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# THE DETERMINATION OF IODINE SPECIES IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES

(Technical Report)

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## The determination of iodine species in environmental and biological samples (Technical Report)

*Abstract:* Iodine is found in organic forms in plants and animals and in inorganic forms in natural water samples. Methods of identification and quantitative determination for iodine species are decided by the types of compound and the matrices in which they occur. Iodine is an essential element and specific radioimmunoassay methods have been devised for physiologically important compounds (thyroxine and related compounds) in which it is found. In addition, marine plants and animals have provided a rich source of varied and unexpected organic iodine compounds that occur, along with other organic halogen compounds, as secondary metabolites. Natural waters, particularly marine waters contain iodine chiefly in the forms of iodide and iodate. Organic iodine compounds in biological samples have been identified by classical natural products chemical techniques following their isolation, and by modern spectroscopic methods. Routine analysis of such compounds can be done by gas chromatography (GC) and high performance liquid chromatography (HPLC). Analysis of inorganic iodine species in waters is carried out by catalytic, electrochemical and spectrometric methods and by GC; possibly HPLC inductively coupled-mass spectrometry (ICP-MS) might be the method of choice in the future.

### INTRODUCTION

Iodine (atomic number 53, atomic weight 126.90447), a halogen, although widely distributed in the biosphere, is usually classified among the rarer elements. It is ranked 62nd in abundance with an average crustal concentration of 0.46 p.p.m. [1,2]. It occurs in seawater at a concentration of 0.05 p.p.m. and is concentrated by some algae [2,3]. Iodine is essential for life and is a component of the thyroid hormones [4,5]. It is used in the manufacture of germicides, antiseptics and dyestuffs, as a catalyst, in analytical chemistry and other aspects of the chemical industry, and in photography. Artificial isotopes of iodine are used in medical, biological and biochemical research [1–3].

### SPECIATION OF IODINE

For chemical species of an element to be estimated individually they must be analysed by techniques that do not require the destruction of the matrix. Such destruction would in almost all cases alter the nature or balance of species originally present. Thus the matrices must be those in which the species of interest are dissolved (natural waters, in particular seawater, in the case of iodine species), or gaseous media (in which the iodine species are also gases), or can be extracted into solution (for example the organic iodine species in marine algae can be extracted into organic solvents). The separation of particulate iodine (iodine in some form adsorbed on to a particle or bound to an insoluble macromolecule) from non-particulate iodine (in solution or gaseous) that might be brought about by a means of filtration was not considered and the few papers dealing with such techniques have been disregarded. Also we were concerned only with chemical species and the considerable body of work regarding the detection and estimation of radioisotopes of iodine has not been consulted. However the chemical species of radioiodine ( $^{131}\text{I}$ ,  $^{129}\text{I}$ ) in the terrestrial and aquatic environments have been studied [6]. Brief mention has been made of the radio-immunoassay methods for the estimation of iodine-containing hormones and related compounds in the animal, particularly the human, body. Even though such methods do not depend, in a strict sense, on the presence of iodine in the molecule, they facilitate the estimation of iodine-containing species in situations where any other available method might be prohibitively laborious or much less sensitive.

## ENVIRONMENTAL AND BIOLOGICAL CATEGORIES

Iodine species in environmental and biological samples have been classified into the categories that reflect their natural occurrence.

- (i) Organic iodine compounds in animals
  - (a) Marine animals
  - (b) Thyroxine and related compounds
- (ii) Organic iodine compounds in marine plants
- (iii) Organic iodine compounds in seawater and organic and inorganic iodine compounds in the atmosphere
- (iv) Iodide and iodate in water (chiefly seawater) samples
- (v) Iodide in miscellaneous matrices

## CHEMICAL FORMS AND THEIR ANALYSIS IN THE VARIOUS CATEGORIES

### (i) Organic iodine compounds in animals:

#### (a) Marine animals

In 1905 Wheeler and Jamieson showed [7], by a comparison with synthetic material, that 'Jodgorgosäure' first isolated [8] by Drechsel in 1896 from a gorgonian coral was 3,5-diiodotyrosine (Table 1, structure **17a**) and not iodoaminobutyric acid as had been assumed by its discoverer. Such an identification had already been questioned by Henze [9]. It was then established [10] that the iodine compound in the ordinary bath sponge (presumably *Spongia officinalis obliqua*) was also 3,5-diiodotyrosine (elemental analysis, mixed mp with synthetic material). Further investigations, particularly those of Ackermann [11,12] and Low [13,14] confirmed the presence of 3,5-diiodotyrosine in sponges and demonstrated the concurrent presence of the dibrominated analogue.

Iodovulone I (**18**) and its bromo- analogue, unprecedented prostanoids, apparently with antitumour activity, were isolated from the stolonifer *Clavularia viridis* by flash silica gel chromatography of ether extracts followed by repeated high performance liquid chromatography (HPLC) on silica gel [15]. Iodovulone I was identified by mass spectrometry (MS) and nuclear magnetic resonance (NMR), infra-red (IR) and ultra-violet (UV) spectroscopy. Routine methods for the analysis of such a compound are only likely to be developed if valuable pharmaceutical activity is confirmed.

#### (b) Thyroxine and related compounds

That iodine is a normal constituent of the thyroid gland was discovered [16] by Baumann in 1895. The organo-iodine compound (thyroxine) contained in the thyroid was successfully isolated and crystallised by Kendall in 1915 [4] although he subsequently put forward an erroneous structure for the compound [17]. The correct structure (3:3':5:5' tetraiodothyronine; Table 1, **19a**), first of the desiodo- compound, then of thyroxine itself (T4) was established by Harington [5,18] in the mid 1920s using classical techniques of analysis, controlled degradation, and synthesis. Subsequently a second active hormone was isolated from the thyroid gland and shown, ultimately by synthesis, to be 3:3':5-triiodothyronine (T3, **19b**) [19].

