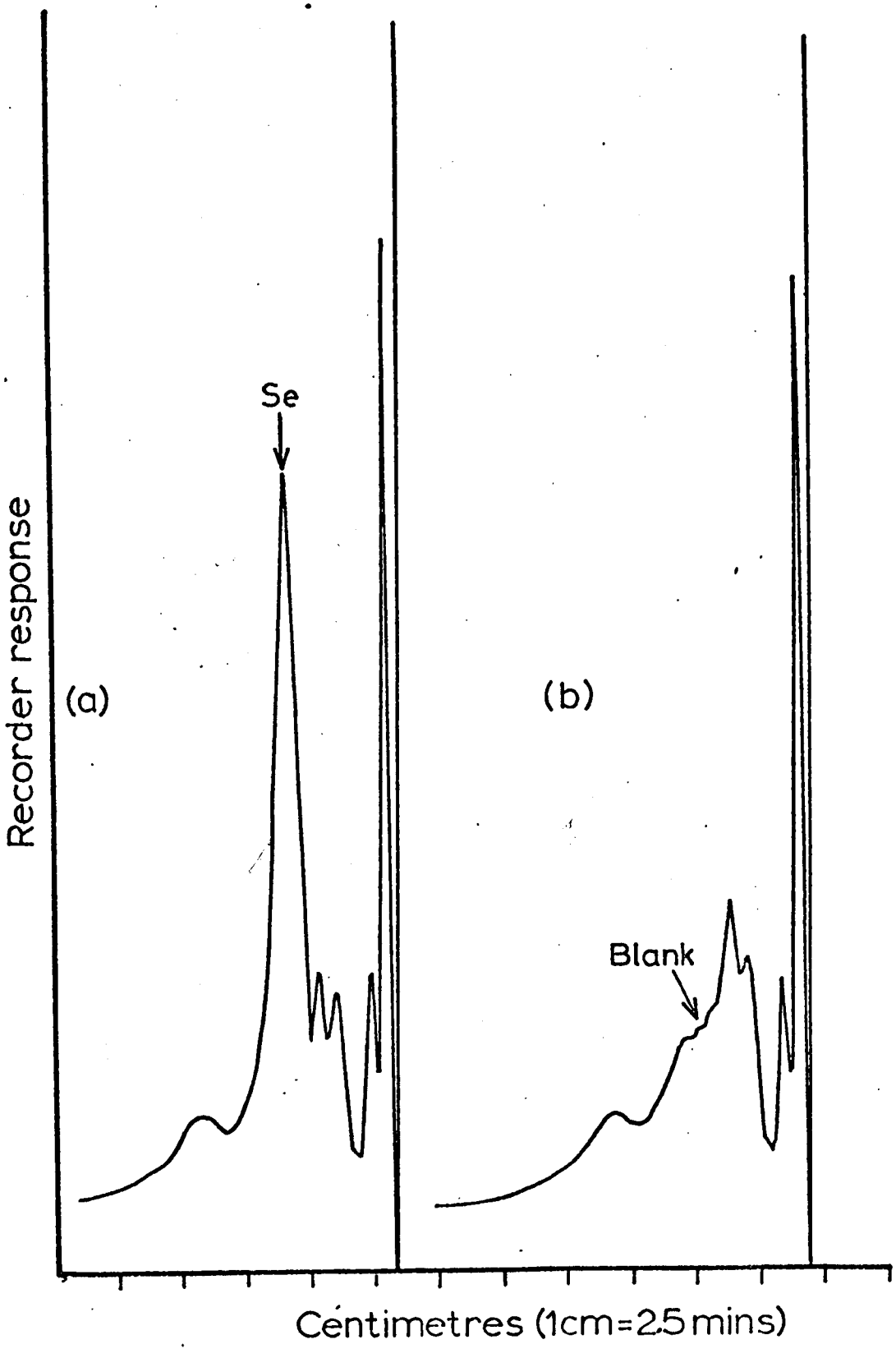
With the instument available the linear range was limited to less than about 240ngSe l^{-1} as the method stands, but this can easily be extended either by reducing the sample size or by increasing the volume of toluene used for the extraction.

4.4.6. Final Method for the Determination of Selenite Using the D.A.D.B.B. Reagent (vis Sect. 3.1.).

A 250ml sample was filtered and washed by shaking with 25ml of toluene to remove any toluene soluble material and to saturate the water with the solvent. On separation the aqueous phase was isolated and transferred to a 500ml separating funnel. 15ml of concentrated, toluene washed, hydrochloric acid and 10ml of the D.A.D.B.B. reagent were then stirred in. The mixture was allowed to stand for 75mins. at room temperature and then extracted into a 1ml aliquot of toluene by shaking vigorously for 2mins.. The organic phase was allowed to separate out and then it was transferred to a 25ml separating funnel in which it was washed twice with 3ml of 40% perchloric acid. A 2µl aliquot was then injected into the gas chromatograph. The organic extract was found to be stable for at least a week if it was kept at $-4^{o}C$.

The gas chromatographic conditions used were as follows. A 2m x 4mm (i.d.) glass column was packed with 3% OV 330 on a support of Chromosorb W (60-80 mesh, A.W., D.M.C.S.). The column oven was maintained at $195^{o}C$ and the detector oven at $265^{o}C$. Argon/methane, (90%/10%), was used as the carrier gas at a flow rate of 65ml min^{-1}. The recorder response was measured using a planimeter.

Figure 12. G.C. Record for Chromatography of (a) 100ngSe l^{-1} and (b) 0ngSe l^{-1} Using D.A.N.B..



4.5. Gas Chromatography of the D.A.N.B. Extract.

The 4-nitro-piazselenol was formed as is described in Section 3.3.2.3., using a selenite solution containing 120ngSe l^{-1}. A 5ul aliquot of the extract was injected into the gas chromatograph, using the conditions recorded in Section 4.1.. Although the piazselenol peak was easily discernable, there was a certain amount of interference from other electron capturing substances. It was decided, therefore, to use the chromatographic procedure described by Measures and Burton (1980a). A glass column, 2m x 4mm (i.d.), was packed with 3% Silar 10C on a support of Chromosorb W (60-80 mesh, A.W., D.M.C.S.). The best results were obtained with the column oven at a temperature of 195°C and a carrier gas flow of 70ml min^{-1} of 90% argon/10% methane. The greatest response from the electron capture detector occurred at a detector oven temperature of 265°C. Washing the extract with 9M hydrochloric acid, 40% perchloric acid or water had no significant effect on the chromatogram.

These conditions were used in all the subsequent work. Typical examples of the electron capture detector response to the extract of a deionised water blank and a 100ngSe l^{-1} standard selenite solution are shown in Fig. 12.

4.6. Evaluation of the D.A.N.B. Method.

4.6.1. Sensitivity and Reproducibility.

The response of the electron capture detector was found to vary from day to day, as it did with the D.A.D.B.B. reagent. The value of the blank was also found to vary with the purity of the D.A.N.B..

Table 17.

Coefficients of Variation of Replicate Analyses (5) for Selenium (IV) Using the D.A.N.B. Reagent.

Conc. of Selenium ngSe l^{-1}.	Mean Planimeter Reading ($\bar{x}$).	Coeff. of Variation.
0	32.5	5
20	55.0	3
40	80.0	2
100	147.5	2
200	239.0	1

Table 18.

Data for Calibration Curves Using the D.A.N.B. Reagent at Selenium (IV) Concentrations Between 0 and 250ng l^{-1}.

Concentration of Selenium (ng l^{-1}).	Planimeter Readings. Curve 1.	Curve 2.
0	30	35
20	55	58
40	78	83
100	145	150
200	265	273
260	313	318

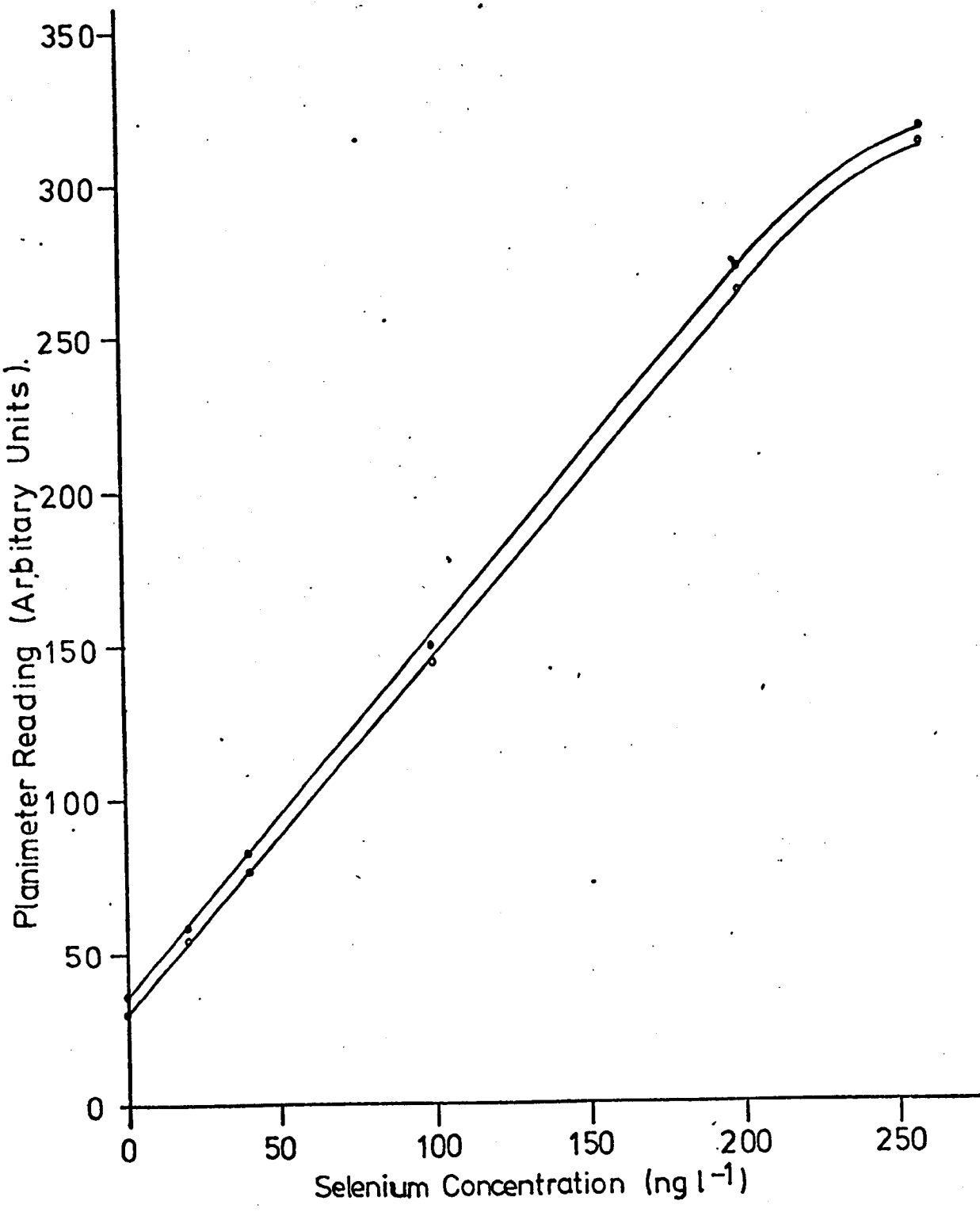


Figure 13. Calibration Curves Using D.A.N.B. Reagent.

Calibration curves and standard deviations were determined by carrying out analyses on samples of deionised water spiked with known amounts of selenite in the range of 0-260ngSe l^{-1}. The procedure described in Section 3.3.2.3. was carried out, 5µl of the extract being injected into the gas chromatograph under the conditions described in Section 4.5.. A planimeter was used to measure the areas of the peaks as this produced the most reproducible results.

6 replicate analyses were performed on standards containing various concentrations of selenium in deionised water so as to be able to determine the standard deviations at selenite levels between 0 and 200ngSe l^{-1} (Table 17) and to estimate the limit of detection.

The detection limit was found to be about 6ngSe l^{-1}, which could be slightly improved by the use of more sensitive equipment.

4.6.2. Calibration Curves.

The data for two calibration curves were collected using the method and the G.C. conditions described above (Sections 3.3.2.3. and 4.5.). The results, shown on Table 18 and Fig. 13, show that the curve deviates from linearity at a concentration of about 220ngSe l^{-1}. This will vary from instrument to instrument and may be extended by increasing the volume of the toluene used for extraction or by reducing the volume of the sample.

4.6.3. Interfering Substances.

As there is a great similarity between the D.A.N.B. and the D.A.D.B.B., and the latter suffered no interference, it was assumed that the former would be free of interference as well.

4.6.4. Conclusions on the D.A.N.B./G.C. Method for the Determination of Selenite.

It can, thus, be concluded that the D.A.N.B./G.C. method described below in Section 4.6.5. is useful for the determination of selenium at concentrations above 6ngSe l^{-1}. The limit of detection could be slightly improved by the use of a more precise peak measuring technique. Interferences are, by implication, negligable (vis Sect. 4.4.) and therefore the method is suitable for measuring the selenite concentration of natural waters.

Although this method is less sensitive than that using the D.A.D.B.B. reagent the fact that D.A.N.B. is commercially available makes its use far more convenient. However, the acid concentration required is well below that needed for many of the methods used for the conversion of selenium (0), (-II) and (VI) to selenite (vis Chapter 7), which limits its usefulness.

4.6.5. Final Method for the Determination of Selenite Using the D.A.N.B. Reagent.

A 250ml sample was washed with 25ml of toluene so as to remove any toluene soluble material and to saturate the water with the solvent. The sample was then transferred

to a 500ml separating funnel, and 5ml of the D.A.N.B. reagent was added. After mixing, the pH of the solution was checked to ensure that the pH lay between 1.75 and 1.95. if it did not it was adjusted with 0.5M hydrochloric acid or 0.5M sodium hydroxide, as appropriate. The sample was then allowed to stand for 120mins. in subdued light. 1ml of toluene was added and the mixture was shaken vigorously for 2mins.. The organic phase was separated off and 5µl were injected into the gas chromatograph. The extract was found to be stable for at least a week if kept at a temperature of $-4^{\circ}C$.

The gas chromatographic conditions were:- A 2m x 4mm (i.d.) glass column packed with 3% Silar 10C on Chromosorb W (60-80 mesh, A.W., D.M.C.S.) at $195^{\circ}C$. The electron capture detector was maintained at a temperature of $265^{\circ}C$. The carrier gas used was 90% argon/10% methane at a flow rate of 65ml min^{-1}.

CHAPTER 5.

5. Direct Fluorimetric Method for the Analysis of Selenite Using 2,3-Diamino-Naphthalene.

5.1. Principle of the Method.

Selenite reacts, in acidic media, with 2,3-diamino-naphthalene, to give the highly fluorescent piazselenol, naphtho-(2,3-d)-selena-1,3-diazol, which can be extracted into cyclohexane and then determined fluorometrically. Although a number of substances interfere with the method, some of them can be removed by masking with E.D.T.A..

5.2. Reagents.

i) D.A.N. Reagent.

2,3-diamino-naphthalene, (D.A.N.), (Aldrich Chemical Co. Ltd.), was recrystallised from deionised water, (about 0.1% w/v). The solution was allowed to cool in the dark. The white precipitate was filtered off, washed with deionised water and dried "in vacuo" , overnight in a refri gerator. A 0.1% w/v solution of the recrystallised D.A.N. was made up in 0.1M hydrochloric acid. This sometimes required gentle heating in a water bath at 50°C. On cooling, the solution was extracted twice with fractionated cyclohexane. The reagent solution was found to be stable for at least a week, if kept at 4°C, in the dark. If kept at room temerature, however, it must be used immediately.

ii) Ethylene-diamine-tetra-acetic Acid (E.D.T.A.).

A 0.1M solution of E.D.T.A. was made by dissolving 1.45g of the compound (disodium salt) (Analar) in 15ml of 2M ammonia

solution and then making this up to 50 ml using deionised water.

iii) Hydrochloric Acid Solutions.

Concentrated hydrochloric acid (M. & B. Lab. Reagent Grade) was diluted with deionised water to give 0.5 and0.1M solutions. These were then washed with fractionated cyclohexane.

iv) Cyclohexane.

Analytical grade cyclohexane (B.D.H. Chemicals) was fractionally distilled, the middle third (B.P. 81°C) being retained for use.

v) Selenite Standards.

Standards were prepared and standardised in the same way as is described in Section 3.2.1. vii)..

5.3. Development of the Method.

As a preliminary to the study of the fluorometric method, tests were carried out using selenium-75 tracer to determine the optimum pH, concentration of D.A.N. and time required for the completion of the formation of the piazselenol.

5.3.1. Optimum pH Conditions for the Formatiom of the Piazselenol.

250ml aliquots of deionised water were transferred to a series of 250ml Erlenmeyer flasks and spiked with 5ml of the selenium-75 solution (selenite) described in Section 3.2. and 3.2.1.. To each solution was added 0.5ml of E.D.T.A. solution and the pH was adjusted to various values between 1.25 and 3.00 with 0.5M hydrochloric acid. A 5ml aliquot

Table 19.

The Effect of pH on the Recovery of Selenium (IV) Using the D.A.N. Reagent.

pH Before D.A.N. Reagent Addition.	%age ^{75}Se Recovered.
1.25	65
1.50	72
1.80	76
2.02	79
2.32	78
2.75	80
3.00	79

Table 20.

The Effect of pH on the Fluorescence Blank of the Direct Fluorometric Method.

pH Before D.A.N. Reagent Addition.	Fluorescence Blank Arbitrary Units (A.U.).
1.80	46
1.91	50
2.01	44
2.20	52
2.35	54
2.45	75
2.60	100

Figure 14. Effect of pH on formation of piazselenol from DAN and on fluorescence of blank.

