The Determination of Chlorine, Bromine and Iodine in Biological Material by Activation Analysis

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The determination of the halogens in biological samples is hampered by the fact that bromine and iodine are almost always present in trace amounts relative to chlorine. This is because of the relative abundance of these elements in the external environment. Thus in igneous rocks the Cl:Br and Cl:I ratios are about 200:1 and 1000:1 respectively, while in sea water they are 290:1 and 380 000:1 (Rankama & Sahama, 1950). While it is relatively easy to determine total halogen in any sample, the result refers almost entirely to the chlorine present, and special methods have to be employed to determine the bromine and iodine. Many halogen compounds are extremely volatile, and great care must be exercised to avoid losses in ashing biological material before determining these elements.

The method of activation analysis described in the present paper overcomes some of these difficulties. It is a fortunate coincidence that the cross-sections for thermal neutrons increase with atomic weight in the halogen series. This is clearly shown in Table 1.

This table shows that activation analysis for the halogens is capable of very great sensitivity, but it also illustrates the main difficulty of the method for these particular elements, which is the very short half-life of most of the nuclides. In particular, no attempt has been made to determine fluorine by this technique, since negligible radioactivity would be left in this element 2 min. after removal from the neutron source. The relatively long half-life of one of the 80 Br isomers gives plenty of time for chemical separation, but the chlorine and iodine must be separated with the least possible delay to

avoid losses in sensitivity. Solvent-extraction methods have been used as far as possible, since these are far quicker than distillation or precipitation techniques.

One great advantage of the activation method is that no ashing need be attempted until the activation process is complete, after which inactive carrier can be added to determine losses in the subsequent chemistry, and contamination is minimized. The method has been tested on seeds and human blood.

EXPERIMENTAL

Tomato plants were grown in sand culture and seeds were collected as described by Bowen (1959). Blood samples were collected from donors at a transfusion centre, in clean polythene beakers, from which weighed samples were transferred to polythene ampoules which could be sealed before activation. Samples of 1 ml. or more were partially dried before sealing. The standards, which were irradiated with the samples, consisted of 10 $\mu\text{g.}$ of Cl, 0.2 $\mu\text{g.}$ of Br and 1 $\mu\text{g.}$ of I as the ammonium salts in solution. These were either sealed in polythene ampoules or absorbed on dry tomato seeds of known halogen content, a micropipette being used for delivery. Samples and standards were packed in a polythene bag inside a nylon or aluminium can for irradiation in the Harwell reactor B.E.P.O. in a flux of about 10^{12} thermal neutrons/cm.²/sec. Activation times of 30 min. were used for chlorine and iodine determinations, and 4 hr. for bromine.

Chemical separation of chlorine. The activated sample (0.05 g. of seeds or 0.02 g. of blood) was transferred to a

Table 1. *Calculated induced radioactivities in halogens after exposure to a thermal neutron flux of 10^{12} neutrons/cm.²/sec.*

Nuclide produced	Half-life	Radioactivity (disintegrations/min./10 ⁻⁹ g.)	
		30 min. exposure	4 hr. exposure
²⁰ F	11 sec.	17	17
³⁸ Cl	37 min.	62	130
^{80m} Br	4.6 hr.	49	180
⁸⁰ Br	18 min.	140	2000
¹²⁸ I	25 min.	980	1800

Fig. 1. Pyrex distillation head.

50 ml. round-bottomed flask containing 10 mg. of chlorine as ammonium chloride, 1 ml. of 90% phosphoric acid and 1 ml. of 16*N*-HNO₃. A distillation head (Fig. 1) was fitted, with a polytetrafluoroethylene sleeve between the cone and socket joint, and distillation was carried out for 5 min. at 150° in a slow current of air. The distillate was collected in 10 ml. of 5% sodium hydroxide solution in a 50 ml. separating funnel.