Since that time it has been necessary, for medical and other reasons, to devise methods to routinely measure the concentration of the various iodine-containing hormones, their amino acid precursors and their protein carriers in serum and other components of the human body. Methods that have been or are being used include radioimmunoassay [e.g. 20] fluorescence immunoassay [21], enzyme immunoassay [22], and competitive protein binding assay [23]. Knapp and Leopold [24] used an automated catalytic method, analogous to that for the determination of iodide in water samples, to detect T3 and T4 eluting after separation on liquid chromatograph columns. They considered that enough free iodine atoms were being split off from the hormone molecules by the oxidative effect of the catalytic mixture to facilitate the detection method. Their system allowed the determination of as little as 0.20 ng of T3 and 0.10 ng of

T4. The use of HPLC [25–27] also allowed the determination of enantiomeric iodinated thyronines. Again the detection was by a catalytic method. Other chromatographic methods have been used for the separation of thyroid hormones - gel chromatography [28,29], ion-exchange chromatography [30,31], GC [32–34] and HPLC [35, 36]. For the analysis of plasma samples by HPLC a prior separation from the serum proteins is necessary. Precipitation with organic solvents such as THF achieves this end and also extracts iodinated thyronines. UV detection after HPLC separation is not sensitive enough for plasma levels of thyroid hormones and the catalytic method of determination as mentioned above is required.

In current clinical practice radioimmunoassay of T3 and T4 appears the method of choice for routine analysis [e.g. 37–41].

### (ii) Organic iodine compounds in marine plants

Generally, the iodinated compounds that have been identified from marine algae are non-polar derivatives of secondary metabolites whereas from marine animals iodinated tyrosine has predominated. Also iodinated compounds from marine algae usually exist as part of a complex of poly-halogenated compounds with bromo- or, occasionally, chloro- analogues. A number of iodine-substituted compounds have been isolated from marine red algae and identified largely by the spectroscopic and spectrometric techniques currently applied in natural products chemistry, particularly to compounds of a non-polar or volatile nature. Thus NMR spectroscopy (both  $^1\text{H}$  and  $^{13}\text{C}$ ) and mass spectrometry, particularly GC-mass spectrometry together with IR and UV absorption spectroscopy have in various combinations been used to identify the compounds illustrated in Table 1. Silicic acid chromatography of an ethanol extract of the red alga *Bonnemaisonia hamifera* yielded 1-iodo-3,3-dibromo-2-heptanone (**20**) as well as brominated analogues lacking iodine [42]. The authors claim this as the first natural product containing both iodine and bromine, and the first iodine-containing natural product other than iodinated thyroxine related compounds (presumably this category includes the iodinated tyrosines identified in marine animals, sponges in particular).

Subsequently a family, including two iodinated derivatives, of multihalogenated gamma-methylene lactones (**21a,b**) were isolated from *Delisea fimbrata* [43]. In this case the compounds were separated from a dichloromethane extract of the alga by open column silica gel chromatography and HPLC. The use of nmr for structural assignments was aided by an X-ray crystallographic analysis of a brominated analogue. *Delisea fimbrata* also yielded a series of halogenated ketones [44] which included two iodinated compounds (**22a,b**); one of these also contained bromine and the other both chlorine and bromine. Two iodobromo aromatic sesquiterpenes (**23,24**) were isolated from a dichloromethane extract of *Laurencia nana* by column chromatography and HPLC [45]. Again mass spectrometry and nmr and IR spectroscopy played major roles in the structural assignments of the compounds.

It seems unlikely that routine methods of analysis will be required for such compounds although rapid screening of algae may be necessary if the antibiotic/antifungal properties claimed for the compounds lead to their acceptance as pharmaceuticals. Presumably HPLC or GC MS would be the most appropriate methods for such investigations.

4-amino-7-(5'-deoxyribos-1' $\beta$ -yl)-5-iodopyrrolo[2,3-d]pyrimidine (**25**), a compound which produced pronounced muscle relaxation and hypothermia in mice, was isolated (fractionation on Florisil followed by HPLC) from extracts of the red alga *Hypnea valendiae* and identified by high resolution mass spectrometry and nmr spectroscopy. Purification of the compound at all stages was monitored by bioassay [46].

A claim has been made [47] for the presence of diiodotyrosine (**17a**) in algal species of the genus *Laminaria* but Coulson, who also offered some (paper chromatographic) evidence for the presence of 3,5-diiodothyronine and thyroxine (**19a**) in marine brown algae, suggested that care would be needed in investigating iodine amino acids in algae and thereby possibly implied that some caution should be exercised in accepting the results [48]. No more definitive reports have been published, to our knowledge, since these early studies although it is quite possible that algae ultimately provide the source of 3,5-diiodotyrosine in marine animals such as sponges.

A number of studies have been carried out on the release by marine algae and other organisms to the sea and/or the atmosphere of small organohalogen compounds (usually including methyl iodide), but these are considered in the next section.

### (iii) Organic iodine compounds in seawater and organic and inorganic iodine compounds in the atmosphere

In 1973 Lovelock detected methyl iodide in the marine atmosphere and in surface seawater of the Atlantic Ocean [49]. Analysis was by GC with a novel electron capture (EC) detector capable of coulometric (absolute) measurements. The concentration of MeI in the atmosphere was  $0.0012 \text{ cm}^3 \cdot \text{m}^{-3}$ , and in surface seawater  $135 \times 10^{-12} \text{ cm}^3 \cdot \text{cm}^{-3}$  water (NTP). Lovelock suggested, because of the large difference between water and air concentrations, that MeI was the natural carrier of iodine between sea and land. He also suggested that algae were the source of MeI in the ocean. Further measurements of MeI in coastal waters of southern England ( $1.2\text{--}2.8 \times 10^{-9} \text{ cm}^3$  of gas per  $\text{cm}^3$  of water) and in Atlantic and Antarctic open ocean, Atlantic and Caribbean, off SW Ireland and adjacent to kelp beds off SW Ireland ( $135, 138, 3400$  and  $12\,000 \times 10^{-12} \text{ cm}^3$  gas per  $\text{cm}^3$  seawater respectively) strongly linked MeI production to an algal source [50].