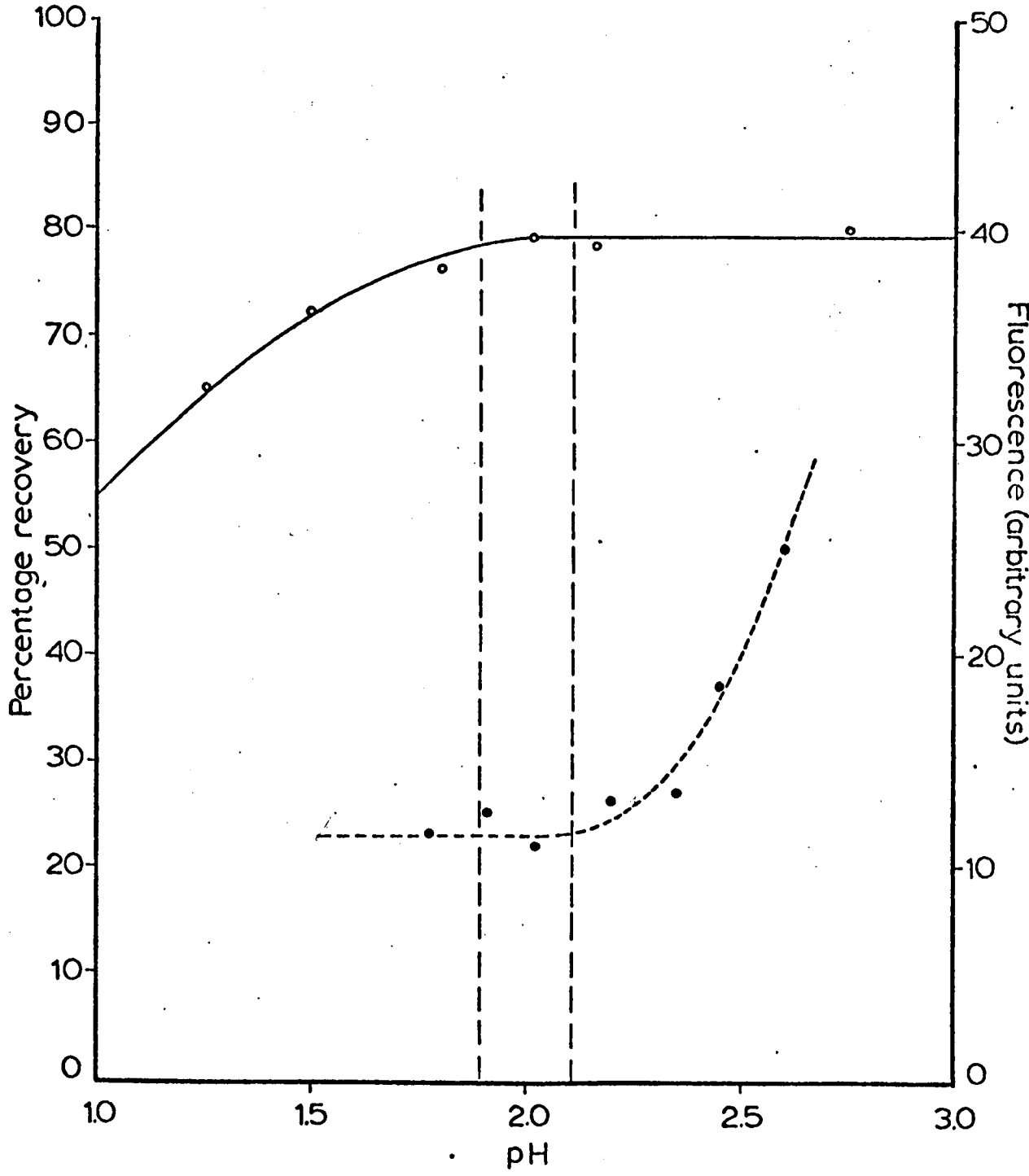
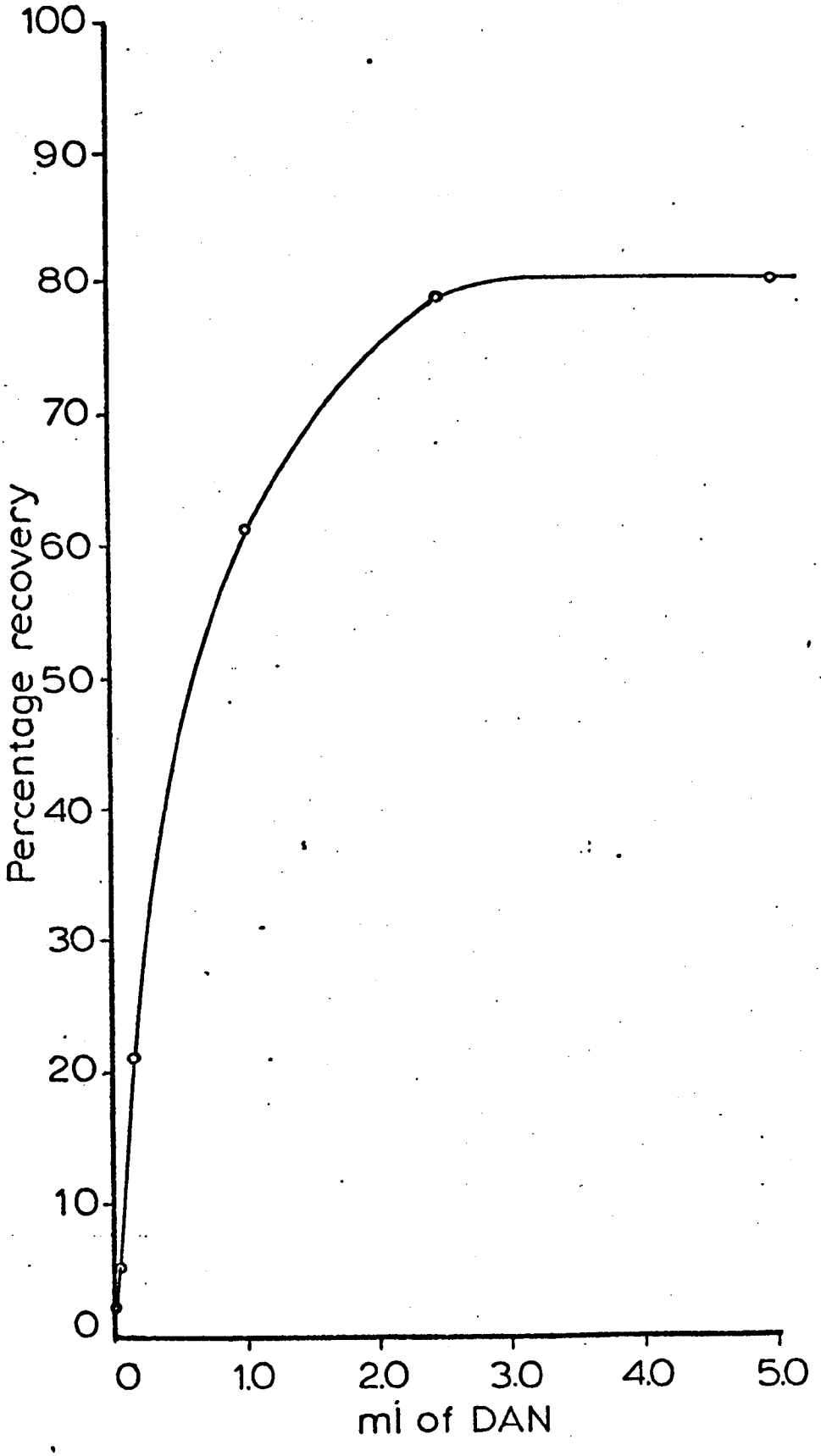


Figure 15. Effect of volume of DAN added on selenium recovery.



of the D.A.N. solution was added and the mixture was heated to 50°C, on a water bath, for 120mins. On cooling, the solution was shaken with 10ml of cyclohexane to extract the piazselenol. The organic phase was isolated and 1ml was counted and compared with the count of an aqueous standard (Sect. 3.2.1.) to determine the percentage extracted (Table 19).

Complete extraction was not achieved, the maximum recovery being about 79% at a pH value of above 1.9 (Fig. 14).

However, it was found that there was a rapid increase in the value of the fluorescing properties of the blank above a pH value of about 2.2 (Table 20, Fig. 14). The optimum pH conditions are, therefore, between 1.8 and 2.2..

An investigation into the reason for this increase in the fluorescence of the blank showed that it is due to decomposition products of D.A.N. as it becomes more unstable with increasing pH rather than to impurities in the reagent being held in solution at higher acid concentrations.

5.3.2. Optimisation of the Quantity of D.A.N. Reagent Required for Maximum Extraction.

As the fluorescence blank is high it is imperative to use the least possible amount of D.A.N. consistant with retaining maximum extraction of selenium. Using conditions otherwise identical to those described in Section 5.3.1., the amount of reagent added was varied between 0.004ml and 5ml. It was found that 5ml of the reagent was necessary for maximum recovery of selenium (Table 21, Fig. 15).

Table 21.

Volume of D.A.N. Reagent Required for Maximum Extraction of Selenium(IV).

ml of D.A.N. Reagent Added.	%age ^{75}Se Extracted.
0.004	2
0.010	5
0.200	21
1.000	61
2.500	78
5.000	80

Table 22.

Reaction Time Required to Achieve Maximum Extraction.

Reaction Time at 50°C (mins).	%age ^{75}Se Extracted.
15	37
30	54
45	64
60	72
75	75
90	76
105	79
120	79

Figure 16. Rate of formation of piazselenol from DAN

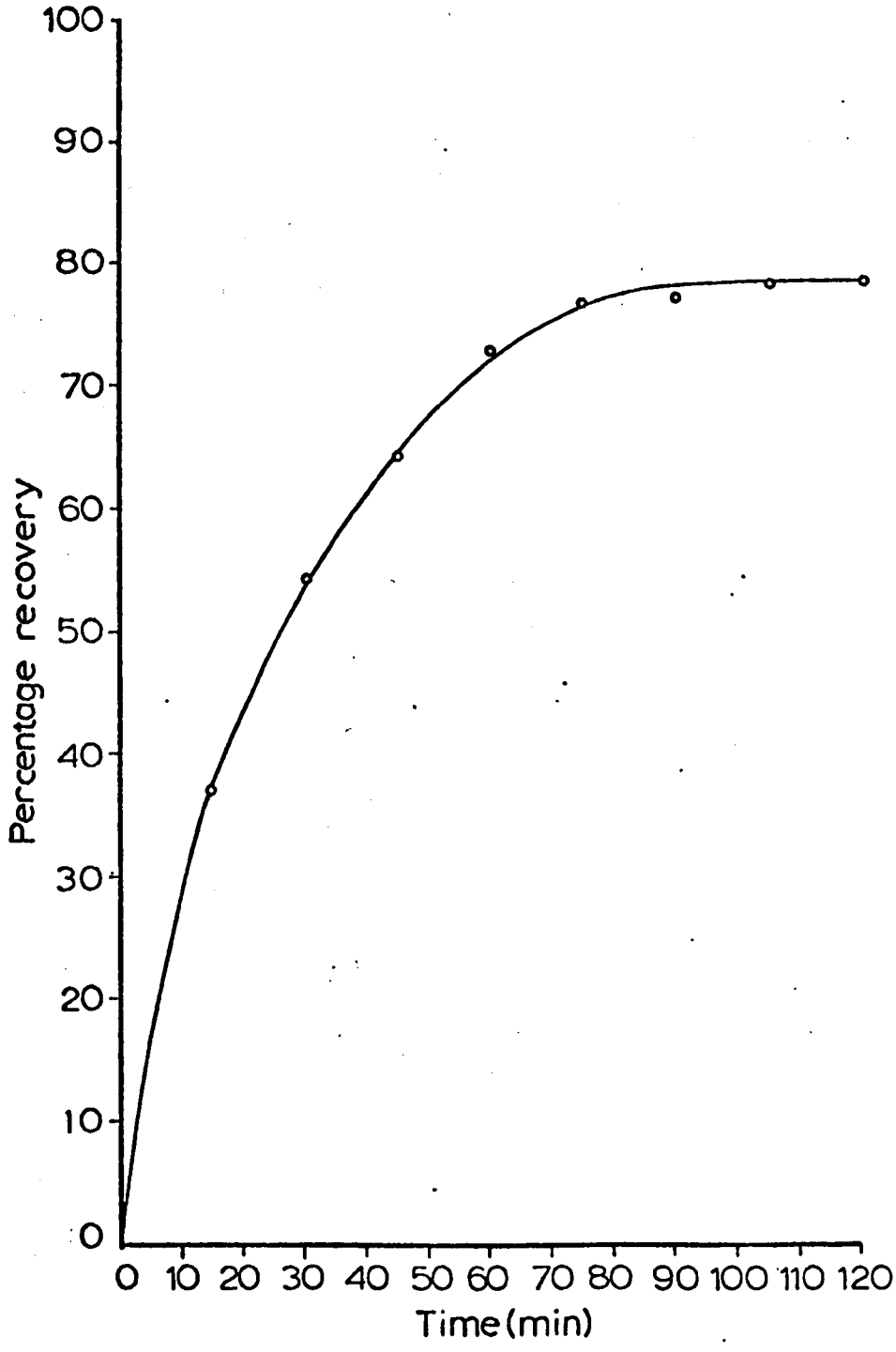


Figure 17.

Excitation Spectrum of D.A.N. Piazselenol.

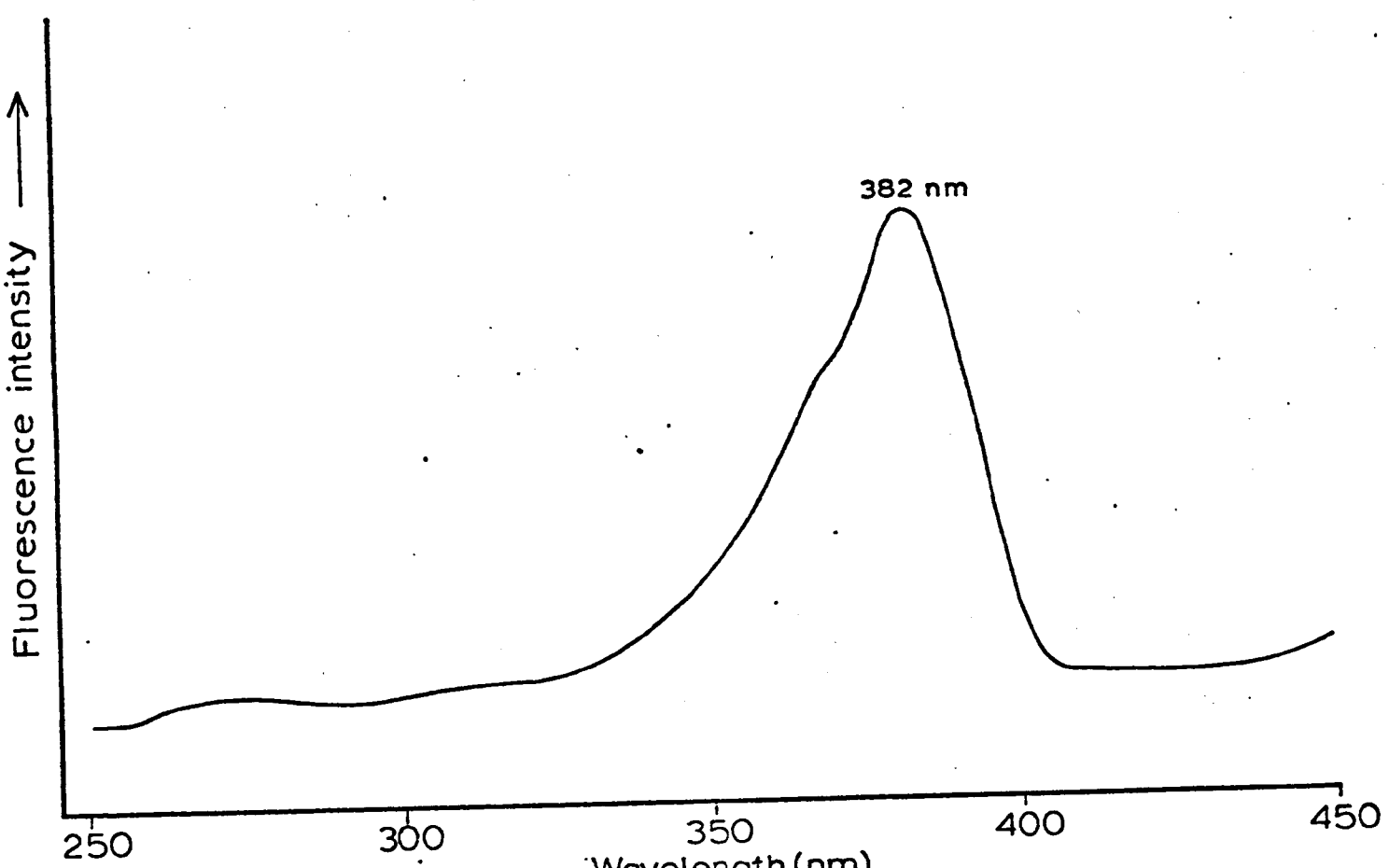
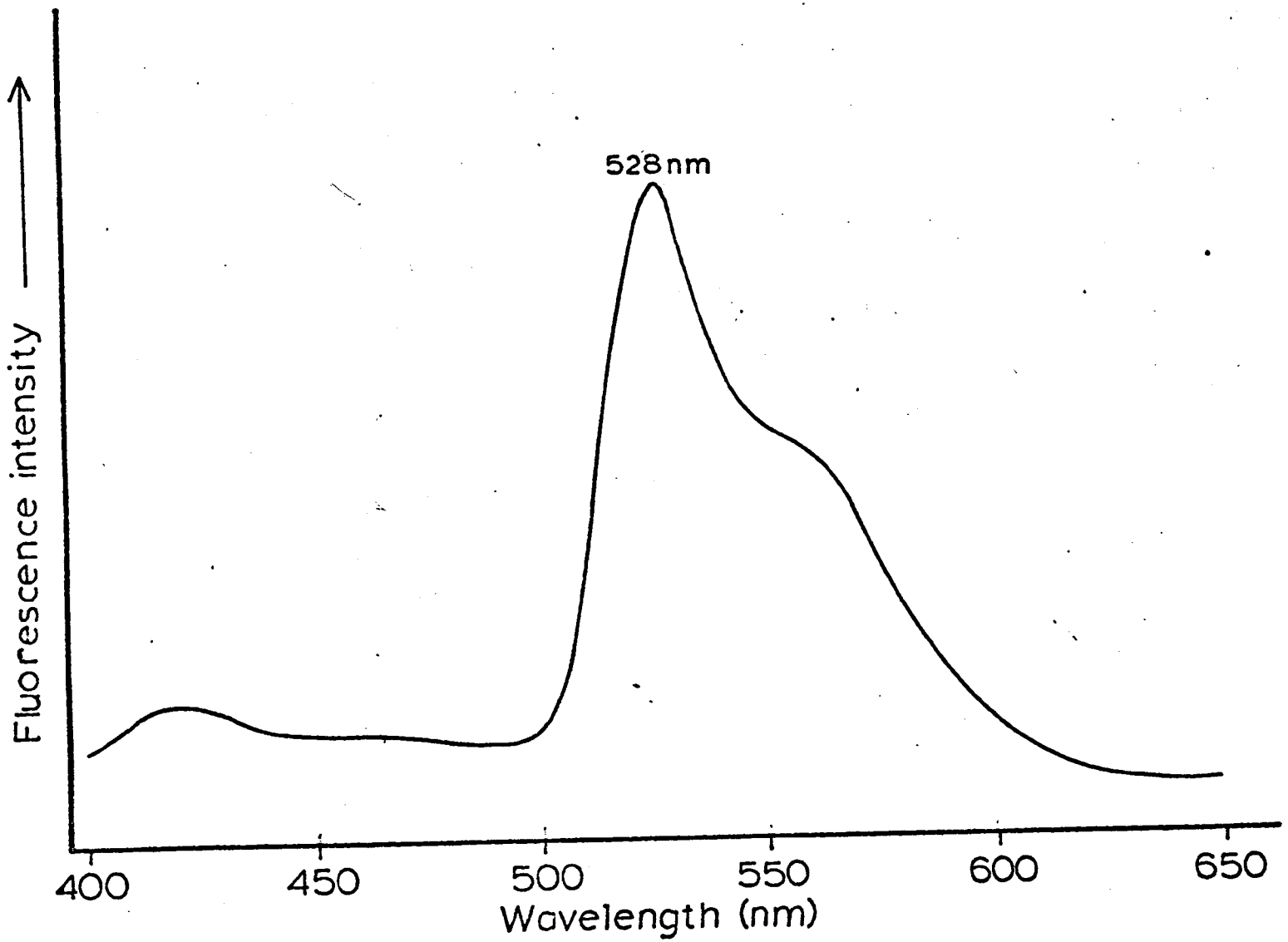


Figure 18.

Emission Spectrum of D.A.N. Piazselenol.



5.3.3. Reaction Time Required for Maximum Extraction of Selenium.

Using condtions, otherwise identical to those described in Sections 5.3.1. and 5.3.2., the time allowed for the reaction to continue was varied from 15 to 120mins.. It was found that the reaction should be allowed to proceed at least 100mins before carrying out the extraction (Table 22, Fig. 16).

5.4. Fluorimetry of the Selenite/D.A.N. Complex.

5.4.1. Equipment Used.

Two fluorimeters were used in the course of the present work, a Baird Atomic Fluorospec., Model SF-100, connected to a Bryans Ltd. X-Y Plotter, Model 21000, and a G.K. Turner Associates Fluorimeter.

5.4.2. Investigation of Excitation and Emission Spectra.

The characteristics of the excitation and the emission spectra of the selenium/D.A.N. complex were investigated in a preliminary study. A quantity of the compound was prepared by adding the D.A.N. reagent to a strong selenite solution. The piazselenol precipitated was filtered off and recrystallised from aqueous ethanol. A known quantity of the dried product was then dissolved in fractionated cyclohexane and diluted to give a concentration of about 0.1ugSe l^{-1}. The excitation and emission spectra were then recorded, using the "Fluorspec.". The excitation and the emission maxima were found to be at 382nm and 528nm respectively (vis Fig. 17 and Fig. 18).

On the basis of these results, suitable Wratten filters

were selected for the Turner fluorimeter. That chosen as the excitation filter was the Wratten 7-60 and that for the emission filter was the Wratten 58A. Unfortunately, although each has a wide bandpass, they are not quite of the required wavelength. This reduces the potential sensitivity and increases the possibility of interference from other fluorescing substances in the extract.

5.5. Method Used for the Reduction of the Blank.

Initially the fluorescence blank was found to be so high that it registered off scale on the Turner fluorimeter. The level of this fluorescence was considerably reduced by carrying out the entire procedure in subdued light. A further considerable reduction was produced by back extracting the cyclohexane with 25ml of 0.1M hydrochloric acid, twice.