Where iodine and bromine contributed appreciably to the activity of the distillate, they were removed as follows: two drops of 5% ammonium iodide, 5 drops of 1% sodium nitrite and 5*N*-nitric acid were added to the funnel till the solution was acid. The solution was extracted three times with 10 ml. portions of carbon tetrachloride to remove iodine, the lower layer being discarded each time. Two drops of 5% ammonium bromide were then added and 2% potassium permanganate was run in drop by drop until its pink colour just persisted. Three further extractions with 10 ml. portions of carbon tetrachloride removed bromine. The aqueous layer was then made alkaline in a centrifuge tube and 5 drops of 10% ferric nitrate were added. The ferric hydroxide was spun off and the supernatant liquid was poured into a fresh centrifuge tube and made acid with nitric acid. Silver nitrate (3 ml. of 4% solution) was used to precipitate silver chloride, which was centrifuged, washed twice with water and once with acetone, and then transferred to an aluminium counting tray and dried. Counting was carried out with an end-window Geiger counter. The weight of the precipitate was used for calculating the chemical yield, and the count relative to that of the standard gave the amount of chlorine in the sample. Self-absorption corrections were negligible. Counting was repeated at 35 min. intervals to establish the radiochemical purity of the separated chlorine. The whole chemical procedure could be carried out in about 35 min. for two samples, or 1.5 hr. for eight samples.

Chemical separation of bromine. The activated sample (0.05 g. of seeds or 0.05 g. of blood) was distilled as described for chlorine, except that the 10 mg. of chlorine carrier was replaced by 20 mg. of bromine as ammonium bromide.

The distillate was made *N* with respect to nitric acid, and 2% potassium permanganate was added drop by drop until the pink colour just persisted. This solution was extracted three times with 10 ml. portions of carbon tetrachloride and the yellow non-aqueous layers were combined in a fresh separating funnel. They were shaken with 10 ml. of 0.5% sodium hydroxide until the yellow colour disappeared, and the lower layer was discarded. The aqueous layer was transferred to a 50 ml. centrifuge tube and made alkaline with sodium hydroxide, and five drops of 10% ferric nitrate solution were added. The ferric hydroxide was centrifuged

and silver bromide precipitated in the same manner as described for the chloride. The separation time was about 1.5 hr. for eight samples.

The silver bromide was not counted until 6 hr. had elapsed after activation. In this time, the activity due to any contaminating halogens had decayed to negligible amounts and the most important activity left was that of ⁸²Br. Traces of the 36 hr. isotope ⁸²Br were also present, but it was unnecessary to correct for them. A small self-absorption correction was needed for precipitates of very different weights.

Chemical separation of iodine. The activated sample (0.5 g. of seeds or 1 g. of blood) was distilled as described above after 20 mg. of iodine had been added as ammonium iodide. If necessary a hot-air blower was used to complete the transfer of iodine to the receiving flask. It was not necessary to add a reducing agent to the sodium hydroxide solution to prevent formation of iodate.

The distillate was made *N* with respect to nitric acid, and bromide and chloride carriers were added. The iodine was twice extracted by shaking with sodium nitrite solution and carbon tetrachloride, and then re-extracted into 10 ml. of 0.5% sodium hydroxide in a fresh separating funnel. This extraction and re-extraction procedure was carried out twice for such samples as seeds with a relatively high iodine content, and three times for samples containing much chlorine. The final solution in aqueous sodium hydroxide was treated successively with two drops of 5% ammonium chloride, 1 ml. of aq. ammonia (sp.gr. 0.88) and 3 ml. of 4% silver nitrate. The precipitate was centrifuged and the supernatant liquid discarded. Washing was carried out with 2*N*-nitric acid, water (twice) and acetone, and the silver iodide was transferred to an aluminium dish and counted without delay. The whole chemical procedure could be carried out in 1 hr. for six samples.

Counting was repeated at 25 min. intervals to establish the radiochemical purity of the separated iodine. Samples were occasionally contaminated with ³⁸Cl. The self-absorption of ¹²⁸I β -particles was negligible.

Losses in the chemical steps were measured radiochemically on four replicates, and the mean chemical yields were 94% for Cl, 76% for Br and 85% for I. The greatest losses of bromine occurred in the permanganate oxidation step (Table 2). These losses are, of course, allowed for by measuring the chemical yield in each analysis.