Other studies have continued the work initiated by Lovelock. Zafiriou was concerned with the generation of MeCl from the reaction of algae-generated MeI (as suggested by Lovelock) with Cl ion [51]. He conducted laboratory experiments with seawater, identifying products by GC MS. Chloroform was the internal standard; the standard, reagent (MeI) and volatile product were separated on an  $8 \text{ m} \times 0.3 \text{ mm}$  wall-coated OV-101 column at  $23 \pm 1 \text{ }^\circ\text{C}$  and were detected by flame ionisation.

McConnell and Fenical (1977) conducted a GC MS study of organohalides extractable from the red alga *Asparagopsis* with various solvents. Their work extended the number of compounds identified (Table 1) and reinforced the notion that algae give rise to oceanic organic iodine (particularly MeI) and thus to atmospheric iodine [52].

Chameides and Davis (1980) were concerned with the photochemistry of atmospheric iodine and produced a theoretical treatment based on laboratory photolysis experiments that indicated that photolysis of MeI would generate I which would interact with atmospheric species such as  $\text{O}_3$ ,  $\text{H}_x\text{O}_y$ , and  $\text{NO}_x$  to produce IO, HOI,  $\text{IONO}_2$  and  $\text{I}_2$ . Production and cycling back to I could cause catalytic removal of tropospheric  $\text{O}_3$  it was postulated [53]. Cicerone (1981) reviewed [54] reports on halogens in the atmosphere and referred to earlier work, particularly that of Duce and co-workers [55]. He divided atmospheric iodine into inorganic iodine gases, organic iodine gases, iodine in particles, and iodine in precipitation, but of quoted work only that of Lovelock [49] gave information on a specific iodine compound (MeI). He also gave a theoretical treatment of the likely key processes and mechanisms for the transformations of iodine compounds in the atmosphere.

Important studies on methyl iodide in the atmosphere and methods for its measurement were carried out in the early 1980s by Rasmussen et al [56] and Singh et al [57]. Rasmussen used EC GC ( $^{63}\text{Ni}$  source operated at  $350 \text{ }^\circ\text{C}$ ; 10% SP2100 on 100/200 mesh Supelcoport  $10 \text{ ft} \times \frac{1}{4}$  in OD, stainless steel; temperature programme  $10 \text{ }^\circ\text{C}$  to  $80 \text{ }^\circ\text{C}$  at  $16 \text{ }^\circ\text{C}/\text{min}$ ) to analyse 100 mL samples of air trapped in a U-tube containing glass beads at the temperature of liquid oxygen. Singh also used EC GC for his study. 400 mL air samples were preconcentrated in a stainless steel trap packed with glass wool and held at the temperature of liquid argon or oxygen. The cryogen was replaced with boiling water and the carrier gas allowed to carry the sample to the GC column ( $10 \text{ m} \times \frac{1}{8}$  in nickel, 20% DC 200 on 80/100 mesh Supelcoport).

Harper (1985) showed that the fungus *Phellinus pomaceus* was able to convert I<sup>-</sup> to MeI. Measurement by headspace GC with flame ionisation detection [58, 59].

In 1985 the release of volatile halogenated organic compounds into seawater by a range of temperate marine algae was demonstrated [60]. Macroalgae (*Ascophyllum nodosum*, *Fucus vesiculosus*, *Enteromorpha linza*, *Ulva lacta* and *Gigartina stellata*) were incubated in jars or in aquaria and after one day 2 L samples were withdrawn and the organo-halogen compounds concentrated by closed loop stripping with Tenax traps used for collection of compounds of interest. Organoiodine compounds on the

traps were detected and identified by GC MS. Detected were methyl-, ethyl-, isopropyl-, n-propyl iodide, chloriodomethane and diiodomethane. Also extracted from algal tissues (as opposed to being detected in the water) were methyl iodide, 1-iodobutane and 1-iodopentane. The authors estimated that about  $10^8$  g I per year entered the atmosphere from macroalgal sources (compared with an estimate of  $10^{12}$  g per year of phytoplankton derived MeI [56]).

Studies on the release of MeI to the environment by kelp, both in axenic culture and with associated microbes and by other macroalgae were taken up by Manley and coworkers [61–63] who made measurements by EC GC with the  $^{63}\text{Ni}$  source operated at 275 °C. Methyl iodide (and other methyl halides) were extracted into nitrogen by equilibrating a seawater sample with the gas and cryogenically concentrating the gas phase for GC injection. Analysis was routinely performed on a stainless steel column (6.1 m long  $\times$  3.2 mm dia) packed with 20% SP 2100 on 80/100 mesh Supelcoport maintained at 25 °C. Peak identification was initially verified by GC-MS and repeatedly verified with standards and with the independent use of another column packed with 10% SP 1000 on 80/100 mesh Supelcoport and operated at 50 °C.

Klick and Abrahamsson (1992) also used EC GC to detect and measure a range of volatile organoiodine compounds in open ocean and coastal surface waters [64]. In the samples 1-iodopropane, 2-iodopropane, chloriodomethane, 1-iodobutane, 2-iodobutane and diiodomethane were determined. Seawater samples were extracted with pentane and the extracts analysed on two connected fused silica capillary columns; first a DB-1701 (25 m  $\times$  0.32 mm; 0.1  $\mu\text{m}$  film), second a DB5 (30 m  $\times$  0.32; 1  $\mu\text{m}$  film). The temperature programme was: 2 min at 40 °C, then to 100 °C at 10 °/min. For low concentrations of the compounds, close to the detection limits, the analytical error was estimated to be between 3 and 12% (relative standard deviations) for the individual compounds. Because samples were taken from open ocean surface waters the authors believed their origin to be phytoplankton rather than macroalgae.