5.6. Final Method for the Direct Fluorimetric Method of Determination of Selenite.

A 250ml sample was washed with 25ml of cyclohexane and transferred to a 250ml Erlenmeyer flask. 0.5ml of E.D.T.A. solution was added and the pH was adjusted to 2.0±0.1 with 0.5M hydrochloric acid. A 5ml aliquot of the D.A.N. solution was added and the mixture was heated to 50°C in a water bath for 100mins. in the dark. On cooling, the mixture was extracted into 10ml of fractionated cyclohexane by shaking for 10mins. on an orbital shaker. The two phases were then separated in a 500ml separating flask. After this the cyclohexane was washed twice by back-extraction with 25ml of 0.1M hydrochloric acid. Finally the cyclohexane was centrifuged

Table 23.

Reproducibility of the Direct Fluorometric Method at Various Concentrations of Selenium(IV).

Concentration of Selenium μgSe l^{-1}.	Turner Fluorimeter.		"Fluorospec".	
	Fluorescence A.U. ($\bar{x}$).	Coeff. of Variation.	Fluorescence A.U. ($\bar{x}$).	Coeff. of Variation.
0.00	17	10	5.00	20.0
0.04	26	8	-	-
0.08	37	7	-	-
0.12	46	6	-	-
0.16	56	5	-	-
0.20	72	5	-	-
0.40	-	-	22.6	5.0
1.00	-	-	34.2	4.0
10.00	-	-	51.2	2.5

n.b. The sensitivity of the "Fluorospec" was altered to bring the response for 10.00ugSe l^{-1} on scale, leading to the apparent discrepency in results.

to remove any remaining aqueous phase. The fluorescence of the solution was then measured on either the Turner fluorimeter or the "Fluorospec".

5.7 Evaluation of the Direct Fluorimetric Method for the Determination of Selenite.

The method was tested for its suitability for use in the analysis of selenite at both low, natural levels (0-200ngSe l^{-1}) and at higher, polluted levels (up to 10µgSe l^{-1}).

5.7.1. Sensitivity and Reproducibility.

The value of the blank, particularly at low levels, was highly variable from day to day. On a number of occasions it was sufficiently large to swamp any signal from the selenium/D.A.N. complex.

Standard deviations were determined at levels between 0.04 and 10µgSe l^{-1} by analysing 250ml aliquots of deionised water spiked with known quantities of selenite standard. Replicate analyses (6) were carried out using the method descibed in Section 5.6.. The readings for selenium concentrations of up to 200ng l^{-1} were obtained using the Turner fluorimeter and those at selenium concentrations exceeding 200ng l^{-1} were obtained using the "Fluorospec". Using both instruments, the detection limit of the method was found to be about 50ngSe l^{-1} (*vis* Table 23).

The sensitivity of the "Fluorospec" was altered to bring the higher readings on scale, leading to an apparent discrepency in the response.

Table 24.

Calibration Curves for the Direct Fluorometric Analysis of Selenium (IV)

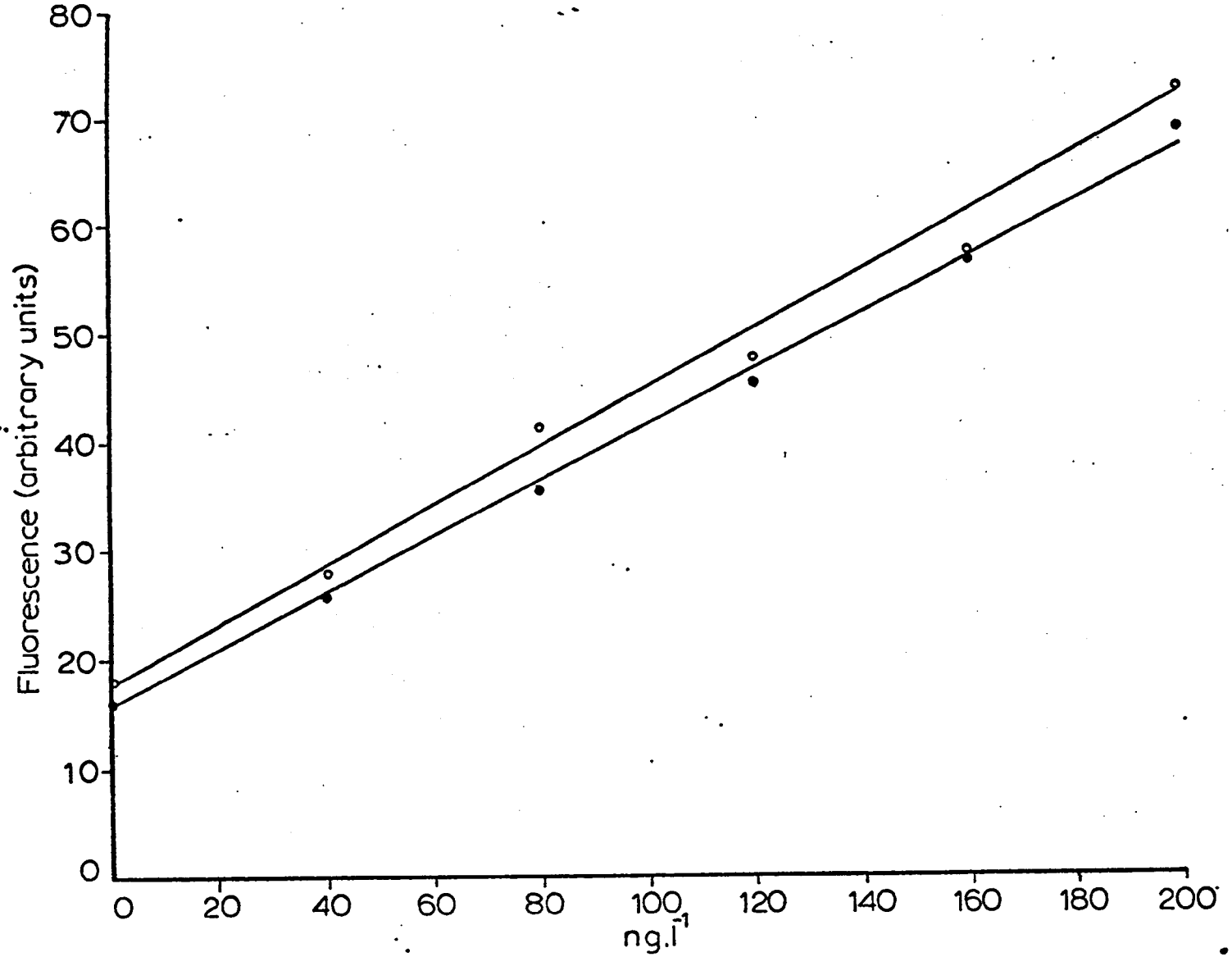
Concentration of Selenium ($\mu g\ l^{-1}$).	Readings on Turner Fluorimeter.		Readings on "Fluorospec".
0.00	17	3	3
0.04	26	-	-
0.08	38	-	-
0.12	46	-	-
0.16	57	-	-
0.20	70	-	-
1.00	-	6	5
2.50	-	12	8
15.00	-	82	51
30.00	-	146	102

Table 25.

Examination of the Effect of Various Substances on Direct Fluorometric Analysis of Selenium at 100ngSe l^{-1} and 1.00µgSe l^{-1}.

Interfering Substance (I)	Concentration of (I) (mg l^{-1})	%age of Fluorescence Without (I)	
		100ngSe l^{-1}	1.0µgSe l^{-1}
Na^{+}	300	106	103
K^{+}	20	100	97
Mg^{2+}	100	104	102
Ca^{2+}	300	108	97
Cd^{2+}	2	160	101
Sr^{2+}	3	96	102
NO_2^{-}	500	70	96
NO_3^{-}	50	70	100
F^{-}	10	106	102
Co^{2+}	5	102	104
Fe^{3+}	1	70	99
Cu^{2+}	5	100	100
Det.	50	104	102
Fulvic Acid	50	99	101

Figure 19. Determination of low levels of selenium by fluorimetry (Turner fluorimeter).



Determination of low levels of selenium by fluorimetry (Turner fluorimeter).

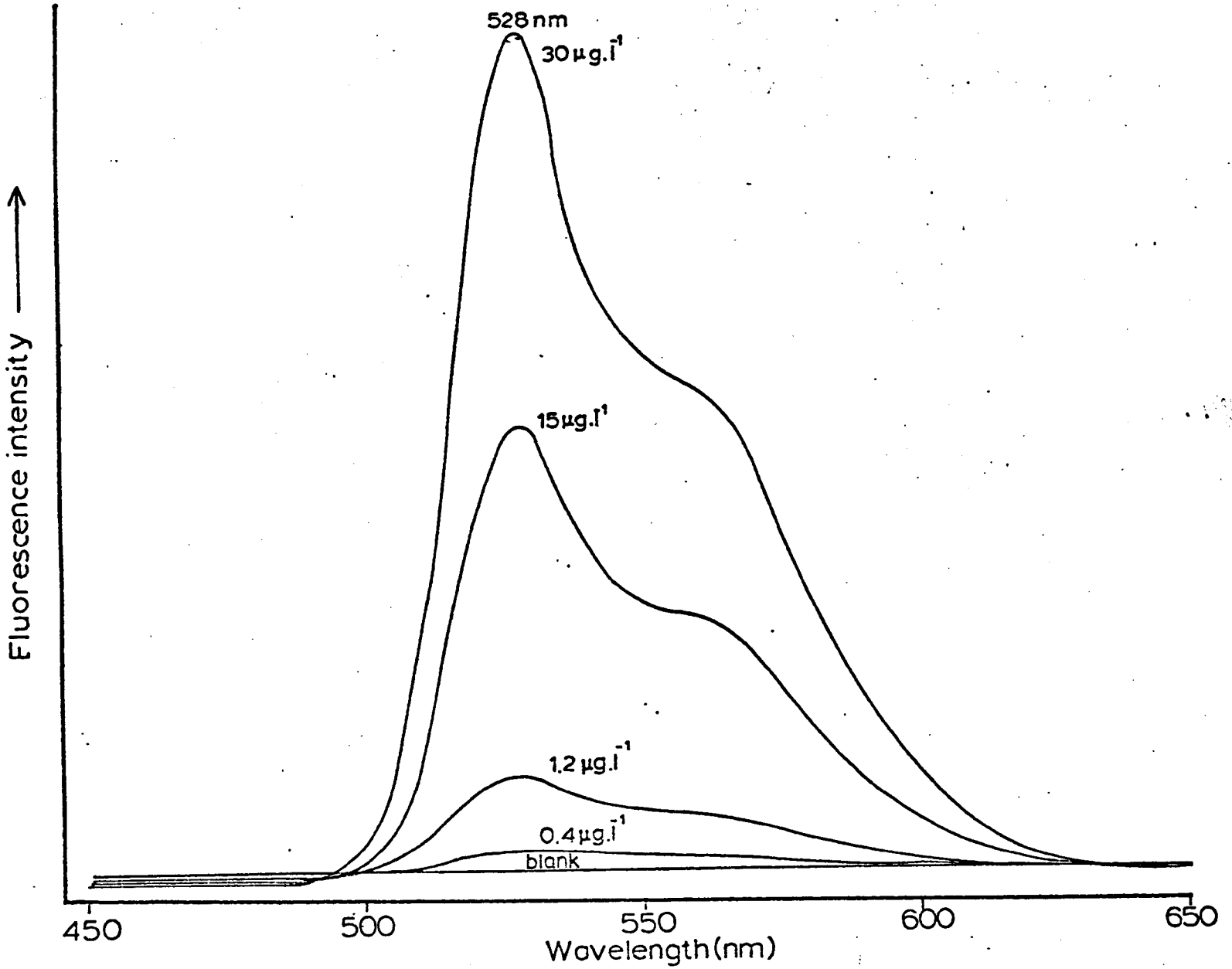
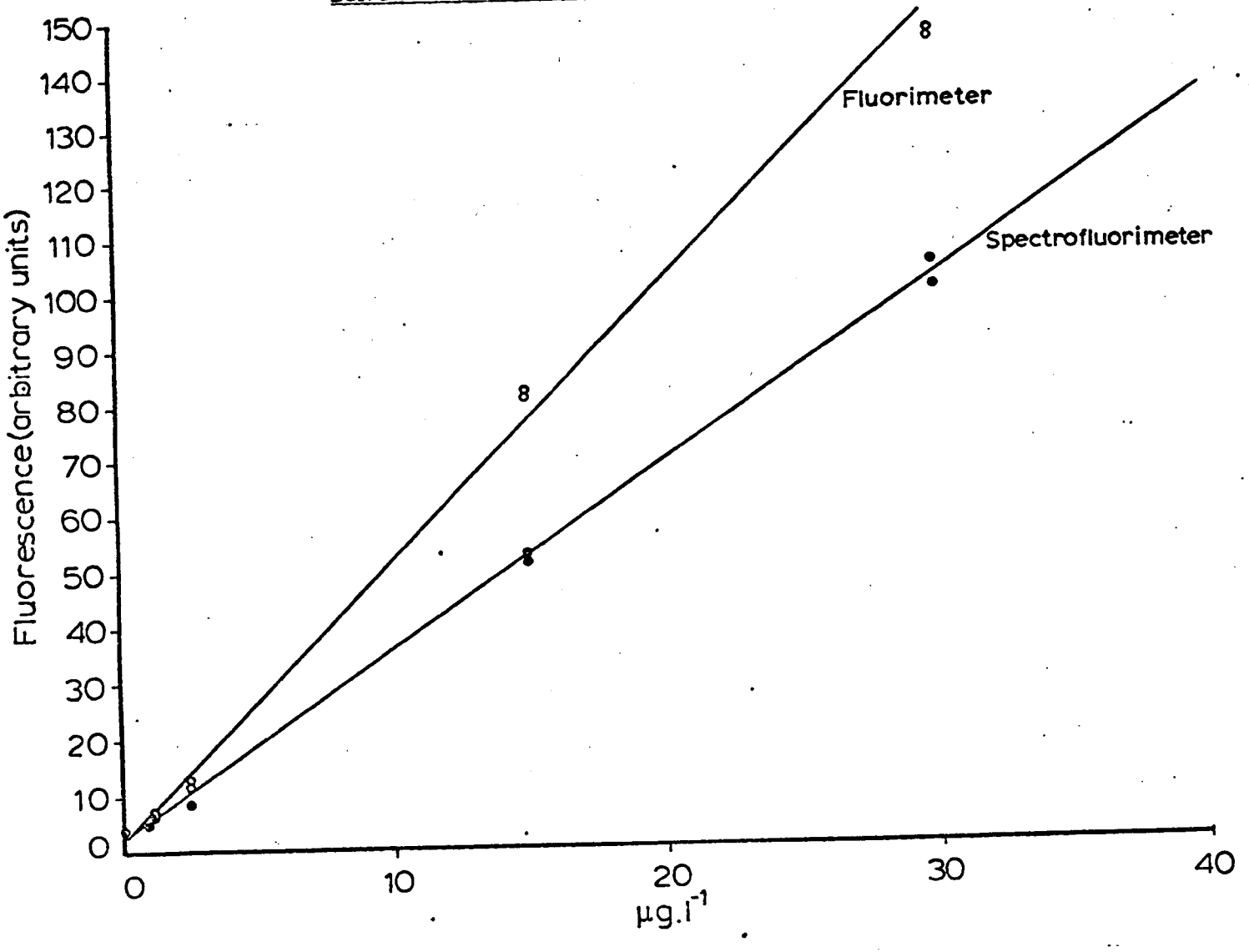


Figure 20. Fluorescence spectra with various levels of selenium (excitation with 382nm radiation) (high level).

Figure 21. Calibration curves for high level selenium obtained with Turner fluorimeter and Baird Atomic spectrofluorimeter.



5.7.2. Calibration Curves.

The data for calibration curves at less than 200ngSe l^{-1} and more than 200ngSe l^{-1} were collected by spiking deionised water with known quantities of standard selenite solution using the method outlined in Section 5.6. (Table 24, Figs. 19, 20 and 21.). The "more than 200ngSe l^{-1}" calibration curve results were obtained from both the Turner and the "Fluorspec" (Fig. 20). In general it was found that the "Fluorspec" gives far more reproducible results.

5.7.3. Testing of Possible Interferences.

The effects of possible interfering substances were investigated using concentrations recommended by the Water Research Centre (Medmenham Laboratory S.C.A. Paper No. 226). Iron (III), nitrate and nitrite were found to supress the signal significantly while cadmium was found to enhance the signal of the extract of a solution containing 100ngSe l^{-1}. At higher selenium concentrations (1µgSe l^{-1}) no significant effect was noted (Table 25).

5.8. Conclusions on the Direct Fluorometric Method for Selenium (IV).

At low (less than 200ng l^{-1}) selenium concentrations the considerable interference from a number of common ions and the high variability of the blank render the method of little use.

At higher (more than 500ng l^{-1}) selenium concentrations the method is a quick, reliable determination of selenium (IV) in natural waters.

CHAPTER 6.

6. Preconcentration of Selenium for the Fluorimetric Method of Analysis.

A study of the literature (Sect. 2.1.) revealed that three methods of preconcentration showed promise whilst also separating selenium from possible sources of interference. These were, the evolution of hydrogen selenide, separation of a selenium complex on a macroreticular resin and co-precipitation.

6.1. Evolution of Hydrogen Selenide Gas.

In acidic, aqueous media, selenite can be reduced by the action of sodium borohydride to give hydrogen selenide gas. Selenate is only reduced to a very small extent, at normal temperature and pressure (Sinemus et al, 1981). The hydrogen selenide formed can then be removed from the solution either by bubbling with nitrogen or by the water being degassed by the hydrogen formed by the decomposition of the sodium borohydride. The gas can then be collected either in an iodine/iodide solution or in a cold trap.

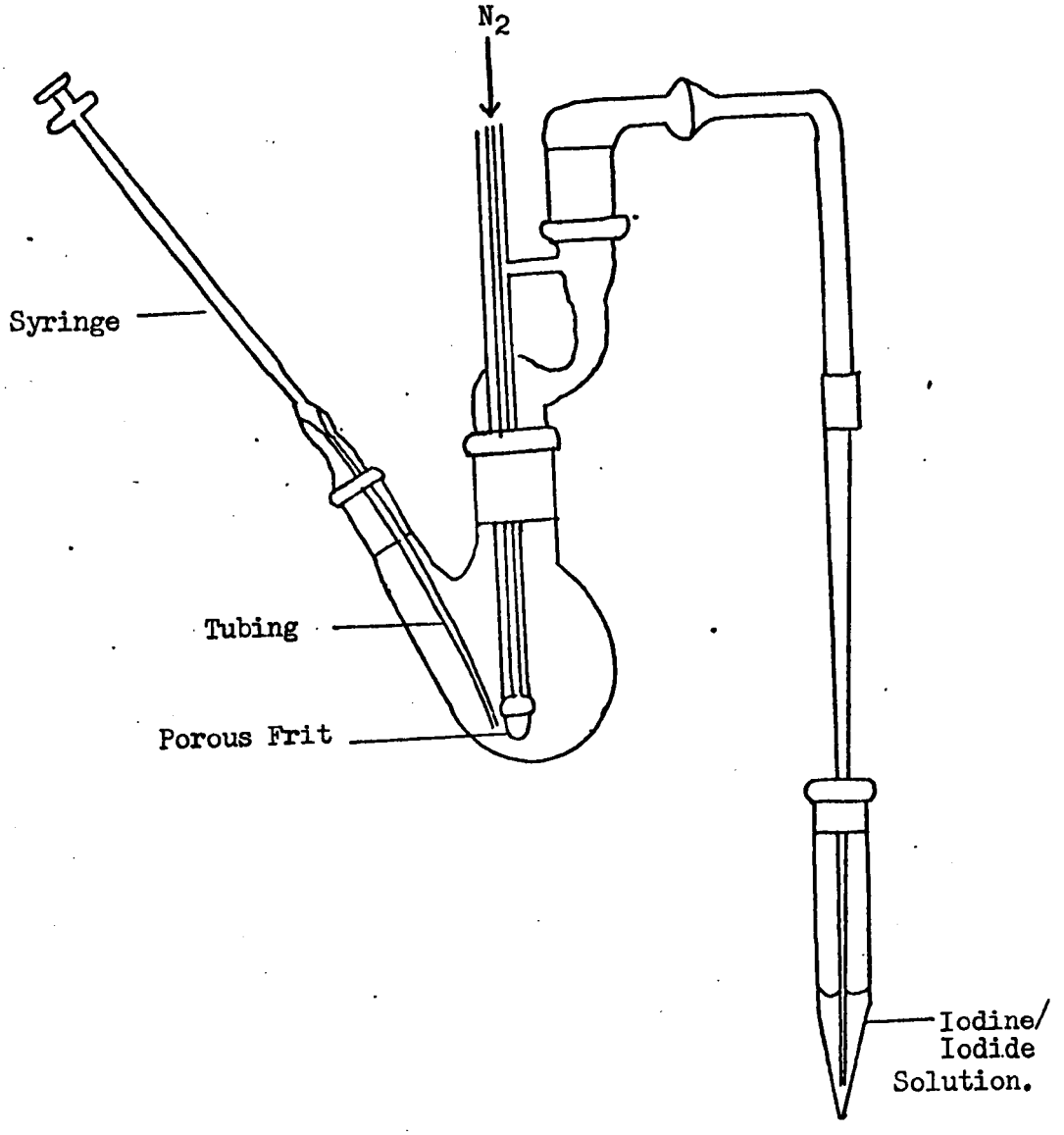
6.1.1. Reagents.

i) Sodium Borchydride Solution.