Decontamination factors can be worked out from Table 2 for the separation of any individual halogen by any combination of chemical steps.

All analytical data reported below are the means of at least two replicates which agreed to within the estimated limits of accuracy of the technique ($\pm 5\%$).

Table 2. *Losses of halogens during separation*

The steps are described in detail in the text.

Chemical step	Loss (%)		
	Cl	Br	I
Distillation	2.4	0.5	3.3
CCl ₄ extraction from aq. HNO ₃	0.11	2.7	1.7
CCl ₄ extraction from aq. KMnO ₄	1.0	15.2	—
Precipitation of Fe(OH) ₃	1.5	0.4	3.3
Silver halide ppt. in aq. HNO ₃	1.4	1.3	5.6
Silver halide ppt. in aq. NH ₃	99.59	7.3	3.2

RESULTS

Chlorine, bromine and iodine were determined in the seeds of tomato (*Lycopersicum esculentum*) and in the nutrient solution on which the tomato plants fed. The culture solution contained ($\mu\text{g./ml.}$) 3.67 Cl, 0.0104 Br and 0.00136 I, giving a Cl:Br ratio of 354:1 and a Cl:I ratio of 2700:1. Fresh seeds (moisture content standardized at 7.32%) contained ($\mu\text{g./g.}$) 66.7 Cl, 1.28 Br and 0.376 I, so that the respective ratios were 52.1:1 and 177:1. The results for blood with Br and I are shown in Table 3. For iodine, the contents in three other patients were ($\mu\text{g./g.}$) 0.0324, 0.0426, 0.0508; mean, 0.0419.

DISCUSSION

Errors in the activation method are different in kind from those normally encountered in analytical work. The neutron flux used for activation was homogeneous to $\pm 2\%$, and statistical counting errors were kept at about this level for the initial counts. There are a few nuclear processes other than (n, γ) reactions which might produce radio-halogens, e.g. the (n, p) reactions on the inert-gas isotopes ^{38}A , ^{80}Kr and ^{128}Xe . If samples containing considerable quantities of uranium were to be analysed by this method, allowance would have to be made for the short-lived fission products, e.g. ^{82}Br , ^{83}Br , ^{132}I , ^{134}I . No difficulty should arise with ordinary biological samples.

Errors whose magnitude is hard to assess could be caused by non-equilibration of active halogen and carrier, and by adsorption of halogen on the walls of the ampoule in which activation is carried out. Thus chlorine present in biological material as chlorate or perchlorate might not distil over during the wet-ashing process. The recovery figures quoted above, though satisfactory, give no information on this point. It is, however, widely assumed that virtually all the halogen in biological material is present either as inorganic or organic halides from which hydrogen halides are readily liberated by acid hydrolysis. Miller & Dodson (1950) have shown that energetic chlorine atoms

produced by recoil after neutron capture undergo a Szilard-Chalmers reaction with solvent molecules or with the container walls. Activated halogens are also strongly adsorbed by polythene and polystyrene, but can easily be washed off Pyrex glassware. Every effort was made in the present work to wash all traces of the sample and standards into the distillation flask after activation.

The formation of interhalogen compounds is not generally a serious difficulty in analytical work. Sometimes, when the determination of iodine and chlorine was being attempted in the same sample, all the iodine distilled over as iodine trichloride rather than as free iodine. This could be largely prevented by using more dilute nitric acid in the ashing mixture or by adding hypophosphite during the distillation.

Neutron-activation analysis has been applied to the halogens in a number of cases (e.g. Brues & Robertson, 1950; Atchison & Beamer, 1956; Cosgrove, Bastian & Morrison, 1958; for bibliography see Gibbons, Loveridge & Millett, 1957). It appears to be quite as sensitive and accurate as conventional methods for chlorine (cf. Johnson, Huston & Ozanne, 1958) and iodine (cf. Johnson & Butler, 1957; Rodgers & Poole, 1958). For bromine the technique is considerably more sensitive than any yet reported in the literature (cf. Ucko, 1936; Conway & Flood, 1936; Neufeld, 1936; Hunter, 1955).