Heumann and co-workers [65, 66] also studied methyl iodide and other organoiodine compounds in seawater and the atmosphere. A purge and trap method was used [65] to remove MeI from samples of Atlantic surface water and prepare them for analysis by EC GC ( $^{63}\text{Ni}$  source). Separation of MeI from Freon 113 and  $\text{CH}_2\text{Cl}_2$  was achieved with two coupled capillary columns of different polarity [first, OV 1701 (50 m  $\times$  0.32 mm; 1  $\mu\text{m}$ ), second, SE 54 (50 m  $\times$  0.32 mm; 5  $\mu\text{m}$ )]. The temperature was held at 40 °C for 3 min and then increased at 3 °C/min to 180 °C. Methyl iodide could be detected in all seawater samples in this study ( $n = 48$ ) with concentrations ranging from 0.22–1.16 ng I/L with a mean of  $0.6 \pm 0.3$  ng I/L.

This work was continued [66] by examining volatile organoiodine (and organobromine compounds) in arctic seawater and air samples. Again the purge and trap method was used to prepare water samples for EC GC but on this occasion GC analyses were carried out on a Restek type Rtx 502.2 (60 m  $\times$  0.32 mm; film 3  $\mu\text{m}$ ) column. The temperature programme was: 10 min at 40 °C, increased to 100 °C at 3 °C/min, 5 min at 100 °C, increased to 240 °C at 3 °C/min. Organohalogen compounds in air samples were absorbed onto Carbosieve S-III and thermally desorbed for GC by heating the tube containing the absorbent to 400 °C.  $\text{CH}_3\text{I}$ ,  $\text{CH}_2\text{I}_2$ ,  $\text{CH}_2\text{ClI}$ ,  $\text{CH}_3\text{CH}_2\text{CH}_2\text{I}$  and  $\text{CH}_3\text{CHICH}_3$  were determined in both in both air and seawater.

Although EC GC is a selective method for halogen compounds, it is not very specific to iodo-compounds, and this means that gas chromatographic separation from other halogen compounds is important. A compilation of measured retention indices (RI) of 221 halogenated aliphatic and alicyclic compounds based on a 1-bromoalkane scale by temperature-programmed GC using a fused-silica capillary column coated with methyl silicone has been established. RIs were given for 16 iodo-alkanes together with other halogenated compounds [67]. Retention times and indices of 30 chloro-, bromo-, and iodo-alkanes on non-polar (SB-1) and polar (Supelcowax-10) wide-bore capillary columns have also been given [68].

A series of filters was used to separate atmospheric particulate iodine, HI and  $\text{I}_2$  (NaOH impregnated filter), HOI (TBAH impregnated filter), and organoiodine (activated charcoal). Analysis of the various fractions was by isotope dilution mass spectrometry [69].

Determination of the chemical forms of iodine in the gas of a nuclear power plant was necessary to facilitate the design of effective processes for its removal. The major form was considered to be  $I_2$  [70]. Iodine species in reactor effluents had been measured previously [e.g. 71]. A significant fraction of the  $^{131}I$  in ventilation air in both boiling water reactors and pressurised water reactors was in the form of hypoiodous acid (HOI) [71]. Air to vegetation transport of HOI has been studied [72].

During atmospheric sampling of trace organic gases, the use of potassium iodide-based ozone traps gave rise to artefact formation of organic iodine compounds not present in the atmosphere [73].

A method for determining methyl iodide in the work-place was given [74] using a diffusive sampler and capillary GC/FID with thermal desorption. A time-averaged concentration of MeI in the range of sub p.p.m. to p.p.m. for 2 to 6 hours were measured by the method.

#### **(iv) Iodide and iodate in water (chiefly seawater) samples**

Iodine species are often measured in natural water samples, including seawater, without any initial separatory (chromatographic) step or any other pretreatment of the sample. When separation has been performed, it has often been on artificial mixtures of anions and there has been sometimes only token attempts to apply the devised method to real samples.

It is usually assumed in most of the methods discussed below that the only iodine species in the water samples are iodide and iodate and, consequently, that iodide + iodate = total iodine. Thus, determination of two of these quantities automatically gives the third.

Iodine is a minor chemical element in seawater with a total concentration of between 400 and 500 nM [75]. It is present in seawater principally as two chemical species, iodate ( $IO_3^-$ ) and iodide ( $I^-$ ) [76]. Iodate is considered to be the thermodynamically stable form in oxygenated waters and theoretically iodide should not be detectable in surface waters [77]. Significant amounts of iodide are, however, often observed in the euphotic zone with a concentration up to 250 nM [e.g. Ref 78]. This thermodynamic equilibrium is thought to result from biological activity [75].

The main methods for analysis are:

- (a) Catalytic
- (b) Electrochemical
- (c) Spectrophotometric
- (d) GC after derivatization
- (e) Miscellaneous—colorimetric, ICP-atomic emission spectrometry (AES), ICP-MS, isotope dilution MS, X-ray fluorescence (XRF), chemiluminescence.

##### *(a) Catalytic*

The catalytic action of iodide on the reaction between arsenious acid and ceric sulfate in sulfuric acid solution can be used to detect and estimate iodine as iodide [79]. This early report (1934) was followed by another (in 1953) describing determination of iodide by its catalysis of the oxidation (and thereby colour loss) of ferric thiocyanate in nitric acid solution [80]. Whether these methods determine only iodide-iodine or total iodine depends upon the oxidation-reduction potential of the reaction mixture [81]. The catalytic acceleration by iodine of both of these reactions has been used to measure iodine in seawater as part of substantial surveys [82–85]. Yonehara (1964) first estimated total iodine by its catalysis of the oxidation of ferric thiocyanate and then removed iodide by oxidising it to elemental iodine and extracting it into carbon tetrachloride; the remaining iodate-iodine was then measured by a repeat of the method used for total iodine [85]. The concentration of iodide was then available by difference.

It was found [86] that the catalytic activity of iodine in nitric acid solution is 20 times that in sulfuric acid and is also far less sensitive towards accompanying ions, making the system far more useful for the determination of traces of iodine.