25g of powdered sodium borohydride was dissolved in 100ml of deionised water. The solution was filtered and then stirred with 2g of calcium hydroxide, warmed to 75°C for 20 mins., cooled and then filtered again. Finally the volume was made up to 250ml with deionised water.

Figure 22.

Apparatus for Generation of H_2Se from Maher (1980).



ii) Iodine/Iodide Solution.

0.25g of Specpure iodine was dissolved in 5ml of a 10% w/v solution of potassium iodide.

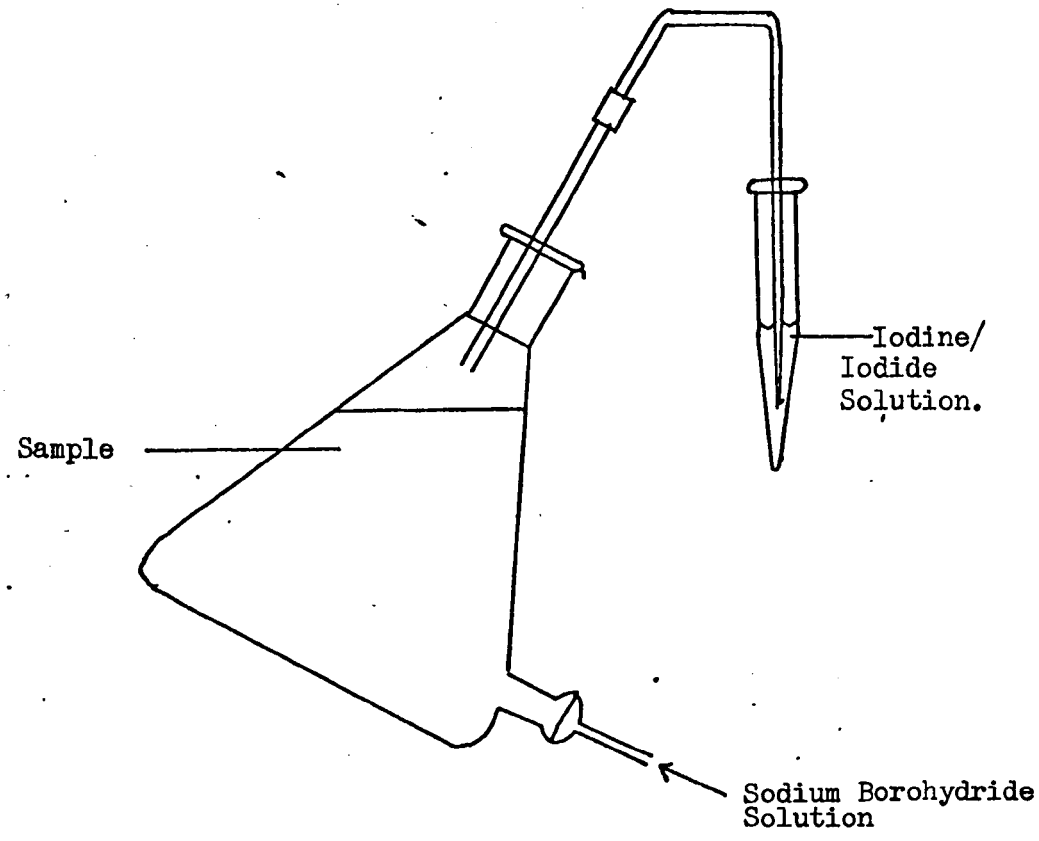
6.1.2. Procedures Using the Iodine/Iodide Trap.

Haywood and Riley (1976) found that a solution of iodine in iodide was capable of absorbing gaseous hydrogen selenide. This approach was used by Maher (1980) in a procedure in which a 50ml sample of the analyte was made 4M with respect to hydrochloric acid and placed in the flask of the apparatus shown in Figure 22. Sodium borohydride solution (1ml) was then injected into the sample. A stream of nitrogen was used to transfer the hydrogen selenide formed to a trap containing 2ml of the iodine/iodide solution.

This procedure was then tested by spiking 50ml of acidified deionised water with a 1ml aliquot of the selenium-75 solution described in Sections 3.2. and 3.2.1., injecting 1ml of the sodium borohydride solution and sweeping the hydrogen selenide out of the sample into a 2ml aliquot of the iodine/iodide solution. The recovery was then determined by measuring the counts from the selenium-75 recovered in 1ml of the trap solution and comparing this to the counts obtained from the aqueous selenium-75 standard. The recovery was found to vary between 10 and 50% with an average of 30% (15 replicates). It was decided, therefore, to abandon this form of generation of hydrogen selenide in favour of a modification of the method for arsenic developed by Haywood and Riley (1976).

Figure 23.

Apparatus for Generation of H_2Se from Haywood and Riley (1976).



The sodium borohydride was gradually introduced to 250ml of acidified sample at a rate of about 15ml hr^{-1} for about 15mins., using the apparatas shown in Fig. 23. The gas es formed were bubbled through 2ml of the iodine/iodide solution.

This method was tested using the radioactive tracer, selenium-75. In the course of 10 analyses only a minimal recovery of 10±5% was recorded and thus, the above method of preconcentration was rejected.

6.1.3. Condersation of Hydrogen Selenide onto a Liquid Nitrogen Trap.

As hydrogen selenide is trapped in its original form, instead of being in the 4+ oxidation state, the method is not compatible with the fluorometric method of determination. This preconcentration technique was, therefore, not tested.

6.2. Adsorption of Selenium/D.D.T.C. Complex on a Macroreticular Resin.

A study was made of the uptake of the selenite/sodium diethyl-dithiocarbamate (D.D.T.C.) complex on the macroreticular resin, Amberlite X.A.D.2 (vis Sect. 2.1.5.).

6.2.1. Reagents.

i) D.D.T.C. Solution.

1g of sodium diethyl-dithiocarbamate was dissolved in 10ml of deionised water.

6.2.2. Procedure.

1ml of the D.D.T.C. solution was added to 5l of deionised water spiked with a 10ml aliquot of selenium-75 solution. (vis Sects. 3.2. and 3.2.1.). The pH of the mixture was adjusted to 4-5, using 1M hydrochloric acid. After stirring,

the mixture was passed through a column (10cms.x 2.5cms. (i.d.)) at a rate of 5ml min^{-1}. After washing with 100ml of hydrochloric acid (pH 4-5), the column was eluted with 200ml of a mixture of 2M nitric acid (190ml) and concentrated perchloric acid (10ml).

The eluate was evaporated down, almost to dryness, and then made up to 10ml using 6M hydrochloric acid (Sugimura and Suzuki, 1977). A 1ml aliquot of the final solution was counted.

The results obtained showed a 40±5% recovery over 6 analyses. Although the degree of concentration was still considerable (200:1) there was sufficient nitrate in the final solution to interfere with the fluorometric determination (vis Sect. 5.7.2.). In addition, the time required for the passage of the solution through the column was about three days, which not only increased the chance of contamination but also, at a pH of 4-5, allows for considerable adsorption of selenite onto the walls of the vessel. For these reasons the method was abandoned.

6.3. Co-precipitation of Elemental Selenium.

When boiled with ascorbic acid in acidic media, both selenite and selenate are reduced to the elemental form of selenium. As auric chloride is also reduced to elemental gold under these circumstances it was decided that this might provide a means of co-precipitating selenium from solution. Boiling with nitric acid should then redissolve the selenium.

6.3.1. Reagents.

i) Ascorbic Acid Solution.

5g of ascorbic acid (Analar) was dissolved in 50ml of deionised water to make a 10%w/v solution.

ii) Auric Chloride Solution.

A 10mg ml^{-1} solution of auric chloride was provided by B.D.H. Chemicals.

6.3.2. Procedure.

5l of deionised water was spiked with 10ml of selenium-75 solution (vis Sects. 3.2. and 3.2.1.). 5ml of the ascorbic acid solution and 0.5ml of the auric chloride solution were added and the pH was adjusted to 4.5, using 0.5M hydrochloric acid. The mixture was then heated to boiling for 10mins.. The gold precipitate was allowed to settle out overnight and then filtered off ,using a GF/C filter. The selenium was then redissolved by boiling the filter with 5ml of concentrated nitric acid. The final mixture was then centrifuged for 15mins. at 3000r.p.m. to separate the residue of the filter. The acid was transferred to a 25ml volumetric flask. The filter was then washed with 5ml of deionised water and centrifuged three times, the washings being transferred to the volumetric flask. The solution was made up to volume and a 1ml aliquot was counted.

The recovery was 50±20% over 10 replicates. The low recovery and the poor reproducibility may be accounted for by failure to remove all the gold from the walls of the boiling vessel because of the strength of the adherence. The addition

of surfactant to the solution appeared to have no effect on either the recovery or the reproducibility (5 replicates). The remains of the filter and the gold showed only insignificant counts in all cases. The method was, therefore, rejected.

6.4. Co-precipitation of Complexed Selenite.

The work described in Section 6.2. prompted an investigation into the co-precipitation of selenite/dithiocarbamate complexes with those of various other metals.

Initially, work was carried out using diethyl-dithiocarbamate complexes of cobalt (II) and copper (II) as carriers. Recoveries, however, were found to be less than 10%. Attention was, therefore, directed to the use of pyrrolidine-dithiocarbamate complexes. The efficiency of copper (II), mercury (II), lead (II), cobalt (II) and zinc (II) as the carrier was investigated.

6.4.1. Reagents.

i) Ammonium Pyrrolidine-dithiocarbamate (A.P.D.C.) Solution.

1.0g of A.P.D.C. was dissolved in 100ml of deionised water, to give a 1% solution.

ii) Metal Ion Solutions.

0.2g of copper sulphate, 0.2g of cobaltous chloride, 0.125g of mercuric chloride, 0.125g of lead nitrate or 0.2g of zinc chloride were dissolved in 50ml of deionised water to give a metal concentration in solution of 2ng ml^{-1}.

Table 26.

Percentage Recovery of Selenium-75 from Co-precipitation with A.P.D.C. Complexes of Various Metals.

Metal Ion (2+).	% Se-75 Recovered (6 replicates).
Cu	100±5
Co	10±4
Hg	63±7
Pb	76±6
Zn	73±8

6.4.2. Procedure.

1l of deionised water was spiked with 5ml of selenium-75 solution (Sects. 3.2 and 3.2.1.) and the pH was adjusted to 4 using 0.1M hydrochloric acid. 10ml of the A.P.D.C. solution was mixed into the solution which was allowed to stand for 4hrs, to allow the precipitate to settle. After filtration, the precipitate was dissolved in 5ml of chloroform. A 1ml aliquot was removed and counted.

The recoveries, using the various metals as carriers are shown in Table 26. The results showed that copper was an efficient carrier and further work was , therefore, carried out on the preconcentration of selenite using the copper/ A.P.D.C. complex as a carrier.

Prior to analysis it was necessary to destroy the complex. Initially, 3ml of nitric acid was used for this purpose. As nitric acid interferes with the fluorimetric determination (vis Sect. 5.7.2.), attempts were made to remove the acid by evapo ration on a water bath. Unfortunately, it was necessary to evaporate the mixture to dryness to reduce the amount of nitrate present to an acceptable level, and this led to a considerable volatilisation of the selenium. The extent of this loss could be reduced by the addition of two milliequivalents of sodium hydroxide, but even so an unacceptable proportion was lost.

It was decided that hydrogen peroxide might be preferable to nitric acid for the decomposition of the complex. To evaluate this approach the precipitate, obtained as is described above, was heated to 60°C on a water bath with

1ml of 30% hydrogen peroxide and 25ml of 10% hydrochloric acid and it was found that the complex was satisfactorily decomposed. The use of selenium-75 tracer showed that none of the element was lost under these conditions. However, it was discovered that a large and highly variable proportion of the selenite was oxidised to selenate by the hydrogen peroxide. Although it would be possible to reduce the selenate to selenite (vis Chapter 7), another long-winded method would be added to an analysis already prone to contamination and interference. It was decided, therefore, to reject this method.

6.5. Conclusions on the Preconcentration of Selenium Prior to Fluorimetric Analysis.

Although a number of methods which were investigated successfully preconcentrated reproducible quantities of selenite from solution, the interferences inherent in the fluorimetric method of determination and the time required rendered all of them impractical to normal use.

CHAPTER 7.

7. Conversion of Selenium (-II), (0), and (VI) to Selenium (IV).

The fluorimetric and gas chromatographic methods for the determination of selenium, described in Chapters 3, 4 and 5, rely on the formation of various piazselenols from 1,2-diamino-aromatic compounds and the oxy-anion, selenite (SeO_3^{2-}). In most natural waters, however, a large proportion of the selenium may be present as selenate (SeO_4^{2-}) (vis Sect. 1.2.), but under reducing conditions it may exist as selenide (Se^{2-}) and the element (Se^0). Before total selenium can be determined it is necessary to convert these forms to selenite, these other species being determined by difference. In most of the methods which were investigated for achieving this conversion it was assumed that if selenate is present the reduced forms will be absent and vice versa, depending on the redox conditions prevailing in the sample.

7.1. Sulphuric Acid/Titanium Trichloride Reduction-Bromine/Bromide Redox Buffer Oxidation.

In this method, which was developed by Shimoishi and Tôei, (1978), all oxidised forms of selenium were reduced with titanium (III) to the element which was then oxidised to selenite using a bromine/bromide redox buffer.

7.1.1. Reagents.

i) "Selenium Free" Sulphuric Acid.

Hydrobromic acid (20ml) was added to 200ml of 50% v/v sulphuric acid and the mixture was boiled until dense white

fumes were evolved.

ii) Titanium Trichloride.

A 20% w/v solution of titanium trichloride was used (B.D.H. Chemicals).

iii) Bromine/Bromide Redox Buffer Solution.

Potassium bromide (2.38g) was dissolved in 50ml of saturated bromine water and the solution was diluted to 100ml with deionised water. This solution was stable if stored at 4°C in the dark.

iv) Hydrazine Hydrochloride Solution.

Hydrazine hydrochloride (7g) (Analar) was dissolved in 100ml of deionised water to give a 1M solution.

7.1.2. Procedure.

A 250ml sample was transferred to a 500ml conical flask and 2.5ml of selenium-free sulphuric acid and 0.25ml of titanium trichloride solution were added. The contents of the flask were then heated to 90°C in a water bath for 10mins. After cooling, bromine/bromide redox buffer was added until the solution was a light straw colour (about 7ml), and reheated to 90°C for 10mins. The solution was cooled and 1ml of hydrazine hydrochloride solution was added to convert any remaining bromine to bromide. Selenium (IV) was determined using the D.A.D.B.B. method described in Section 4.4.4..

7.1.3. Testing the Method.

The efficiency of the reduction was investigated using the radioactive tracer selenium-75 (vis Sect. 3.2.). To do this 25ml of the selenium-75 (IV) working standard was oxidised

to the (VI) state by means of potassium permangenate. This solution was tested for selenite content using the D.A.D.B.B. method. It was found to contain no selenite and it was, therefore, assumed that oxidation had been quantitative. Aliquots of the standard containing 2.8 and 14 ngSe(VI) were added to 250ml samples of deionised water. Reduction was then carried out, followed by the D.A.D.B.B. extraction method. It was found that at levels of 11.2 and 56ng Se l^{-1}, 43.5±2.5 and 39.5±2.5% of the selenium was recovered respectively (6 replicates).

As the recovery of selenium was so low, work on this method was discontinued and other methods were investigated.

7.2. Hydrobromic Acid Reduction-Bromine Oxidation.

In this procedure, as in the above, the conversion of total selenium to the 4+ state is achieved in two stages. Selenium (VI) and (IV) are reduced to the elemental form by boiling with hydrobromic acid and hydrochloric acid; this is then reoxidised to the 4+ state with bromine water (Uchida et al, 1980).

7.2.1. Reagents.

i) Hydrobromic Acid.

Hydrobromic acid (47%) (Aristar) was provided by B.D.H. Chemicals.

ii) Bromine Water.

Aristar bromine (1ml) was dissolved in 100ml of deionised water to give a 3%w/v solution.

Table 27.

Coefficients of Variation of the Analysis of Selenium (VI) Using the Hydrobromic Acid Reduction/Bromine Oxidation D.A.D.B.B. Method.

Concentration of Selenium (VI) ($ngSe\ l^{-1}$).	Planimeter Reading ($\bar{x}$).	Coeff. of Variation (%).
0	21	3.0
20	43	4.0
60	89	2.0
120	140	2.5
200	221	2.0

iii) Hydrazine Hydrochloride.

See Section 7.1.1.

7.2.2. Procedure.

A 250ml sample was transferred to a 500ml conical flask and 25ml of hydrochloric acid and 12.5ml of hydrobromic acid and 0.25ml of bromine water were added. The mixture was boiled gently for 15mins and allowed to cool. Hydrazine hydrochloride solution (1ml) was added and the D.A.D.B.B. extraction method was then carried out.

7.2.3. Testing the Method.

Samples (250ml) of deionised water were spiked with 2.8 and 14ng of selenium-75 (VI) solution (vis Sect. 7.1.3.). The procedure described in Section 7.2.2. was then carried out, the piazselenol formed being extracted into 2ml of toluene, a 1ml aliquot of the extract being counted. The counts obtained were compared with those of 1ml aliquots of the aqueous standard diluted to 50% strength with water.

A 95±2% recovery was found at both levels of 11.2 and 56ngSe l^{-1} (six replicates in both cases). The method is quick (about 1½hrs) and can be used with samples of up to 1l and is, therefore, suitable for routine analysis. Further characteristics of the process were, therefore investigated.

7.2.4. Performance Characteristics of the Method.

Replicate analyses (6) of samples containing 0-200ngSe l^{-1} were carried out using the gas chromatographic, D.A.D.B.B. method. The results (Table 27) show that the method does not affect the reproducibility of the D.A.D.B.B. extraction

Table 28.

Data for Calibration Curve of the Analysis of Selenium (VI) Using the Hydrobromic Acid Reduction/Bromine Oxidation/ D.A.D.B.B. Method.

Concentration of Selenium (VI). ($ngSe\ l^{-1}$).	Planimeter Reading.
0	21
20	41
40	60
80	101
120	139
140	160
160	179
240	260
280	292

Figure 24.

Calibration Curve for Selenate by HBr/Br and G.C..