There are few strictly comparable results for the halogen content of seeds, but the values found in the present work do not appear to be unreasonable. Johnson, Stout, Broyer & Carlton (1957) found 0.03 $\mu\text{g. atom}$ of chlorine per tomato seed, corresponding to approximately 350 $\mu\text{g.}$ of Cl/g. (dry wt.). The content of leaves is very much higher. Beeson (1941) has summarized published determinations of bromine in vegetables, which range from 1 to 14 $\mu\text{g./g.}$, and the Chilean Iodine Educational Bureau (1952) list a large number of figures for iodine in tomatoes which range from 0.1 to 1.6 $\mu\text{g./g.}$ (dry wt.). Hence only our chlorine figures fall outside the rather wide range of published results. This is not surprising as Johnson *et al.* (1957) analysed commercial seeds, while ours were specially grown on solutions containing very little chlorine.

It is therefore particularly interesting that the Cl/Br and Cl/I ratios should be so widely different in the seeds and in the nutrient solution which fed them. This phenomenon has long been known for marine algae, but has not previously been demonstrated for higher plants. The sand supporting the roots of the tomato plants was extensively purified with nitric acid and water washed before the experiment and is unlikely to have retained appreciable halide after this treatment. The plants were

Table 3. Chlorine and bromine contents of blood of seven patients

Patient	Cl ($\mu\text{g./g.}$)	Br ($\mu\text{g./g.}$)	Cl/Br ratio
A	3050	3.38	902
B	2890	1.85	1560
C	2800	1.66	1690
D	2720	1.56	1740
E	2590	3.95	657
F	2370	3.22	736
G	2700	3.65	740
Mean	2730	2.75	993

grown in a closed greenhouse without filtered air, in a locality 100 miles from the ocean. It is difficult to conceive of any method by which extraneous bromide and iodide could enter these seeds other than by the normal process of translocation. The result suggests that bromine and iodine may be essential elements for the tomato plant, as has recently been demonstrated for chlorine (Broyer, Carlton, Johnson & Stout, 1954).

Recent work by Lehr, Wybenga & Rosanow (1958) indicates still more clearly that iodine is probably essential, but evidence for the essentiality of bromine is still very tenuous (Newton & Toth, 1952; Ulrich & Ohki, 1956; Ozanne, Woolley & Broyer, 1957).

The analytical figures for the halogens in blood give confidence in the reliability of the method, since they, too, agree with the rather wide range of published results. Thus Spector (1956) quotes 2500–3100 $\mu\text{g.}$ of chlorine/g. and 0.03–0.13 $\mu\text{g.}$ of iodine/g. as the range for normal human blood: these figures are based on a critical selection of a large number of published determinations. Most workers agree that the normal content of bromine in blood lies between 1 and 10 $\mu\text{g./g.}$, with occasional high values for patients who have received bromides in medicine (Ucko, 1936; Conway & Flood, 1936). Our figures are somewhat lower than most others (e.g. Hunter, 1955), but agree with the determinations of Ucko (1936).

SUMMARY

1. Methods are described for the determination of chlorine, bromine and iodine in biological material by neutron-activation analysis. The ultimate limits of detection of the method are approximately 10^{-8} g. for chlorine, 10^{-9} g. for bromine and 10^{-10} g. for iodine.

2. The three halogens have been determined in seeds of the tomato (*Lycopersicum esculentum*) and in the nutrient solution on which the plant subsisted. Both bromine and iodine are concentrated relative to chlorine in the seeds, suggesting a physiological need for these elements.

3. The mean values for the three halogens in whole human blood were found to be 2730 $\mu\text{g./g.}$ (Cl; 7 patients), 2.75 $\mu\text{g./g.}$ (Br; 7 patients) and 0.0419 $\mu\text{g./g.}$ (I; 3 patients).

4. The method is particularly recommended for the determination of bromine, since it is more sensitive and less subject to interference by other halogens than are other current methods.

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