Another reaction catalysed by iodine, that of mercury (II)-4-(2-pyridylazo)resorcinol and 1,2-cyclohexanediamine-N,N,N',N'-tetraacetic acid, has been used as the basis for the estimation of iodide in rainwater [87]. Concentrations as low as  $10^{-8}$  M could be determined. Inorganic iodine in rainwater has also been determined using the catalytic effect of iodide on the oxidation of thiocyanate ions [88].

A method for the determination of total iodine and iodate-iodine in freshwater based on the catalytic effect of iodine on the reaction between ammonium cerium(IV) sulfate and arsenic(III) oxide [89] showed that total iodine, present at 0 to 5  $\mu\text{g/L}$ , could be resolved to within  $\pm 0.1$   $\mu\text{g/L}$  ( $\pm 2 \times$  standard error) at the 95% confidence level (11 replicate samples). The iodate-iodine in the sample was determined by the same procedure after any iodide-iodine had been extracted into chloroform as an ion pair with the tetraphenylarsonium cation. The analysis of sets of six replicate samples encompassing the 0 to 0.5  $\mu\text{g/L}$  range of iodate-iodine concentrations and containing also 10  $\mu\text{g/L}$  of iodide-iodine returned essentially the same standard errors as for the total iodine determinations. Analysis for total iodine could be conducted at a rate of approximately 50 samples per hour and that for iodate-iodine at about 20 per hour.

A throughput rate of 20 samples per hour was achieved [81] for an automated system using a Technicon AutoAnalyser and based upon the catalytic effect of iodide on the destruction of thiocyanate by the nitrite ion. Two automated systems were employed; in the first the oxidation-reduction potential of the solution was adjusted so that iodate was not reduced to iodine or iodide. Thus free iodide only was estimated. This was achieved by reducing the concentration of nitric acid in the ammonium iron(III) sulfate reagent, by reducing the concentration of the sodium chloride reagent, and by reducing the concentration of the potassium thiocyanate reagent. These changes resulted in a loss of sensitivity. So total inorganic iodine was determined in a number of drinking waters over the range 0.2 to 5.0  $\mu\text{g/L}$  and free iodide over the range 0.4 to 5.0  $\mu\text{g/L}$ . The authors tested possible interfering ions and concluded that interferences would not present any major problems in the analysis of drinking waters for either total inorganic iodine or free iodide. The general precision of the total inorganic iodine method was of the order of 3% and that of the free iodide method was of the order of 10%. In the absence of certified reference material, accuracy was estimated by recoveries from spiked samples. The mean recovery of iodide by the total inorganic iodine method and the free iodide method were  $100 \pm 5.4\%$  and  $102 \pm 7.5\%$  respectively.

A thermometric (rather than colorimetric) technique for measuring the reaction rate of the iodide-catalysed cerium(IV)-arsenic(III) reaction has been employed for the determination of iodide in seawater and urine [90].

The catalytic method for determining iodide based upon its catalysis of the reduction of ceric ions by arsenious acid is the basis of the method recommended in Standard Methods for the Examination of Water and Wastewater [e.g., 17th edn, Ref 91] for samples containing less than 80  $\mu\text{g/L}$ . Results obtained by this method were reproducible (Los Angeles source waters) and reported to be accurate to  $\pm 0.3$   $\mu\text{g/L}$  [92, 82].

A novel acetone- $\text{H}_2\text{O}_2$ - $\text{ClO}^-$  chemiluminescence system was applied to the determination of iodide in river water and other samples [93]. The above system is catalysed by iodide ion. The detection limit was reported as  $2 \times 10^{-11}$  g/mL I<sup>-</sup>; the linear dynamic range was  $4 \times 10^{-10}$  g/mL I<sup>-</sup>, and the variation coefficient at an iodide concentration of  $5 \times 10^{-9}$  g/mL was 4.6%.

A flow injection method was devised for the spectrophotometric determination of trace amounts of iodide based upon its catalytic effect on the oxidation of 4,4'-bis(dimethylamino)diphenylmethane (tetrabase) by chloramine T in weakly acidic solution [94]. Iodide in water samples could be determined at a rate of 85/h with 300  $\mu\text{L}$  injections. The detection limit was 0.1  $\mu\text{g/L}$  and the calibration graph for iodide was linear up to 2  $\mu\text{g/L}$ . Free iodine showed a lower catalytic effect but iodate showed no effect up to at least 50  $\mu\text{g/L}$  as iodine. Both showed the same effect as iodide upon being reduced with thioacetamide in acidic solution. The method was applied to the determination of iodide in natural water samples and to iodine impurities in distilled and redistilled water samples. It was also used to examine iodine in ambient air.

A method combining both kinetic and potentiometric features for the determination of iodide and based upon the use of a coated wire Sb(V) ion-selective electrode to study the iodide-catalysed reaction

between hexachloroantimonate(V) and hydroxylamine has been proposed [95]. Under the selected experimental conditions of  $1 \times 10^{-6} \text{ mol dm}^{-3} \text{ SbCl}_6^-$ ,  $8 \times 10^{-2} \text{ mol dm}^{-3} \text{ NH}_2\text{OH}$ ,  $1 \times 10^{-1} \text{ mol dm}^{-3} \text{ HCl}$  and  $25 \pm 0.1^\circ$ , iodide could be determined in the range 0.01 to  $0.40 \mu\text{g cm}^{-3}$  with good selectivity.