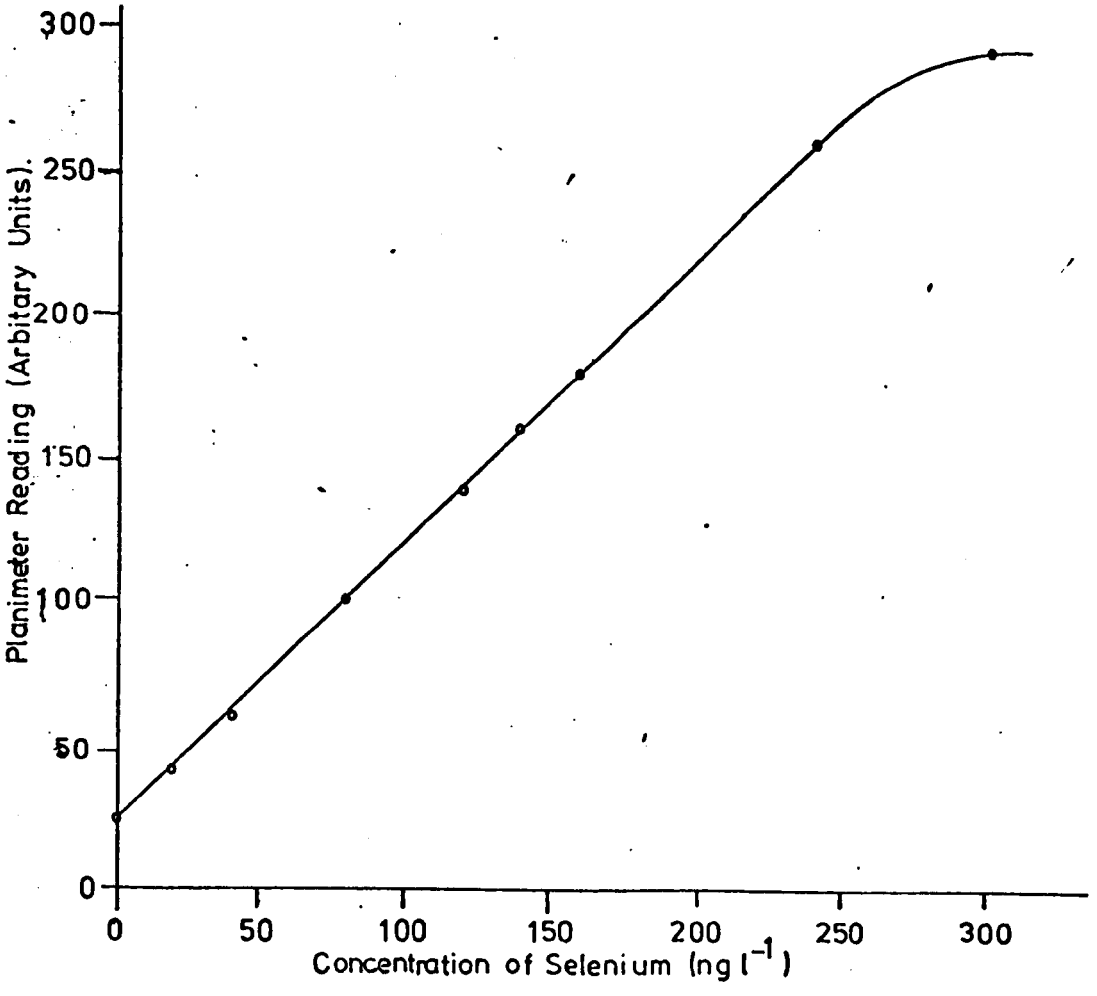
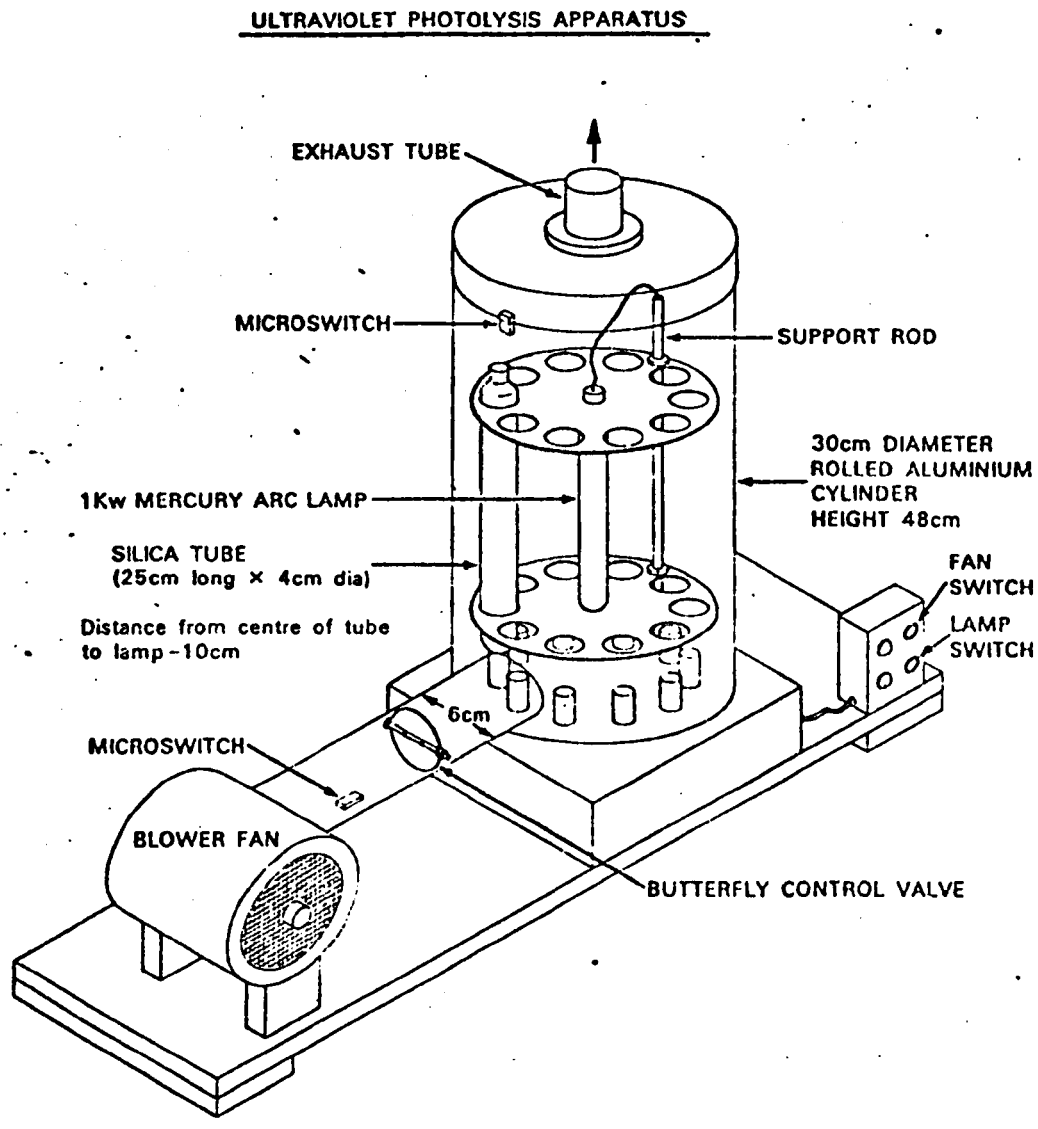


Figure 25.


ULTRAVIOLET PHOTOLYSIS APPARATUS
EXHAUST TUBE
MICROSWITCH
SUPPORT ROD
30cm DIAMETER
ROLLED ALUMINIUM
CYLINDER
HEIGHT 48cm
1Kw MERCURY ARC LAMP
SILICA TUBE
(25cm long × 4cm dia)
Distance from centre of tube
to lamp - 10cm
FAN
SWITCH
LAMP
SWITCH
6cm
MICROSWITCH
BLOWER FAN
BUTTERFLY CONTROL VALVE

and raises the blank only slightly, if at all. The detection limit remains at 1-2ngSe l^{-1}.

Calibration curves were determined by spiking deionised water with 0-280ngSe(VI) l^{-1} and carrying out the reduction prior to analysis. The results (Table 28, Fig.24) show that linearity is maintained up to 260ngSe l^{-1} as with selenite (Sect. 4.4.2.).

7.2.5. Conclusions of the Method.

The investigations showed that the HBr/Br_2 method converts selenium (VI) to selenium (IV) reliably and almost quantitatively and is suitable for routine use.

7.3. Reduction by U.V. Irradiation.

The basis of this method has been discussed in Section 2.3.3.. When selenite and selenate are irradiated with U.V. light, at a pH of 8.1, in the presence of hydrogen peroxide, an equilibrium is set up between the two oxidation states, selenite being predominant (vis Fig. 25).

7.3.1. Reagents.

i) Borax Solution.

A 1M borax solution was prepared by dissolving 3.81g of disodium tetraborate (Analar) in water and diluting to 100ml with deionised water.

ii) Hydrogen Peroxide.

A 100 volume (30%) hydrogen peroxide solution provided by B.D.H. Chemicals was used

7.3.2. Procedure.

The pH of a 100ml sample was adjusted to 8.1 by the

addition of about 2ml of the borax solution and the solution was transferred to a fused silica tube. After the addition of 50µl of hydrogen peroxide the sample was irradiated by a 1KW, medium pressure, mercury lamp for 5hrs.. After cooling, the sample was analysed by the D.A.D.B.B. procedure which had been scaled down for use with a 100ml sample (vis Fig. 26).

7.3.3. Testing the Method.

Samples of deionised water (100ml) were spiked with 2.8 and 14ng of selenium-75 (VI) (vis Sect. 7.1.3.) and the above procedure was carried out with the D.A.D.B.B. extraction method using a 2ml aliquot of toluene for the extraction, a 1ml aliquot being used for counting. The results were compared with those obtained with 1ml aliquots of the aqueous standard containing 1.4 and 7ngSe ml^{-1}. It was found that selenium (IV) yields of 85±4% and 90±3% were obtained at levels of 28 and 140ngSe l^{-1} respectively (6 replicates): however it was noted that the pH of the sample was a critical factor in gaining reasonable reproducibility. This, along with the limited volume of sample which could be treated, as well as the time required, led to the conclusion that this method was not as suitable for routine analysis as the hydrobromic acid/bromine method (Sect. 7.2.).

7.4. Oxidation of Selenium (0) and (-II) with Bromine.

As has been shown in Section 7.2., bromine oxidises selenium (0) to selenium (IV). It is reasonable to assume that any naturally occuring inorganic selenium (-II) compounds will also be oxidised by this method.

Table 29.

Coefficients of Variation of the Analysis of Selenium (0) Using the Bromine Oxidation/D.A.D.B.B. Method.

Concentration of Selenium (0).	Planimeter Reading ($\bar{x}$).	Coeff. of Variation (%).
0	21	4.0
20	45	2.5
60	79	2.0
180	103	2.0

7.4.1. Reagents.

See Section 7.2.1..

7.4.2. Procedure.

A 250ml sample was acidified with 15ml of concentrated hydrochloric acid and 1ml of bromine water was added. After shaking for 5mins, 2ml of hydrazine hydrochloride were mixed in to convert the excess bromine to bromide. The normal D.A.D.B.B. procedure was then followed.

7.4.3. Testing the Method.

Colloidal selenium (0) standards were prepared by reducing aliquots of selenium (IV) standards with sulphur dioxide in hydrochloric acid (vis Sect. 3.1.2.). Aliquots of the well mixed colloid were added to 250ml samples of deionised water, which were degassed with nitrogen to drive off any remaining sulphur dioxide. The procedure in Section 7.4.2. was then carried out on standards containing 0 to 180ngSe(0) l^{-1}.

The results show that recovery was quantitative at all levels tested (Table 29), the coefficients of variation being comparable to those obtained for the D.A.D.B.B. method for selenite (Table 12).

This oxidation, therefore, provides a convenient method for the determination of the reduced forms of selenium in natural waters without having to make assumptions based on the prevailing redox conditions.

7.5. Determination of Organo-Selenium Compounds.

The efficiency of theU.V. and bromine methods for the oxidation of organic compounds containing selenium was tested. Piazselenol, seleno-DL-methioine, seleno cystamine dihydrochloride

and seleno-urea were used. The U.V. technique was found to break down the piazselenol and so it was assumed that any naturally occuring seleno- compound would also be broken down. The bromine oxidation was found to break down seleno-DL-methionine quantitatively, partially (about 30%) destroy the seleno-cystamine dihydrochloride and to leave the seleno-urea intact.

7.6. Conclusion.

The toxicity and geochemical behaviour of selenium depend on its oxidation state, and in such studies it is obviously important to have a knowledge of the balance between the various forms present.

Use of combinations of the methods for selenite and those described in Sections 7.2. and 7.4. makes it possible to determine the contribution of each oxidation state to the total selenium concentration.

8. Conclusions on the Determination of Selenium in Natural Waters.

Selenite can be preconcentrated from natural waters by the formation of piazselenols from 1,2-diamino aromatic compounds, followed by extraction into a solvent. The aromatic compound can be chosen to give a high sensitivity by various methods of analysis. Dibromo- and nitro- substituted diamino-benzenes were found to give a satisfactory response on an electron capture detector attached to a gas chromatograph giving limits of detection of $1ngSe^{4+}\ l^{-1}$ and $6ngSe^{4+}l^{-1}$ respectively. Diamino-naphthalene was found to give a high response in fluorimetric analysis, giving a detection limit of $50ngSe^{4+}l^{-1}$. However, this method was found to suffer from serious interference from various common constituents of natural waters, such as iron, nitrate and nitrite, unless selenite was present in concentrations in the range of $1ug\ l^{-1}$. A number of methods of preconcentration were investigated, none of which proved to be satisfactory.

A number of methods for the determination of total dissolved selenium were investigated, the most satisfactory of which involved a simultaneous reduction by hydrobromic acid and oxidation by bromine. This method could only be used in connection with the dibromo-diamino-benzene without masking pH adjustment. Ultra violet photolysis can also be used for the conversion of total selenium to selenite and is suitable for use with the nitro- reagent. It was, however found to be less reliable than the chemical reduction mentioned above.

Inorganic, reduced forms of selenium and some organic forms were found to be selectively oxidised to selenite by bromine in

acidic conditions. All organic forms were converted to selenite by ultra violet photolysis at pH 8.1.

Therefore by using a combination of the dibromo- reagent, bromine oxidation and hydrobromic acid reduction it was possible to analyse selenite, selenate and reduced forms of selenium in tap water, estuarine water and sea water.

APPENDIX.

THE DETERMINATION OF CHROMIUM IN RIVER, ESTUARINE AND COASTAL WATERS BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROPHOTOMETRY.

A.1. Introduction.

Chromium is an element which is essential to life at its natural levels (Underwood, 1977), but toxic at higher levels, especially when present as chromium (VI).

Most of the chromium available for crustal weathering exists in the form of chromium (III)-substituted spinels, silicates and aluminosilicates (Burns and Burns, 1975) and this factor, along with the reducing abilities of organic matter means that the major input of the element into the hydrosphere is in the (III) state (Morgan and Sibley, 1975). Thermodynamically, the metal would be expected to be oxidised almost entirely to chromium (VI) in oxic waters, but remain mainly as chromium (III) in anoxic waters (Elderfield, 1970). However, because of kinetic effects chromium (III) often exists metastably in oxic waters (Earley and Cannon, 1965) in which it may comprise 3 to 95% of the total chromium (Pankow et al, 1977, Shuman and Dempsey, 1977 and Cranston and Murray, 1980). Recorded chromium concentrations in natural waters range from less than 100ngCr l^{-1} to 100µgCr l^{-1} and vary widely with location, temporal variation and the analytical method used (Cranston and Murray, 1980)

Solubility data for chromium (III), however, suggests

that its concentration cannot exceed the solubility of the mineral chromite ($FeCr_2O_4$) which is about 5ngCr l^{-1} (Hem, 1977).

In estuarine waters the concentration of dissolved chromium (III) reaches a maximum at a salinity of about $9^{o}/_{oo}$ and then drops rapidly to a minimum at a salinity of about $15^{o}/_{oo}$ above which it rises slowly to sea water concentrations, suggesting that redox controlled partitioning between the particulate and dissolved phases is occuring (Cranston and Murray, 1978 and 1980). Chromium (VI) concentrations, however, appear to be determined only by conservative mixing (Cranston and Murray, 1980).

The total chromium concentration in sea water varies between 0.1 and 0.5µgCr l^{-1} (Brewer, 1975, Cranston and Murray, 1978 and Campbell and Yeats, 1981). Chromium (III) comprises only a few percent of the total except for a peak at the primary nitrite maximum, suggesting that it is related to the biological activity. Chromium (VI), however, correlates very closely with the profiles of dissolved silica, suggesting that at least some of it is associated with the particulate material (Cranston and Murray, 1978).

If the toxicity and the redox chemistry are to be investigated it is essential to use a method of analysis which can differentiate between the oxidation states. Chromium (III) is liable to be removed from solution by the walls of storage containers, futhermore, the oxidation state of the element is prone to change rapidly. If reliable analyses

for dissolved chromium are to be carried out it is essential to preconcentrate as soon as possible after sampling.

A.2. Determination of Dissolved Chromium.

It was decided that atomic absorption spectrophotometry with electrothermal atomisation would provide the most specific and sensitive means of determining chromium. However, the concentration of the element in unpolluted waters (less than 1μgCr l^{-1}) is below the detection limit of the instrument without the use of a preconcentration stage. Even if evaporation was used to preconcentrate fresh water there would still be no discernment between the oxidation states, and the effects of evaporation of saline waters would be uncertain, and the final solution would be too saline for atomisation.

A survey of the literature showed that two approaches to preconcentration have been recently used. The first of these was adopted by Cranston and Murray (1978) and subsequently used in an extensive survey of the distribution of the element in the eastern tropical Pacific (Murray et al, 1983). Total chromium is preconcentrated by treating the sample with a suspension of hydrous iron (II) oxide. The hydrous iron (II) oxide reduces chromium (VI) to chromium (III) and is, itself, aerially oxidised to hydrous iron (III) oxide which co-precipitates the chromium (III). The precipitate is separated by filtration and redissolved with hydrochloric acid. The resulting solution is made up to volume with water and the chromium content is determined by electrothermal

atomisation atomic absorption spectrophotometry. Chromium (III) alone can be determined using a suspension of hydrous iron (III) oxide instead of that of iron (II).

Campbell and Yeats (1981) adopted another approach for the determination of chromium in Atlantic ocean water. Chromium, having been quantitatively oxidised to the 6+ state, is converted to an ion pair with Alamine-336 (which consists mainly of tri-n-octylamine). Toluene was used to extract the complex which was then analysed for chromium using electrothermal A.A.S.

It was decided that the first of these approaches was preferable as it is simpler, more suitable for routine work and can be used for the determination of both chromium (III) and total chromium (and hence, chromium (VI) by subtraction). As a preliminary to this a study was made of the optimum conditions for electrothermal atomisation atomic absorption spectrophotometry (E.T.A.A.S.) of chromium and for the co-precipitation of chromium (III) and total chromium.

A.3. Investigation of Optimum Conditions for E.T.A.A.S. of Chromium.

A.3.1. Instrumentation.

All work was carried out using a Perkin Elmer 2280 atomic absorption spectrophotometer fitted to a Model HGA 400 heated graphite atomiser and a Model A.S.1. auto-sampler equipped with a 10-20µl syringe. The read out was recorded on a Perkin Elmer Model PRS 10 printer-sequencer. A hollow cathode

Table A.1.

Manufacturers Recommended Conditions For E.T.A.A.S. of Chromium.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)
Drying	110	10	25
Charring	1200	20	5
Atomisation	2700	1	2 (miniflow)
Cleaning	2700	1	2

Figure A.1.

Comparison of Ordinary and Pyro-coated Tubes.

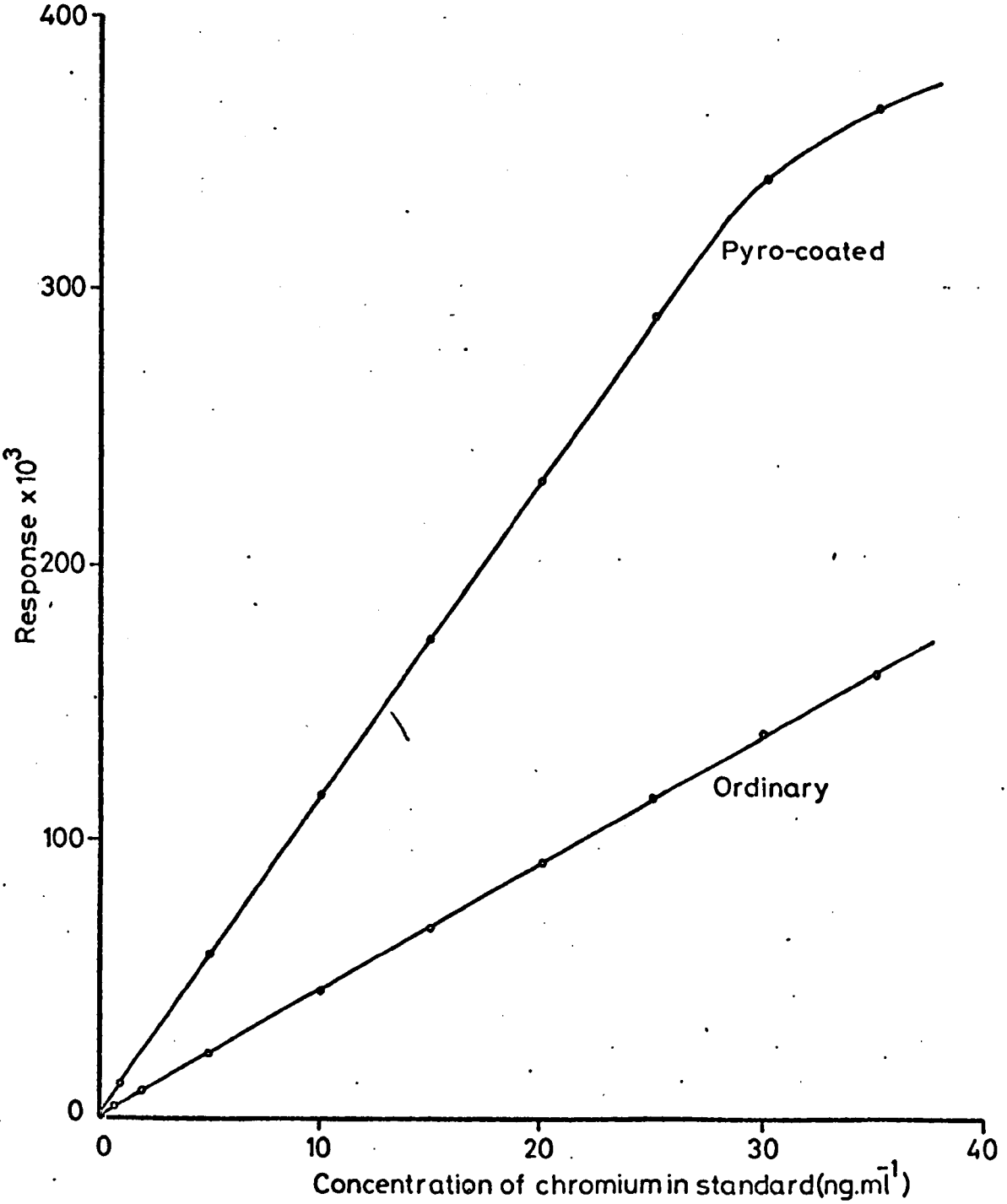
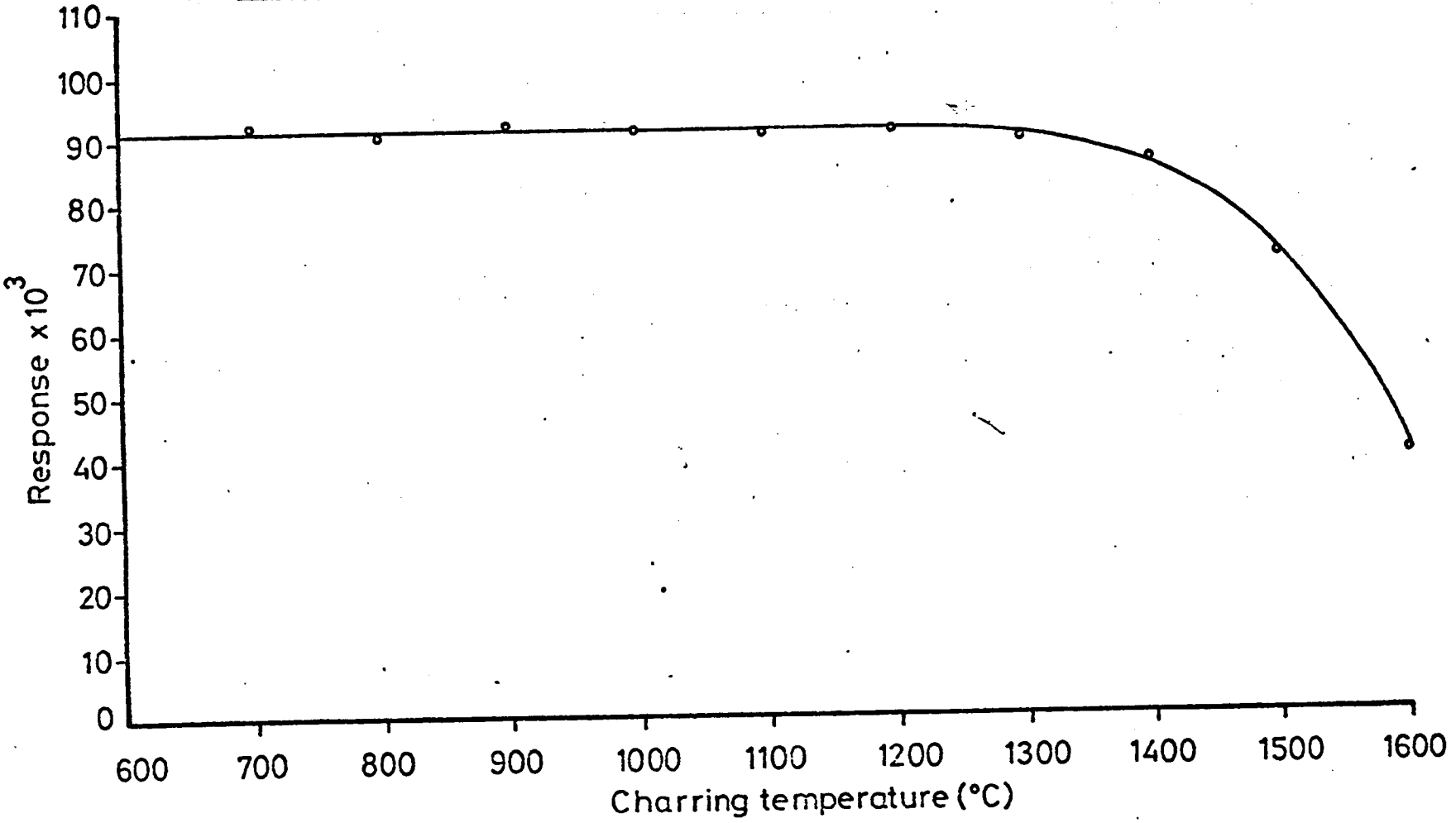


Figure A.2.

Effect of Charring Temperature on E.T.A.A.S. Response to Chromium.



lamp run at 10mA was used as the light source and measurements were made at a wavelength of 357.9nm, using background correction.

A.3.2. Optimisation of Electrothermal Atomisation Conditions.

The temperature conditions recommended in the manufacturer's manual were used as guidelines when optimising the conditions for the nebulisation of the sample (Table A.1.).

Development work was carried out by injecting 20µl aliquots of 5%v/v hydrochloric acid which was made 3×10^{-3}M with respect to iron (III) and containing 10.3 $Cr^{3+} ml^{-1}$.

A.3.2.1. Selection of Graphite Tube.

Calibration curves for chromium were run using new, ordinary and pyrolytically coated tubes. They showed (Fig.A.1.) that the latter gave a somewhat better than two-fold greater sensitivity than the former. This type of tube was used in all further work. It must be noted that, as the tube ages, the sensitivity deteriorates.

A.3.2.2. Optimisation of Charring Temperature.

Maintaining all other conditions as are described in Sect. A.3.2., runs were carried out in which the charring temperature was varied from 600°C to 1600°C. These showed (Fig. A.2.) that loss of chromium did not occur unless the charring temperature appreciably exceeded 1200°C. This temperature was used in all further work.

A.3.2.3. Optimisation of Atomisation Temperature.

The effect of varying the temperature of atomisation in the range 1200°C to 2800°C was next examined while maintaining the other conditions constant. It was found that

Figure A.3.

Effect of Atomisation Temperature on E.T.A.A.S. Response to Chromium.

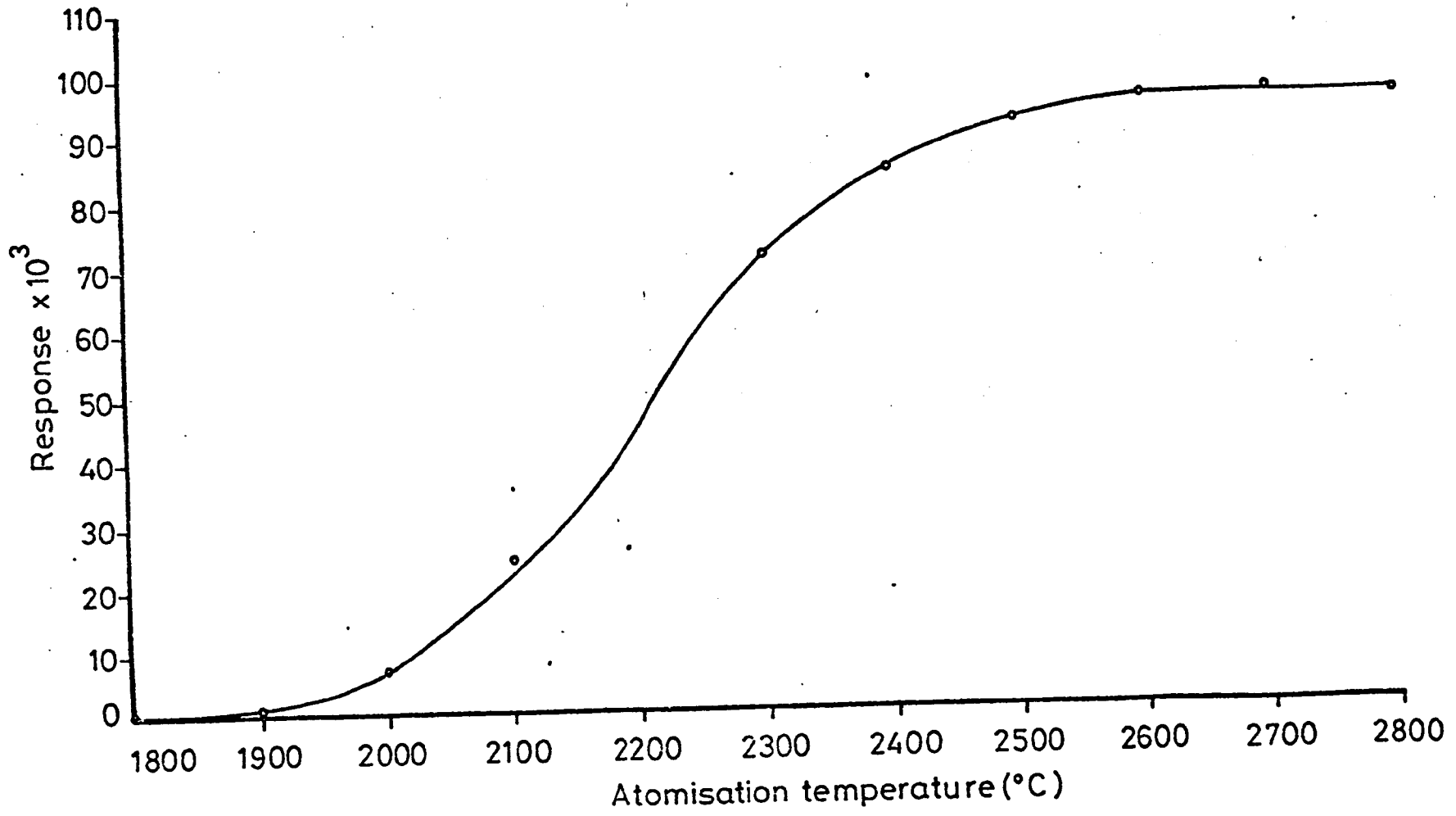


Table A.2.

Effect of Iron Concentration on Instrumental Response of 10ng ml^{-1} Chromium.

Iron Molarity (x 10^3)	Response
0	92
0.3	91
0.6	92
1.2	89
1.8	92
2.4	88
3.0	88
4.5	91
6.0	92

Table A.3.

Effect of Hydrochloric Acid Concentration on Instrumental Response of 10ng ml^{-1} Chromium.

HCl Concentration (% v/v)	Response
0	88
1	92
2	91
3	89
4	92
5	88
6	91
7	89
8	92

(Fig. A.3.) maximum response was obtained at about 2570°C, above which the response remained constant. A temperature of 2630°C was employed in all further work.

A.3.2.4. Effect of Iron on Instrumental Response.

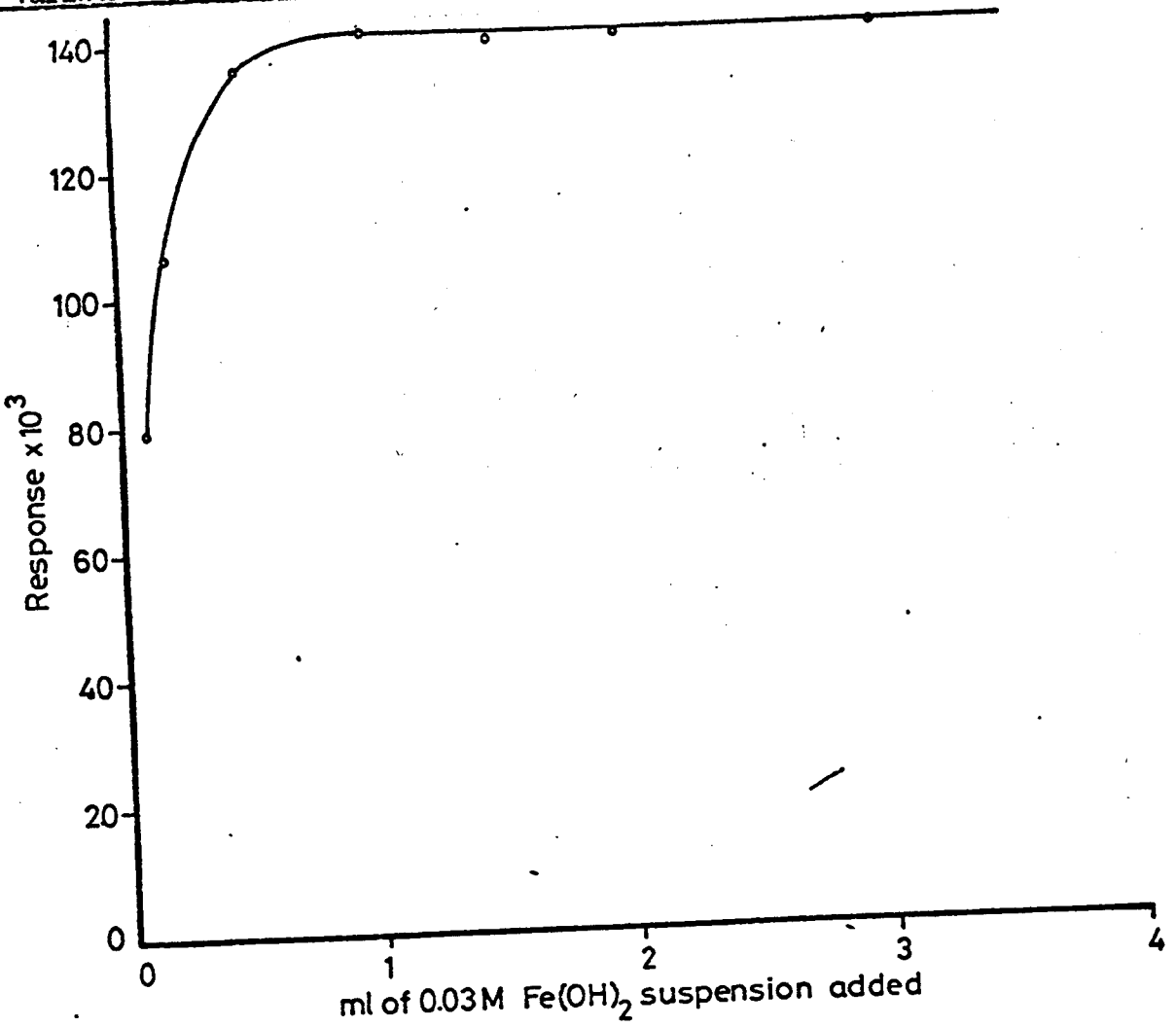
As iron (III), which has been used as a co-precipitant, is present in the solution injected into the furnace it was thought essential to check its effect on the response of the instrument to chromium. Accordingly, 20µl aliquots of standard chromium solutions ($10ngCr^{3+}ml^{-1}$) containing various concentrations of iron were injected into the graphite tube and analysed. It was found that the presence of up to 6.0 $x10^{-3}$M iron had no effect (Table A.2.). This concentration of iron represents six times that used in the procedure developed by Cranston and Murray (1978).

A.3.2.5. Effect of Hydrochloric Acid Concentration on Instrumental Response.

In the procedure used, the hydrous iron oxide precipitate carrying the co-precipitated chromium is dissolved in hydrochloric acid before injection into the graphite furnace. It was decided, therefore, to check the effect of acidity on the instrumental response from a solution containing $10ngCr\ l^{-1}$ and 0.003M iron. The instumental response was found to be independant of hydrochloric acid concentration up to at least 10%v/v (Table A.3.). However, to minimise corrosion of the atomiser and to prolong the life of the graphite furnace it was decided to use a final acid concentration of 5%v/v.

Figure A.4.

Effect of Variation of Amount of Hydrous Iron (II) Oxide on Recovery of Chromium.



A.3.3. Investigation of the Optimum Conditions for the Co-precipitation of Chromium.

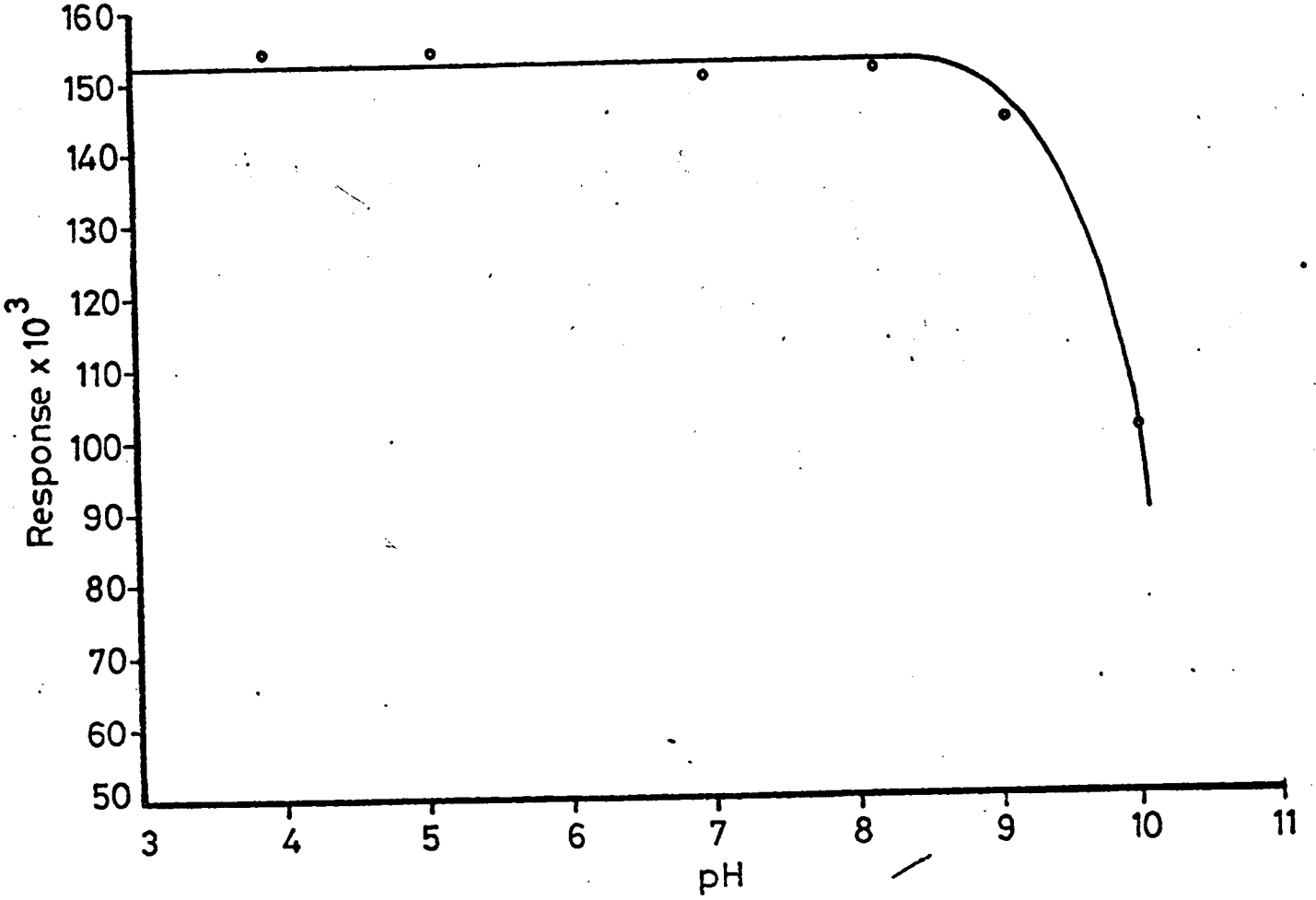
After the optimum conditions for the determination of chromium had been established the optimum conditions for the preconcentration stage were investigated. In this procedure (Sect. A.4.) a 250ml aliquot of sample was treated with a 0.03M suspension of hydrous iron (II) oxide and allowed to reach equilibrium for 2hrs. The precipitate was then recovered by filtration, dissolved in 0.5ml of concentrated hydrochloric acid and the solution was made up to 10ml with water ready for E.T.A.A.S.. The two principal variables in the co-precipitation were the pH and the amount of hydrous iron (II) oxide used.