The catalytic acceleration by iodide of the reaction between chlorpromazine and hydrogen peroxide has also been proposed [96] as a method for the determination of trace amounts of iodide. In the presence of iodide, chlorpromazine is oxidized by hydrogen peroxide in sulfuric acid solution to form a red intermediate which is further oxidized to a colourless compound. The maximum absorbance (at 525 nm) was obtained on an absorbance-time curve at a given reaction time. Since the maximum value increased with an increase in the iodide concentration, this value was used for the iodide determination. Under optimum experimental conditions ( $1.0 \times 10^{-3} \text{ M}$  chlorpromazine, 1.5 M sulfuric acid, 2.0 M hydrogen peroxide,  $30^\circ$ ) iodide in the range 0.2 to  $10 \mu\text{g/L}$  could be determined. The relative standard deviations were 0.8, 2.6 and 4.2% for 6.0, 2.0 and  $0.6 \mu\text{g/L}$  iodide respectively. Iodate showed the same catalytic effect as iodide at the same concentration of iodine but the catalytic effect of free iodine was lower. The technique was applied to river waters and to rainwater.

### (b) Electrochemical

A differential pulse polarographic method for the determination of iodine species in seawater [97] had significant advantages over other methods; the technique was simple, rapid and specific, and the lack of sample manipulation before analysis was considered by the authors as more likely to yield true speciation than procedures involving more complex chemical processing. Iodate was measured directly; a separate aliquot of the sample was oxidised (UV irradiation) and the iodate concentration remeasured. On the assumption that only iodate and iodide were significant species in the samples and that iodide was oxidised completely to iodate by this means, total iodine and thereby iodide-iodine were measured. Seawater samples collected from the Santa Barbara Basin off southern California were studied. The precision of the iodate determination was 2.5% which corresponded to a standard deviation of  $1.0 \mu\text{g/L}$  for an iodate concentration of about  $40 \mu\text{g/L}$ . Because iodide was derived from the difference of two iodate determinations, the standard deviation for iodide was approximately  $1.4 \mu\text{g/L}$ .

The same technique was used [98] to study iodine species in estuarine water and seawater. However it was found that UV irradiation was an unsatisfactory method for the oxidation of iodide to iodate, and consequently the oxidation was carried out by the addition of chlorine water.

Cathodic stripping square wave voltammetry allowed direct determination of iodide in seawater, freshwater and brackish water at subnanomolar concentrations [99]. Precision was typically  $\pm 5\%$ . The minimum detection limit was 0.1 to 0.2 nM (12 parts per trillion) at a deposition time of 180 s. The authors claimed that the method was 1 to 2 orders of magnitude more sensitive than previous methods. In a survey of iodine species in Chesapeake Bay waters [100] the differential pulse polarographic method of Herring and Liss [97] was used to measure iodate directly, and total iodine after oxidation of susceptible species to iodate with hypochlorite. Iodide-iodine was assumed to constitute the difference between total iodine and iodate-iodine.

The reduction of iodate to allow determination of total iodine as iodide by cathodic stripping square wave voltammetry was accomplished by the use of sodium sulfite under acidic conditions (pH 1–2) [101]. The pH of the sample was raised to 8–9 before iodide was measured. However for an extensive survey of changes in iodine speciation across coastal hydrographic fronts in southeastern United States continental shelf waters the same workers [102] used the differential pulse polarographic method of Herring and Liss [97] to measure iodate directly and total iodine was measured by the same method after oxidation of iodide to iodate by the hypochlorite method [103]. Again the difference in concentration between total iodine and iodate was considered to be the concentration of iodide. The precision for the determination of iodate and total iodine were both about  $\pm 5\%$  and the detection limit was about  $0.2 \mu\text{M}$ .

A recent survey of iodine speciation in the northwest Mediterranean Sea [75] used cathodic stripping square wave voltammetry to measure iodide, and differential pulse polarography to determine iodate. The difference between total iodine measured in this way and iodide + iodate was taken to be the concentration of organically-bound iodine.

A number of other studies have used electrochemical methods for the estimation of iodine species in water samples including piezoelectric methods [104, 105], methods using ion-selective electrodes [106–109], and a flow-through electrode system [110]. Electrochemical methods have also been used as sensitive detectors following chromatographic separation of iodide in mixtures of anions [111].

### (c) Spectrophotometric

A Technicon Auto-Analyser was used for the automated spectrophotometric determination of iodate- and total iodine in seawater [112]. Iodate was measured by reaction with acid and excess added iodide to give the iodonium ion ( $I_3^+$ ) which was detected spectrophotometrically (350 nm). Total iodine was determined by the same procedure after all iodide had been converted to iodate by oxidation with bromine water. A related technique was also reported by Utsumi *et al.* [113] who later [114] converted iodide to iodine (hydrogen peroxide), extracted it into carbon tetrachloride, then back extracted it into acidic solution containing Bindschedler's green leuco base forming a complex with an absorption maximum close to 720 nm.

Other spectrophotometric techniques have been described [115–121].

A spectrophotometric method was developed for the measurement of iodate and periodate and applied to their analysis in freshwater [122]. Triphenyltetrazolium chloride reacted with iodate or periodate and the complexes formed, after extraction procedures were carried out, were measured (255 nm for iodate, 295 nm for periodate).

A substantial survey of iodine cycling in the Sargasso Sea and Bermuda inshore waters [123] used, essentially, the method of Truesdale and Spencer [124]. Thus iodate-iodine was measured spectrophotometrically as the iodonium ion and iodide-iodine was converted to iodate by oxidation. However, in this case photo-oxidation was again employed. The precision of the iodate analyses, based on replicate analyses within a single batch of samples was in the range 1 to 5%. Replicate analysis of a single bulk stored sample by two analysts working independently gave a precision of  $\pm 0.02 \mu\text{M}$  or 5.5% for 7 determinations. The precision (1 standard deviation) of the total iodine method was assessed in the same way, and was estimated to be  $\pm 0.02 \mu\text{M}$  or 4%.

A survey of the distribution of dissolved iodine species in the Irish Sea carried out in 1977 used a catalytic method based on the cerium (IV) – arsenic (III) reaction to determine total iodine, and a spectrophotometric method for iodate-iodine based on its conversion to the iodonium ion [125].