A.3.3.1. Effect of Variation of Amount of Hydrous Iron (II) Oxide Used for Co-precipitation of Cr^{6+}.

A series of 250ml aliquots of chromium-stripped sea water (S = 18.0$^{o}/_{oo}$) were spiked to give a concentration of 800ngCr l^{-1} and the pH was adjusted to 8.0±0.5 as described in Section A.4.. The solutions were then treated with various volumes of (0.03M) hydrous iron (II) oxide suspension and shaken for two hours before being allowed to settle overnight and then filtered. The precipitate was then dissolved in hydrochloric acid made up to volume and analysed. The results of these experiments indicated that the recovery of chromium reached a maximum when 0.75ml of the suspension was added and thereafter remained constant (Fig. A.4.). In all subsequent work 1.0ml of the suspension was used.

Figure A.5.

Effect of pH on Recovery of Chromium.



A.3.3.2. Effect of pH on Recovery of Chromium.

The experiments described above were repeated using 1.0ml of the suspension but varying the pH for the equilibration between 3.9 and 10.0. The data gathered (Fig. A.5.) showed that the recovery was constant over the pH range 3.9 to 8.5. However, it fell rapidly as the medium became more alkaline.

A.3.3.3. Recovery of Chromium by Co-precipitation.

Although this co-precipitation technique gave a reproducible recovery of chromium, comparison of the instrumental response with that given by chromium standards indicated that the recovery was not quantitative. It amounted to 91.2±1.3% for both chromium (III) and chromium (VI) and, within the range of the method, was independent of chromium concentration. Although the reason for this shortfall was uncertain, the fact that standards were taken through the whole procedure made up for the 9% loss.

A.4. Final Method.

A.4.1. Reagents.

As this method is very subject to errors caused by contamination, all reagents must be of ultrapure grade and each new batch must be checked for purity. Reagent solutions and suspensions should be stored in polyethylene bottles which have been cleaned by soaking overnight in 10%v/v hydrochloric acid and washing copiously with water.

i) Water.

The water used for determination of the blanks and preparation of standard and reagent solutions must have a negligible chromium content. In the present work, water from a Fisons "Fi-Stream" still was purified by passing it through a Sybron Barnstead "Nanopure" system. Water redistilled from an all silica still was also found to be suitable.

ii) Hydrochloric Acid.

The 10% v/v solution of hydrochloric acid used for the cleaning of apparatus was prepared from Analar grade acid.

Aristar hydrochloric acid was used for dissolution of the iron oxides.

iii) 10% Ammonia Solution.

Aristar ammonia solution (50±2ml) was diluted to 500ml with water.

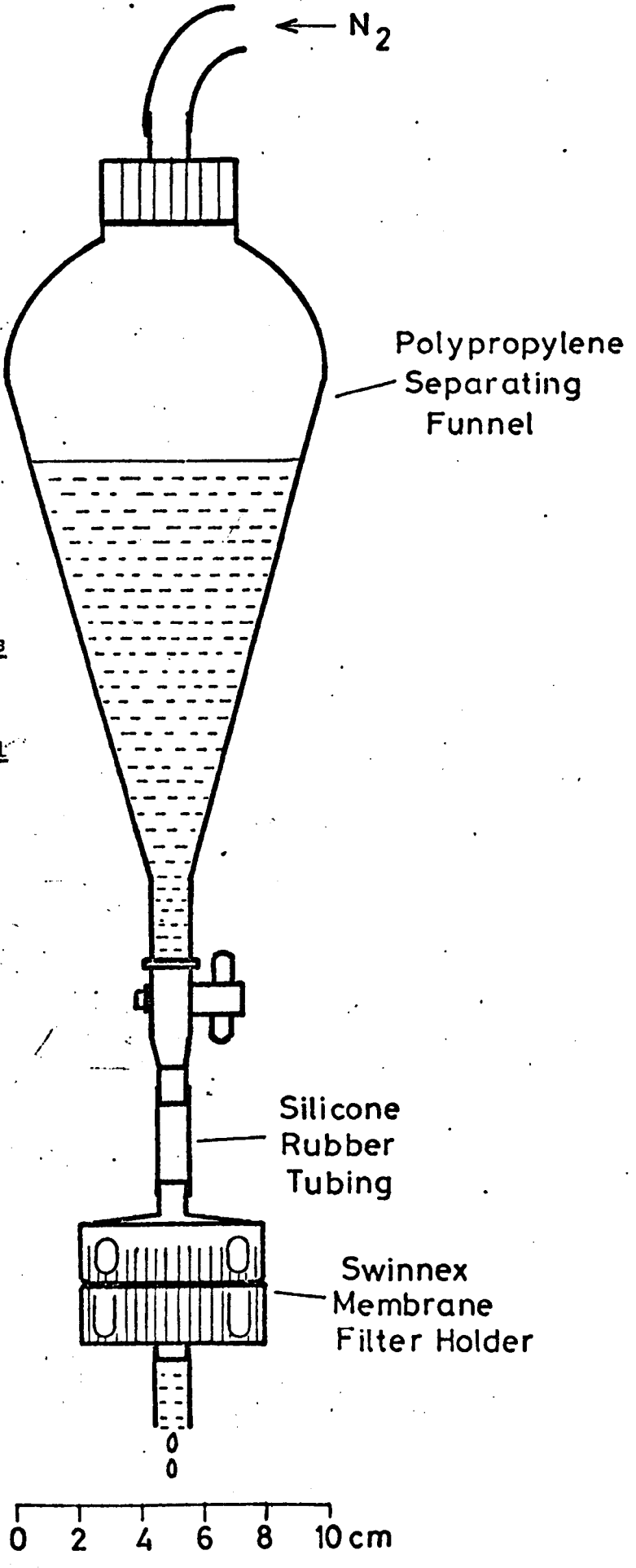
iv) Iron (II) Hydroxide Suspension.

Specpure ammonium iron (II) sulphate (0.6±0.05g) was weighed into a 100ml glass beaker, dissolved in 50±2ml of water and then brought to pH 8 by adding 10% ammonia solution with care (about 1.5ml). The suspension was stirred continuously and used within 10 minutes of preparation.

v) Iron (III) Hydroxide Suspension.

The above suspension of iron (II) hydroxide was stirred for at least 24 hours while making periodic checks to ensure that the pH remained within the range of 7.8 to 8.2. This suspension was stable for at least a month.

Figure A.6.



Filtration Apparatus for Separation of Particulate Material from Sample.

vi) Chromium (III) Standard (100ugCr ml^{-1})

Chromium (III) potassium sulphate (0.0960±0.0002g) was dissolved in 200±10ml of 1% v/v hydrochloric acid. This solution was diluted with water to 1l in a calibrated flask. This solution was stable for at least three months.

vii) Chromium (VI) Standard (100ugCr ml^{-1})

Potassium dichromate, dried at 120°C, (0.02828±0.0002g) was dissolved in 500±20ml of 0.04% v/v hydrochloric acid and diluted to 1l in a volumetric flask using water. This solution was stable for at least three months.

A.4.2. Apparatus.

A.4.2.1. Glassware.

Glassware was reserved solely for chromium determinations. It was soaked in 10% v/v hydrochloric acid overnight and washed thoroughly with water before use.

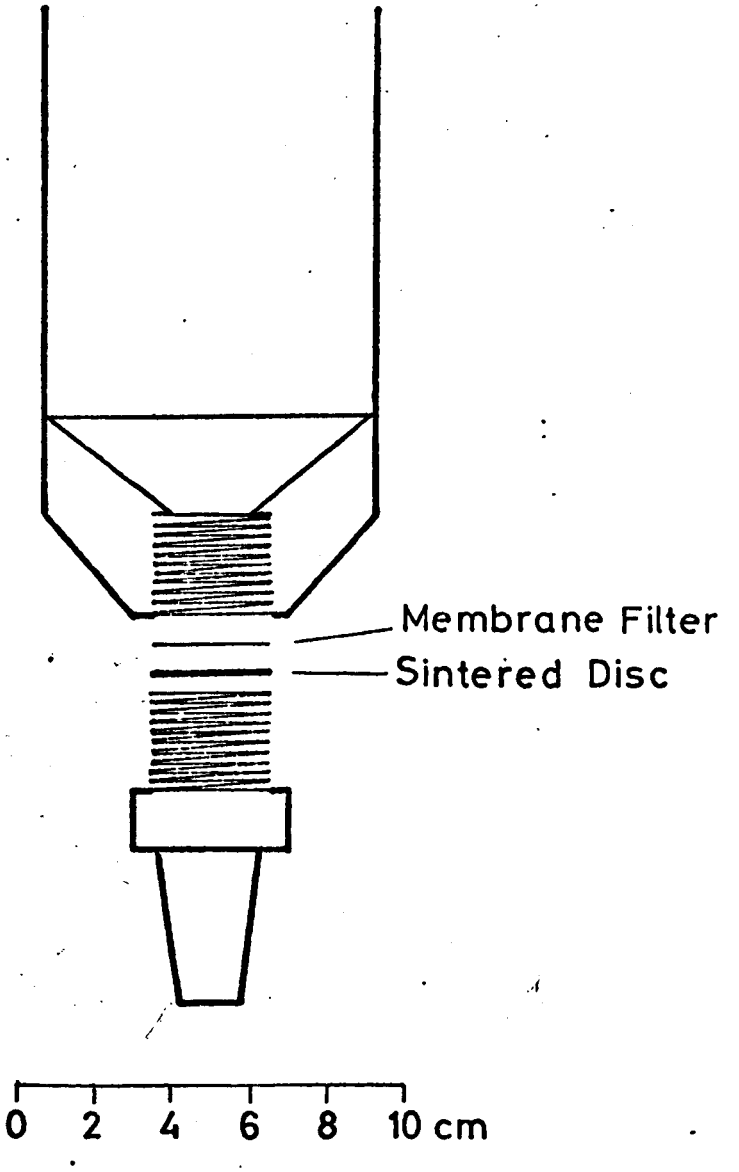
A.4.2.2. Filtration Apparatus.

i) Filtration of Samples.

The filtration apparatus shown in Fig.A.6. was used to remove particulate matter from samples. It consisted of a 500ml plastic separating funnel fitted with a P.T.F.E. stop-cock. This was connected via silicone rubber tubing to a 47mm Millipore Swinnex, in-line filter holder fitted with a Millipore H.A. 0.45µm filter. The filter was washed prior to use with 100ml of 10% v/v hydrochloric acid followed by 150ml of deionised water. The sample was forced through the filter by the application of about 1 bar pressure of filtered nitrogen to the top of the funnel.

Figure A.7.

Filtration Apparatus for Separation of Iron (III) Hydroxide.



ii) Filtration of Hydroxide Precipitates.

Figure A.7. shows the perspex apparatus used for the filtration of the hydroxide precipitates. The holder was fitted to a 1l filter flask and the sample filtered using suction from a water pump. Nuclepore 25mm filters of pore size 0.4μm were found to be the only suitable filters as they contain a negligible amount of chromium and are insoluble in concentrated hydrochloric acid.

Screw Top Polypropylene Bottles.(250ml).

These bottles were obtained from Azlon.

Screw Top Polystyrene Bottles.(30ml).

Sterelin 128A bottles were used.

Atomic Absrorption Spectrophotometer.

See Section A.3..

A.4.3. Sample Collection and Preservation.

Samples were filtered within 2-3 hours of collection using the filtration apparatus described in Section A.4.2.2.. The filter was subsequently washed with 2x10ml of water, dried at 60°C in a polypropylene Petri dish, weighed and stored in the Petri dish for the determination of particulate chromium (Sect. A.6.). The analysis of the filtrate of chromium (III) was commenced immediately as there is a high risk that changes will occur in the redox state of the element. If samples to be analysed for total chromium could not be co-precipitated at once they were preserved by acidification to pH2±0.1 with hydrochloric acid. The total chromium content preserved in this manner was found to remain stable.

Table A.4.

Determination of Total Chromium in 10% Sea Water Containing 10mg l^{-1} of Fulvic Acid or 10mg l^{-1} Di-sodium E.D.T.A. After UV Irradiation for 3 Hours.

Complexing Agent	pH	Chromium (III) Added (ng l^{-1})	Chromium (III) Found (ng l^{-1})
Di-Na EDTA	4	0	105
"	4	800	891
"	8	0	117
"	8	800	925
Fulvic Acid	4	0	161
"	4	800	777
"	8	0	259
"	8	800	1109

The unspiked, unirradiated sample was found to contain 109±6 $ngCr^{3+}$ l^{-1} in the absence of the complexing agent.

The fulvic acid sample used contained 15μgCr g^{-1}, this adds 150ngCr l^{-1} to both spiked and unspiked samples.

A.4.4. Analytical Procedure.

A.4.4.1. Pretreatment.

Pretreatment was found necessary only when a high concentration of dissolved organic material is present. All the samples reported in the present work did not need pretreatment. Application of this procedure may be advisable for total chromium only.

The sample (250ml) was transferred to a silica photolysis tube and the pH was adjusted to 8±0.3 using ammonia or hydrochloric acid solution as appropriate. The tube was loosely stoppered with a bulb stopper and irradiated for 2-3 hours in the apparatus described in Section 7.3. and allowed to cool. The sample was then transferred to a 250ml polypropylene bottle together with the washings from the tube. Blanks were carried out using water instead of the sample.

The method was tested by adding E.D.T.A. (10mg l^{-1}) and fulvic acid (10mg l^{-1}) to stripped sea water spiked with a known quantity of chromium and carrying out the above process. The results (Table A.4.) showed that pH8 was the most advantageous pH to use.

A.4.4.2. Co-precipitation of Total Chromium.

The filtered sample (250±2ml) was placed in a 250ml polypropylene bottle. If necessary the pH was adjusted to 8.0±0.3 using 10% v/v hydrochloric acid or ammonia solution as appropriate. Using a pipette, 1.0ml of the iron (II) hydroxide suspension was added to the sample. The bottle was tightly closed and shaken for 1 hour on an orbital shaker.

The precipitate was allowed to settle overnight and then filtered off using a 0.4μm Nuclepore filter in the apparatus shown in Figure A.7.. The bottle was washed out with 2x10ml of water and the sides and the shoulder of the filtration apparatus were washed down with a jet of water to ensure that all the precipitate was transferred to the filter.

Using nylon forceps the filter was transferred to a 30ml polystyrene tube which was then stoppered. The procedure can be interrupted at this point and the precipitate stored indefinitely.

The precipitate was dissolved in 0.5±0.02ml of hydrochloric acid and allowed to digest for 1-2 minutes, 9.5±0.05ml of water were then added, using an automatic pipette and the solution was mixed well and stoppered.

The atomic absorption spectrophotometric procedure described in Section A.4.4.4. was then performed.

Blanks and calibration standards were carried out simultaneously with the samples in the same way.

A.4.4.3. Co-precipitation of Chromium (III).

The filtered sample (250ml) was transferred to a 250ml polypropylene bottle. If necessary the pH was adjusted to 8.0±0.3 using 10% v/v hydrochloric acid or ammonia solution as appropriate. and 1ml of the iron (III) hydroxide suspension was added with a pipette. The bottle was then stoppered and shaken for at least an hour on an orbital shaker.

Blanks and calibration standards were prepared in 250 ±2ml of water and run simultaneously.

A.4.4.4. Atomic Absorption.

The atomic absorption spectrophotometer was set up using a hollow cathode chromium lamp, the monochromator being set to measure the 357.9nm line. Pyrolytically coated graphite tubes were used and argon was employed as the purge gas. The auto-sampler was set to inject 20µl into the tube. Although a slight loss in sensitivity was noted it was decided that it was advisable to use the automatic background corrector, especially when the furnace tube becomes aged. The optimum settings for the instrument used (Sect. A.3.) were as follows:-

Drying step.	Ramp time	10s
	Temperature	110°C
	Hold Time	25s
Pyrolysis step.	Ramp time	20s
	Temperature	1200°C
	Hold time	5s
Atomisation step.	Ramp time	1s
	Temperature	2630°C
	Hold time	2s
	Miniflow.	
Cleaning step	Ramp time	1s
	Temperature	2700°C
	Hold time	2s

Table A.5.

Results of Total Cr Analyses of Liverpool Tap Water Which Had Been Spiked with Cr^{6+} (5 degrees of freedom.).

Added Chromium ($ng\ l^{-1}$)	Total Chromium Found ($ng\ l^{-1}$)	Standard Deviation
0	202	14
100	317	18
250	440	18
500	690	18
750	917	32
1000	1186	39

Table A.6.

Results of Total Chromium Analyses of Stripped Estuarine Water Which Had Been Spiked with Cr^{6+} (5 degrees of freedom).

Added Chromium ($ng\ l^{-1}$)	Total Chromium Found ($ng\ l^{-1}$)	Standard Deviation
0	21	2
40	64	5
100	132	5
200	223	8
400	419	13
600	618	18
900	931	23
1200	1218	39
1400	1410	43

Table A.7.

Results of Analyses for Chromium (III) in Mersey Water Which Had Been Spiked with Either Cr^{3+} or Cr^{6+} (5 degrees of freedom).

Added Chromium ($ng\ l^{-1}$)	Chromium (III) Found ($ng\ l^{-1}$)	Standard Deviation
Chromium (III)		
0	192	7
200	388	10
400	585	18
600	798	23
800	987	22
Chromium (VI)		
0	192	8
200	199	9
400	198	10
600	201	7
800	203	10

The purge gas (argon) was maintained at a flow rate of 100ml min^{-1}.

A.5. Performance Characteristics of the Method.

The method can be used to determine all forms of dissolved chromium in fresh, estuarine and sea waters.

A.5.1. Standard Deviations.

The above methods were used to determine the chromium concentration of i) Liverpool tap water, spiked with various quantities of chromium (VI) ii) Mersey estuary water (salinity about $18^{o}/_{oo}$).

A.5.2. Results.

i) Liverpool Tap Water.

The method for the determination of total chromium was used to analyse Liverpool tap water both spiked with various quantities of chromium (VI) and unspiked (vis Table A.5.).

ii) Mersey Estuary Water.

Mersey estuary water (salinity about $18^{o}/_{oo}$) was stripped of chromium by co-precipitation with iron (II) hydroxide. Analyses, using the method for chromium (III) were then carried out on the water both spiked with various quantities of chromium (III) and chromium (VI) and unspiked. Analyses were also carried out using the method for total chromium on the water unspiked and spiked with chromium (VI) (vis Table A.6. and Table A.7.)

A.5.2.1. Limit of Detection.

The standard deviation of the distilled water blank was

investigated and found (6 degrees of freedom) to be about 7ngCr l^{-1}, giving a limit of detection ($4.6x\sigma$) of 32ngCr l^{-1}. This figure will vary with the purity of the reagents, the instrument used and the age of the graphite furnace tube.

A.5.2.2. Linearity of the Calibration Curve.

The linearity of the calibration curve for the instrument used was checked by preparing standard chromium (VI) solutions containing 0.25, 0.50, 1.00, 1.25, 1.50 and 1.75µg l^{-1}. These were then analysed for total chromium as described in Section A.4.4.. The mean response for three injections was then plotted against concentration (µg l^{-1}). The linear range was found to extend to about 1.5µgCr l^{-1}. This, however, will vary with the instrument used and the age of the graphite furnace tube.

A.5.3. Extension of the Concentration Range of the Method.

If the chromium concentration of the sample was thought to be likely to exceed 1.5µg l^{-1} a smaller aliquot. sample was taken for analysis and made up to 250ml with water before starting the determination.

A.5.4. Calibration Standards.

The sensitivity of the spectrophotometer was found to vary with fluctuations in current and as the furnace tube aged. It was, therefore, essential to intersperse the sample concentrates with those of standards and blanks.

A.5.5. Interferences.

The effect of possible interfering substances was determined by carrying out analyses on water which had been spiked with known amounts of the substance, both alone and in the

Table A.8.

Effect of Other Substances on the Determination of 500ng l^{-1} Chromium (VI).

Other Substance	Other Substance Added as	Conc. of Other Substance ($\mu g\ l^{-1}$)	Effect in ng l^{-1} of other Substance (a)
Alumium	ammonium sulphate	1000	+ 5
Antimony (III)	potassium antimonyl tartrate	50	+ 13
Cadmium	nitrate	100	- 18
Cobalt	nitrate	100	+ 5
Copper	sulphate	10000	+ 9
Lead	nitrate	5000	- 22
Manganese (II)	sulphate	5000	- 19
Mercury (II)	chloride	10	+ 13
Molybdenum (VI)	ammonium molybdate	1000	+ 20
Nickel	sulphate	5000	- 27
Selenium (IV)	sodium selenite	50	+ 15
Tin (II)	chloride	2000	- 22
Vanadium (V)	ammonium vanadate	1000	- 27
Zinc	sulphate	2000	- 22

a) If other substance did not interfere the effect would be expected (95% confidence) to lie within the range 500$\pm$ 40$\mu g\ l^{-1}$

presence of a known amount of chromium. It was found that most analytical grade chemicals contained sufficient chromium to give absorbances significantly greater than those of the blank when present at concentrations greater than 100µg l^{-1}. An indication of the extent of possible interference was obtained from the difference between the absorbance readings obtained with the samples spiked with chromium and those that were left unspiked. The results (Table A8) show that none of the substances tested showed significant interference. It should be noted that no interference tests were carried out on sodium, potassium, magnesium, strontium, chloride, bromide, sulphate or boric acid as the same response was obtained with sea water as with distilled water.

A.5.6. Effect of Sample Filtration.

It is well known that the filter or filtration apparatus may cause changes in concentration of some dissolved trace metals either by adsorption or contamination. Tests were, therefore, carried out to determine whether filtration through a 47mm Millipore HA filter caused changes in the total chromium content of water samples.

Two bulk water samples from the Irish Sea were diluted to a salinity of about $18^{o}/_{oo}$ and partially stripped of chromium by treatment with hydrous iron (II) oxide followed by filtration through a 0.45µm membrane filter. Six replicate determinations were carried out using the method described in Section A.4.. Six replicate analyses were also carried out on the same sample after it had been passed through either an unwashed or acid washed (10% v/v HCl) 47mm Millipore HA filters.

Table A.9.

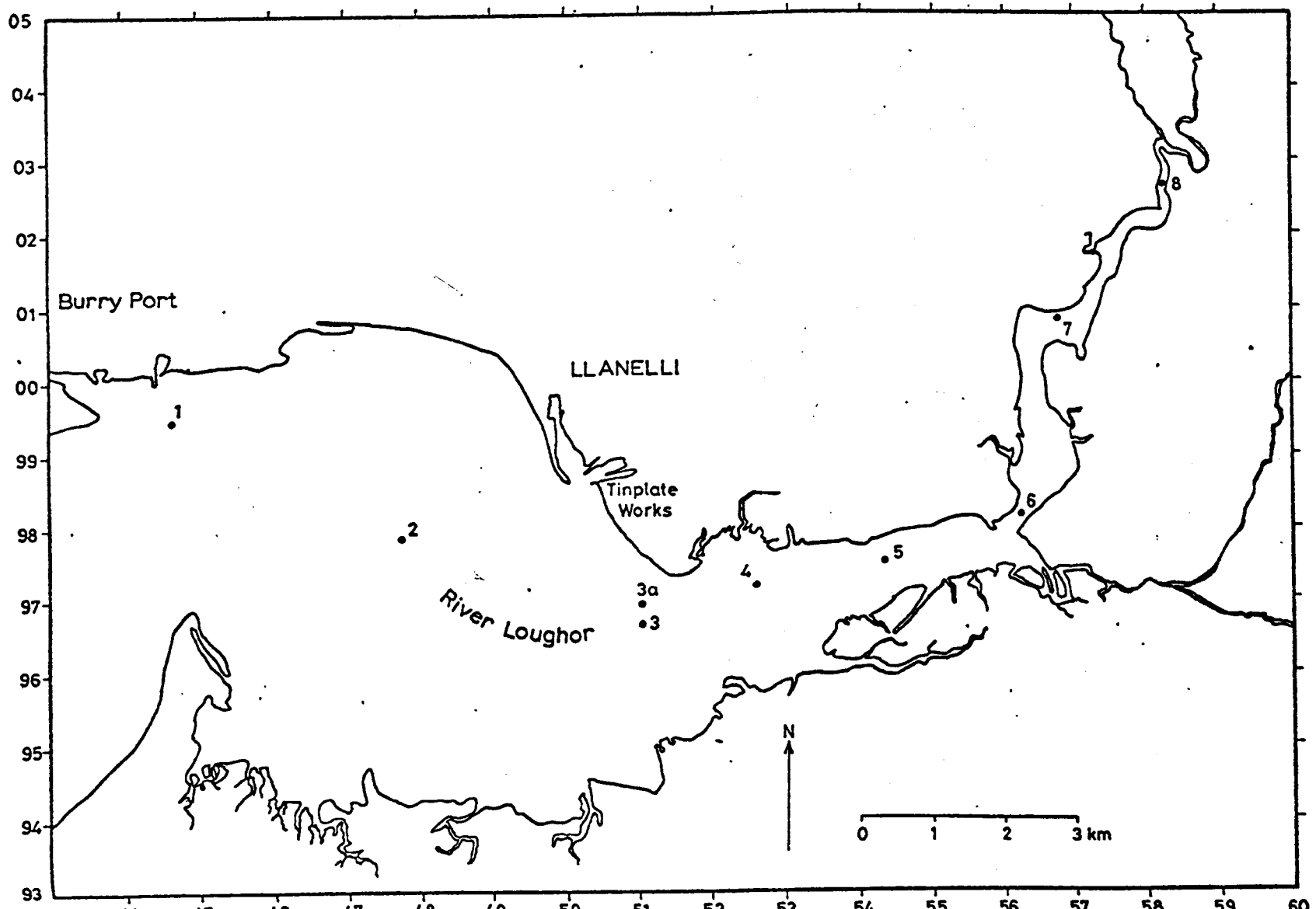
Effect of Filtration Through Acid-Washed Millipore HA Filters on Chromium Content of Mersey Estuary Water.

	Total Cr ($\mu g\ l^{-1}$)	Cr^{3+} ($\mu g\ l^{-1}$)
Initial filtrate	1.55 ± 0.02	1.34 ± 0.01
Second filtrate (Through particulates)	1.51 ± 0.03	1.37 ± 0.02
Third filtrate (Through fresh filter)	1.56 ± 0.03	1.31 ± 0.01

The sample (32 ± 8ngCr l^{-1}) which was refiltered through the unwashed filter gave a filtrate containing 30 ± 3ngCr l^{-1}. That containing 80 ± 7ngCr l^{-1} which was filtered through acid washed filters gave a filtrate containing 80 ± 7ngCr l^{-1}. Therefore filtration caused no change in the total chromium concentration of the sample.

A further test was carried out to determine if the ratio of Cr^{3+}:Cr^{6+} remained constant before and after filtration. In this, a sample of water (Salinity about $28^{o}/_{oo}$) was collected from the Mersey at the Pier Head. On return to the laboratory it was well mixed and divided into four 800ml portions, each of which was filtered through an acid washed filter. A 250ml aliquot of each filtrate was then analysed for both total chromium and chromium (III). The remainder of the filtrate was then refiltered through the same filter while it was still coated with the particulate matter. A 250ml aliquot was again analysed and the remainder was refiltered through a new acid washed filter and 250ml of the filtrate was analysed. The results (Table A.9) showed that filtration caused no significant change to either the concentration of total chromium or the concentration of chromium (III).

Figure A.8. The Loughor Estuary and Sampling Stations.



A.6. Dissolved Chromium in the Loughor Estuary.

The preceding methods for the determination of total chromium and chromium (III) were applied to the waters of the Loughor estuary in South Wales (Fig. A.8.). The survey was organised in conjunction with the Welsh Water Authority in an attempt to assess the effect of a considerable chromium outfall from a tinplate works in Llanelli. The effluent was heavily charged with hydrous iron (II) and iron (III) oxides.

Considerably more information will be required before a valid interpretation of the results obtained can be made. As the discharge is only periodic, information as to the normal distribution is required. Data on the loading and chromium content of the particulate material and its rate of adsorption of chromium are also needed.

Many of the samples contained a considerable quantity of suspended hydrous iron oxides of mixed oxidation states. These may have reduced and adsorbed the dissolved chromium thus altering the sample considerably between collection and the start of the analysis, and leading to changes in both the concentration of dissolved chromium and its oxidation state.

The results, however, show that the method can be used for a wide range of chromium concentrations and is quick and efficient when in use in the field (Table A.10).

Table A.10.

Results of Chromium (III) and Total Chromium Analyses of Loughor Estuary Samples.

Station	Time	Salinity ($^{o}/_{oo}$)	Cr^{3+}(ug/l)	Cr^{6+}(ug/l)	Cr_{tot}(ug/l)
1	1327	17.294	2.98	-	2.98
2	1013	29.161	2.40	-	2.40
2	1316	13.205	3.19	0.75	3.94
3	0610	21.916	1.47	0.26	1.73
3	0804	25.378	2.04	0.08	2.12
3	0915	23.785	5.98	0.93	6.91
3	1005	20.250	3.62	0.19	3.81
3	1103	12.449	1.59	-	1.59
3	1205	11.209	3.62	-	3.62
3	1310	9.388	3.74	0.01	3.75
3	1355	8.125	3.24	0.06	3.30
3	1502	6.984	0.41	0.61	1.02
3	1605	10.377	4.18	0.19	4.37
3	1655	22.175	0.93	0.64	1.57
3	1803	27.573	2.26	0.11	2.37
3A	0912	24.578	3.20	0.62	3.82
3A	1003	21.736	10.11	48.50	58.61
4	0800	23.395	1.92	-	1.92
5	0754	15.578	4.51	1.08	5.59
6	0600	17.429	0.72	0.66	1.38
6	0707	20.870	0.43	1.16	1.59
6	0748	20.146	1.90	0.08	1.98
6	0803	18.481	1.52	0.02	1.54
6	0907	13.073	3.91	1.02	4.93
6	1109	3.951	1.78	0.64	2.42
6	1303	1.65	1.78	-	1.78
6	1509	0.78	3.08	0.13	3.21
6	1720	0.58	2.98	-	2.98
7	0742	11.138	1.27	0.02	1.29
7	1405	1.65	1.08	0.55	1.63
8	0736	1.11	0.91	0.12	1.03
9	0730	<0.1	6.74	143.30	150.04

APPENDIX B.

B. Determination of Particulate Chromium.

B.1. Introduction.

The concentration of chromium in the particulate matter of unpolluted streams and rivers is normally within the range of 30-150μgCr g^{-1}. Rivers polluted by the element (e.g. with effluent from chromium plating works) may contain suspended matter having a far higher chromium level. This is usually the result of reduction to chromium (III) followed by hydrolysis and co-precipitation with hydrous iron (III) oxide. The toxicity of chromium raises the possibility that particulate matter containing high concentrations of chromium may be harmful.

B.2. Development of Method of Digestion.

Unlike many other metals chromium was found to be only partially leached from particulate material by nitric acid or aqua regia. For example, even after prolonged digestion in aqua regia (2 days) Mersey Mud was found to yield only 56% of the chromium present. Quantitative recovery could only be achieved by complete dissolution of the solids by heating them for 10days with a mixture of nitric acid and hydrofluoric acid in a P.T.F.E. beaker. The process was found to be speeded up if the material was heated with nitric acid for 36-48hrs before the addition of the hydrofluoric acid.

After the dissolution process had been completed it was necessary to eliminate the fluoride ion from the solutions because of the possibility of it causing interference in the

A.A.S. determination of chromium. In addition, there was a risk of corrosion of the A.A.S. burner if it was not removed. This difficulty was obviated by repeated evaporation with nitric acid. The final residue was made up to 10ml in a calibrated flask and analysed by atomic absorption spectrophotometry (A.A.S.) using flame or electrothermal atomisation, as appropriate.

B.3. Final Method.

B.3.1. Reagents.

i) Water.

See Section A.4.1.

ii) Nitric Acid.

Analytical grade nitric acid was redistilled from a silica still before use. This was also used to make a 0.1M nitric acid solution by adding 6.3±0.2ml of the distillate to 1ℓ of water.

iii) 40% Hydrofluoric Acid.

Ultra-pure hydrofluoric acid was used.

iv) 20%w/v Ammonium Chloride Solution.

Analytical grade ammonium chloride (20.0±0.5g) was dissolved in water and made up to 100ml±3ml.

v) Standard Chromium (III) Solutions.

Standard chromium solutions were made up as in Section A.4.1. using nitric instead of hydrochloric acid.

B.3.2. Apparatus.

i) Glassware.

All glassware was soaked overnight in 10% v/v nitric acid and washed thoroughly with water before use.

ii) Filtration Apparatus.

See Section A.4.2.2.

iii) Polytetrafluoroethylene (P.T.F.E.) Beakers (50ml).

P.T.F.E. beakers (50ml), with lids made of the same material were washed well with teepol, rinsed and boiled with 10ml of concentrated nitric acid. They were then rinsed well with water before use.

vi) Hotplate.

v) Polystyrene Containers. (30ml).

See Section A.4.2.4.

vi) Atomic Absorption Spectrophotometers.

An I.L. Model 350 spectrophotometer fitted with an air/acetylene flame atomiser was used as well as the Perkin Elmer Model described in Section A.3..

B.3.3. Sample Collection and Preservation.

See Section A.4.3.

B.3.4. Analytical Procedure.

The weighed filter was transferred to a P.T.F.E. beaker, and the container was rinsed out with a few ml of water if necessary. Nitric acid (10ml±0.5ml) was added, the beaker was covered and heated to just below the boiling point of the acid on a hot plate and allowed to cool for a few minutes before being made up to 5ml with nitric acid and then 5.0±0.3ml of hydrofluoric acid was added. The beaker was reheated until all the particulate material had been dissolved.

The cover was removed and the acid was evaporated almost to dryness. Nitric acid (5ml) was added and then evaporated

almost to dryness. This procedure was carried out at least a further two times until the residue could be completely dissolved in 0.1M nitric acid. The solution was made up to 10ml in a calibrated flask containing 1ml of 20% ammonium chloride solution (vis Sect. B.5.). If the particulate loading was small or the particulate matter was poor in chromium, flameless atomic absorption was used, making the addition of the ammonium chloride unnecessary. The solution was transferred to a dry polystyrene container (30ml) for storage prior to analysis by atomic absorption (Sect. B.3.5.)

Blank determinations were carried out by using the above procedure on membrane filters similar to those used to collect the suspended matter and which had been washed with 100ml of water passed through them.

Calibration standards were carried out simultaneously by carrying out the above process in a beaker containing 10μgCr.

B.3.5. Atomic Absorption Spectrophotometry.

The flame atomisation atomic absorption spectrophotometer was set up according to the manufacturer's instructions using the 357.9nm absorption line. The air/acetylene ratio was adjusted to give a flame which verged on luminosity. Although a more oxidising flame would have improved the sensitivity the interference effects were minimised by using a lean flame. This instrument was used for the analysis of samples containing more than 1μgCr.

The procedure was calibrated by aspirating chromium standards containing 0.1, 0.2, 0.5, 1.0, 2.0, 3.0 and 4.0μgCr ml^{-1}

Calibration standards, carried through the whole process, were also analysed but were not found to deviate from the calibration curve. Prior to every reading the instrument was zeroed against 0.1M nitric acid.

This method of analysis was found to be satisfactory for samples containing more than 1μg of chromium. Those containing less than 1μg of chromium were analysed using electrothermal atomisation as described in Section A.4.4.4. with a 10μl injection and a drying temperature of 130°C.

B.4. Performance Characteristics of the Method.

The above technique was used to carry out sextuplet analyses of 12-36mg portions of a washed, dried (110°C), finely ground and homogenised sample of Mersey mud. These showed a chromium content of 90.3±1.7μgCr g^{-1}. Six replicate analyses of the National Bureau of Standards sample of Indiana Harbour Canal sediment (N.B.S. 1645) showed a chromium content of 3.04±0.15% which is in satisfactory agreement with the certified value of 2.96±0.28% of Cr. As a final check on the method a 20ℓ bulk sample of water was collected from the Mersey. This was well shaken and four 1ℓ aliquots were filtered. The filters were dried, weighed and analysed for chromium using both flame and electrothermal atomisation, after dilution. Using flame atomisation the chromium content was found to be 162±10μg g^{-1} and using electrothermal atomisation it was found to be 164±10μg g^{-1}. The comparatively large standard deviation emphesises the difficulty of obtaining representative samples of particulate matter, as the standard deviation between

Table B.1.

Concentrations of Chromium in Mersey Particulates Found by Flame and Flameless Atomic Absorption Spectrophotometry ($\mu g\ g^{-1}$).

Digest Number.	1	2	3	4
Flame AAS	158	164	152	175
Electrothermal AAS	157	169	153	176

Table A11.

Effect of other substances on determination of chromium by flame (air-C_2H_2) atomization atomic absorption spectrophotometry.

Other substance	Other substance added as	Concentration of other substance (mg/l)	Effect * in µg/ml Cr of other substances at a chromium concentration of (2.50 µg/ml)
Aluminium	nitrate	500	-0.04
Calcium	chloride	500	0.10
Cobalt	nitrate	10	-0.05
Copper	nitrate	10	-0.02
Iron	nitrate	500	-0.08
Magnesium	nitrate	200	+0.05
Manganese	nitrate	10	-0.03
Nickel	nitrate	5	-0.03
Potassium	chloride	200	-0.02
Sodium	chloride	1000	$\pm$0.00
Zinc	nitrate	5	-0.02
Bromide	potassium salt	10	-0.04
Phosphate	di-potassium salt	2.5	+0.03
Sulphate	sodium salt	100	+0.02

* If the other substance did not interfere the effect would be expected to lie (95% confidence) between 0 $\pm$ 0.10 µg/ml at 2.50 µg/ml chromium.

Contains. 2% NH_4Cl.

Table A12

Effect of other substances on determination of chromium by electrothermal atomic absorption spectrophotometry.

Other substance	Other substance added as	Concentration of other substance (µg/ml)	Effect * in ng/mlCr of other substance at a chromium concentration of 100 ng/ml
Aluminium	nitrate	500	+1
Calcium	chloride	50	+5
Cobalt	nitrate	5	±0
Copper	nitrate	20	-1
Iron	nitrate	50	+5
Magnesium	nitrate	20	-1
Manganese	nitrate	50	0
Nickel	nitrate	5	-1
Sodium	chloride	100	0
Zinc	nitrate	5	-2
Bromide	potassium salt	10	-1
Phosphate	di-potassium salt	10	0
Sulphate	sodium salt	10	0

* If the other substance did not interfere the effect would be expected to be (95% confidence) between 0 ± 8 ng/ml at 100 ng/ml chromium.

the chromium concentrations found by the two techniques on individual digests was much less ($\pm 2\mu g\ g^{-1}$) (Table 10).

B.5. Interferences.

The effect of possible interferences was determined by carrying out analyses of standards containing $2.5\mu gCr\ ml^{-1}$ in 0.1M nitric acid and 0.1M nitric acid which had been spiked with the substance under investigation. No interference was found with electrothermal atomisation (Table 11). Flame atomisation, using a lean flame, was found to be very prone to suppression by iron (up to 60% suppression of signal). However, this effect was found to be eliminated if 2% of ammonium chloride was present in the final solution (Table 12).

B.6. Constraints on the Method.

It should be noted that the method probably only dissolved that chromium which was present adsorbed on the particulate material or which was present in the lattices of the silicate minerals. It will not attack chromite and, probably, other oxide minerals. However, these compounds are of little ecological significance and constitute only a very small component of the total suspended load.

C. Conclusions.

Chromium can be determined in natural waters by concentrating it by co-precipitation with hydrous iron oxide, and dissolution of the precipitate in hydrochloric acid followed by atomic absorption spectrophotometry. Either total chromium or chromium (III) can be estimated with satisfactory precision down to levels of about 32ng l^{-1}. Samples to be analysed for total chromium can be stored at pH 2, however, determination of chromium (III) must be commenced immediately after collection of the sample. Ultra violet photolysis provides an efficient means of breaking down organic complexes of chromium- e.g. those with fulvic acid

The normal analytical method of leaching trace elements from suspended particulate material by digestion in aqua regia is inefficient for chromium. For this reason it is necessary to resort to dissolution of the sample with a mixture of nitric and hydrofluoric acids before carrying out atomic absorption spectrophotometry.

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