### (d) Gas chromatographic

Gas chromatographic estimation of inorganic halides, including iodide, after derivatization by alkylation [126]; by phase transfer catalysis [127]; as HI [128]; and as pentafluorobenzyl iodide [129]. Electron capture appeared to be the detection method of choice [e.g. 128, 129]. The method can be sensitive. Wu *et al.* [128] reported a detection limit of about 1 ng of iodide ion in 0.10 mL of aqueous sample; interferences were found to be minimal and it was reported that the method was applicable to the determination of iodide in spring water.

Probably the GC method that has been applied to the greatest range of samples involved the conversion of iodide (and bromide) to acetone derivatives and their subsequent detection by electron capture [129]. The technique was used for measuring iodide in rainwater, drinking water, river water, seawater, oil brine, common salt, cow's milk, and human blood serum. The relative standard deviation at  $10^{-8}$  M concentration was 3.0% using a 10 mL sample for the determination without preconcentration.

### (e) Miscellaneous methods

Chromatographic separation of iodide when it is part of a mixture of inorganic anions has been achieved by ion chromatography with electrochemical and conductometric detection [130], HPLC with a micellar mobile phase and UV detection [131], ion-pair chromatography on a reversed phase column with UV detection [132], anion-exchange chromatography with postcolumn fluorescence detection [133], non-suppressed ion chromatography with indirect UV detection [134], by ion chromatography with UV detection [135], or with UV and amperometric detection [136, 137], by anion exchange chromatography

with subsequent colorimetric determination by the leuco crystal violet method [138], by HPLC with precolumn derivatization and UV detection [139], and by reversed-phase ion-pair chromatography with amperometric detection [140]. In addition, a mixture of seven anions, including iodide and iodate, was successfully separated by capillary zone electrophoresis [141].

Probably the most promising method in terms of sensitivity and minimum sample pretreatment used HPLC ICP-MS [142]. Six inorganic anions including iodide and iodate were successfully determined. The absolute detection limit for the iodine species was 25 pg. The method was applied to drinking water, to soup, and to human urine. Probably the sensitivity of the method is in excess of what is required for the routine analysis of environmental samples, but is likely to have major application in metabolic studies where the number of iodine species under investigation will be extended beyond iodide and iodate.

Other methods that have been applied to the determination of iodide include isotope dilution mass spectrometry [143, 144], ICP-AES (for iodide and iodate) following solvent extraction [145], ICP-AES using a membrane gas-liquid separator [146], and by chemiluminescent methods coupled with dynamic gas extraction [147]. In addition total iodine in brines and seawater samples has been determined by atmospheric-pressure helium microwave-induced plasma atomic emission spectrometry with continuous-flow gas-phase sample introduction [148]. Colorimetric methods for determining iodine species in water in the 0.1 to 1.0 mg/L range or greater that were particularly applicable to potable water and swimming pools where iodine was used as a disinfectant have been reported [149]. XRF was used [150] to determine iodide (and chloride and bromide) in snow after the precipitation of their silver salts. The detection limit was 0.5 µg.

The chemical forms of radio-iodine in various water samples have been characterized by using either isotope exchange / solvent extraction or anion exchange column chromatography with detection by NaI (TI)-scintillation counting [151]. Most of the  $^{131}\text{I}$  in rain water collected after the Chernobyl accident existed in dissolved form,  $59 \pm 4\%$  being in the iodate form and  $35 \pm 4\%$  being in the iodide form.

#### (v) Iodide in miscellaneous matrices

Some studies involved the determination of iodine species in water and other matrices; for example Salov *et al.* [142] used HPLC ICP-MS to examine soup and human urine in addition to water, and Maros *et al.* [129] employed GC to analyse salt, cow's milk and blood serum as well as a water samples of different origins. Both studies are mentioned in the previous section. Also in this category is a report on the use of ion-chromatography to determine iodide in fruit juices and mineral waters [152].

Ion-chromatography with electrochemical detection was also used to determine iodide in dairy products and table salt [153]. On the other hand an ion-selective electrode has been employed to measure iodide in milk-based infant formulae after the precipitation of proteins [154, 155]. Iodide in cow's milk and in infant's powdered milk (and in pharmaceutical products and iodinated salts) has also been determined by a kinetic method involving the inhibitory effect of iodide on the Pd(II) catalysed reaction between ethylenediaminetetraacetic acid (EDTA) – Co(III) and the hypophosphite ion [156]. Iodide was determined in the range 2–28 ng/mL.

A kinetic method based on the catalytic effect of iodide on the chlorpromazine-bromate reaction was applied to the determination of iodide in rat thyroid and in iodinated salts [157]. The reaction was followed spectrophotometrically (525 nm) and under optimal conditions ( $10^{-4}$  M chlorpromazine hydrochloride, 1.4 M phosphoric acid,  $5 \times 10^{-4}$  M potassium bromate, 40 °) iodide was determined between 5 and 70 ng/mL by the tangent method.

Also the catalytic effect of iodide on the cerium(IV) – arsenic(III) reaction has also been successfully applied to the measurement of the conjugation of an iodine-containing hapten to ovalbumin and human serum albumin [158]. A variation on the use of the catalytic effect of iodide on the cerium(IV) – arsenic(III) reaction was proposed [159] whereby probe photometry was used as a monitoring technique for determinations by continuous addition of iodide to a reference solution. Iodide could be determined in the range 10 to 100 ng/mL with a relative error usually lower than 4 to 5% and relative standard deviations of 4.6 and 4% for 9.98 and 79.92 ng/mL respectively. The method was applied to the determination of iodide in table salt samples.

## SUMMARY

The analytical techniques that have been applied to the various iodine compounds in the environmental and biological categories, or are appropriate for their analysis, are different. They may be summarized as follows:

(i) (a) Relatively complex organic iodine compounds (iodo-amino acids) in some marine animals (e.g. sponges). Most of this work was done before 1950 and classical techniques of structure determination were used to establish the nature of the compounds. Any future work might well employ HPLC methods.

(i) (b) Complex organic compounds and hormones in animals, particularly mammals, and, for medical reasons, specifically humans, Classical natural product chemical techniques to establish structures have given way to routine determinations using radioimmunoassay techniques.

(ii) More complex organic iodine compounds in marine algae – secondary metabolites. Analysis has been directed towards establishing structures by the techniques of natural product chemistry (NMR spectroscopy, MS, etc.). If routine methods of analysis are required (and such development might depend upon some pharmacological or other use being found for the compounds) HPLC is likely to be the method of choice, possibly with ICP-MS as an element-specific detector.

(iii) Small simple organic compounds (e.g. MeI) in seawater and the atmosphere. Analysis by EC GC after use of a suitable stripping technique.

(iv) Iodate and iodide in waters, particularly seawater. Many different methods of analysis have been employed; polarographic and voltammetric methods appear favoured for the analysis of real samples, particularly for extensive surveys. Possibly HPLC ICP-MS might be the method of choice in the future but its great sensitivity might be largely redundant.

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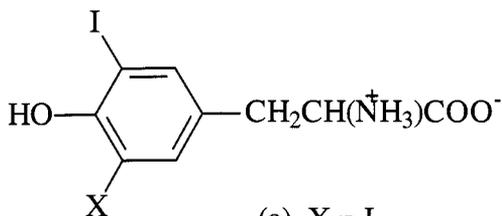
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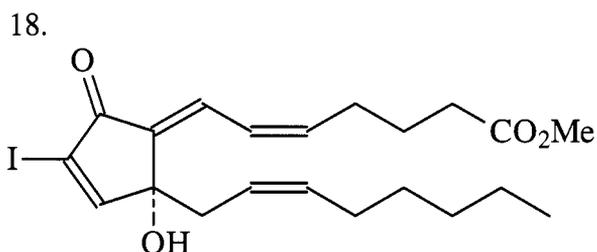
**APPENDIX: Table 1. Iodine species encountered in environmental and biological samples.**

- |     |                     |  |
|-----|---------------------|--|
| 1.  | $I^-$               | iodide                                     |
| 2.  | $IO_3^-$            | iodate                                     |
| 3.  | HI                  | hydriodic acid (hydrogen iodide)           |
| 4.  | $I_2$               | elemental iodine                           |
| 5.  | HOI                 | hypoiodous acid                            |
| 6.  | I                   | Postulated to exist in the                 |
| 7.  | IO                  | atmosphere as a result of                  |
| 8.  | $IONO_2$            | reactions of MeI with $O_3$ etc. [Ref. 53] |
| 9.  | $CH_3I$             |  |
| 10. | $CH_2I_2$           |  |
| 11. | $CH_2ClI$           |  |
| 12. | $CHBr_2I$           |  |
| 13. | $CH_3CH_2CH_2I$     |  |
| 14. | $CH_3CHICH_3$       |  |
| 15. | $CH_3CH_2CH_2CH_2I$ |  |
| 16. | $CH_3CH_2CHICH_3$   |  |
| 17. |                     |  |

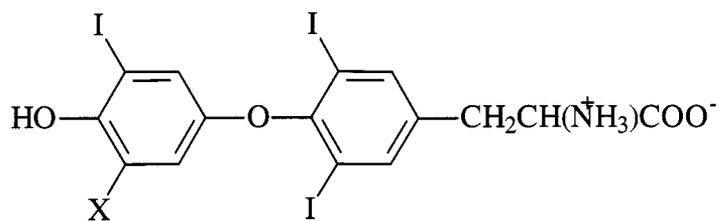


(a)  $X = I$

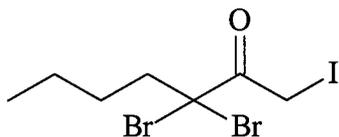
(b)  $X = H$



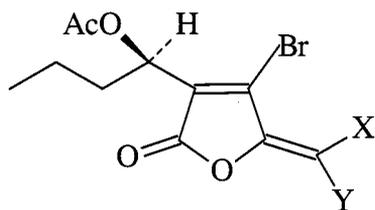
19.

(a) X = I (T<sub>4</sub>)(b) X = H (T<sub>3</sub>)

20.



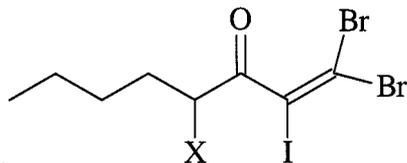
21.



(a) X = I, Y = H

(b) X = H, Y = I

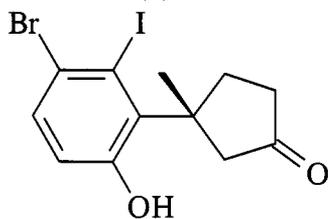
22.



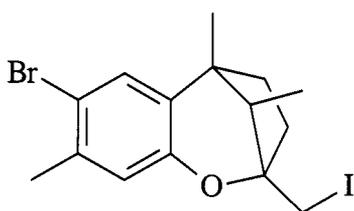
(a) X = H

(b) X = Cl

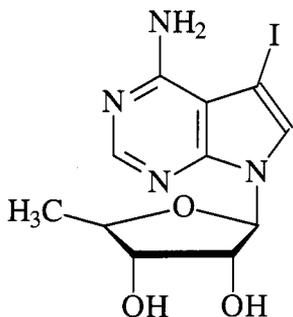
23.



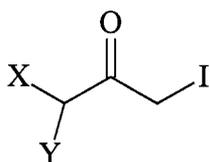
24.



25.

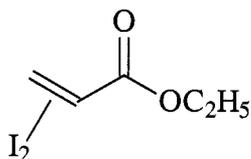
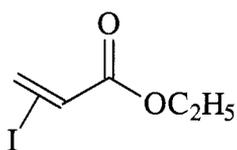
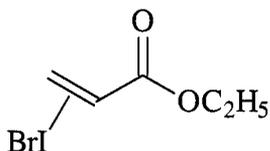


26.



(a) X = H, Y = Cl [See Ref.52]  
 (b) X = Y = Br

27.



Present in alga as acrylic acids,  
 esterified during ethanol extraction.

[See Ref. 52]