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A rapid spectrophotometric method for the determination of bromine in seawater and in the ash of marine algae

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KURZFASSUNG: **Eine spektrophotometrische Schnellmethode zur Bestimmung von Brom in Meerwasser und in der Asche mariner Algen.** Zur quantitativen Bestimmung von Brom in organischen Substanzen bei Anwesenheit anderer Halogene wird eine einfache spektrophotometrische Methode beschrieben. Bei 400 nm ist die Absorption in Chloroform der Bromkonzentration im Bereich zwischen 0 und 0,6 g/l proportional. Als Beispiele werden Bestimmungen des Br-Gehalts in Seewasser und in der Asche verschiedener mariner Rhodophyceen mitgeteilt.

INTRODUCTION

Since the discovery of bromine in seawater (BALARD 1826), a number of techniques for the quantitative estimation of this element in organic matter have been developed. These methods involve the liberation of bromine from its bromides with a simultaneous colour change in some suitable compound added to the solution, e.g. fuchsin (INDOVINA 1935), strychnine and persulphate (BINKLEY 1948), fluorescein (BERKOVICH & LUZINA 1950) and rosaniline (HUNTER & GOLDSPINK 1954). Other methods require the removal of other halogens prior to the liberation of bromine and its subsequent titration with sodium thiosulphate (PINCUSSEN & ROMAN 1935, SHIRAHAMA et al. 1944).

SEGI & TAKATORI (1952) described a method for the colourimetric determination of bromine in marine algae, where the solutions obtained after ashing, are acidified with dilute sulphuric acid and a small volume of chlorine water, causing the liberation of molecular iodine. Subsequently a larger amount of chlorine water is added and colourless iodic acid is formed, the solution again becoming colourless as a result. Further addition of chlorine water liberates bromine which is shifted into chloroform and determined using a Dubosq colourimeter.

As the absorbance spectra in chloroform of bromine and iodine do not overlap significantly, liberated bromine can be readily determined using a spectrophotometer without the serial additions of chlorine water. A rapid, accurate modification of the method of SEGI & TAKATORI (1952) for the determination of bromine in the presence of other halogens is described.

MATERIAL AND METHODS

Preparation of standard solutions: Solutions of known bromine concentrations were prepared using analytical reagent grade KBr. Reaction mixtures consisted of 20.0 ml KBr solution, 5.0 ml H_2SO_4 (16 N), 5.0 ml chloroform and 1.5 ml chloramine T (15 g/l). This mixture was shaken vigourously and the chloroform phase, upon separation, was used for spectrophotometric analysis.

Spectroscopy: All spectroscopy was carried out with a Beckman SP 800 spectrophotometer using 1 cm silica glass cuvettes. Chloroform was used to zero the instrument. The Br-containing chloroform solution was then inserted and an absorbance spectrum run from 700—350 nm. Absorbances were read at 400 and 650 nm. Absorbance at 650 nm was used to allow for slight differences in the turbidity of the samples and was subtracted from the absorbance at 400 nm. If absorbance at 400 nm exceeded 1.5, the original KBr solution was diluted correspondingly.

Preparation of seawater and algal ash samples: Seawater samples were collected in small, tightly-sealing McCARTNEY flasks and centrifuged in these flasks at 3000 rpm for 45 minutes to remove suspended material. Subsequent procedure as for the KBr solutions. Algal material was freshly collected, cleaned of sand and epiphytes, briefly rinsed twice in distilled water and oven-dried at 65° C for 24 hours. A dried algal powder was produced by grinding in a Casella Grainmill (0.12" mesh size sieve) and the powder dried for a further 4 hours at the same temperature. Small amounts (generally 1—3 g) of the powder were weighed into silica dishes and 10.0 ml of 10 % Na_2CO_3 added to prevent volatilization of bromine. Ashing took place in a Gallenkamp muffle furnace at 400—440° C for 8 hours. After cooling, small quantities of distilled water were added and the dishes warmed over a water bath. The contents were then filtered using Whatman No. 541 paper and washed repeatedly with cold distilled water and finally made up to volume (generally 100 ml). These solutions were then treated like the KBr solutions.

RESULTS

Calibration curve

The relationship between the concentration of the standard KBr solutions, the calculated Br concentration and the measured absorbance is given in Table 1. Plotting of these values showed that the relationship between Br concentration and absorbance is not strictly linear, deviating slightly at the higher Br concentrations.

A new Br calibration curve was prepared each time new stock solutions of KBr or the other reagents were prepared as some slight variation (less than 1.5 %) was found in the absorbance with some batches of new stock solutions.

Care must be taken that the absorbance spectra are completed within 15 minutes of adding the chloramine T as the excess Cl_2 will in chloroform, combine with Br_2 to form the yellow Cl-Br, with the resultant change in absorbance.

Table 1

Relationship between bromine concentration and absorbance in chloroform

Concentration of KBr solution (%)	Bromine (g/l)	Abs.$_{400\ nm}$–Abs.$_{650\ nm}$
0.0750	0.5036	1.490
0.0625	0.4197	1.310
0.0500	0.3357	1.135
0.0375	0.2518	0.910
0.0250	0.1679	0.690
0.0125	0.0839	0.339
0.00	0.000	0.00

Presence of iodine

In chloroform, iodine forms a purple solution whose absorbance spectrum shows a single maximum at 480—505 nm. At 400 nm however, there is no absorbance due to iodine (see difference spectrum in Fig. 1). Bromine forms a brown solution in chloroform with a fairly broad maximum at 410 nm. At 410 nm, iodine still shows a little absorbance but as bromine absorbance is still near its maximum at 400 nm, this wavelength is used in bromine determinations as it excludes any absorbance due to iodine.

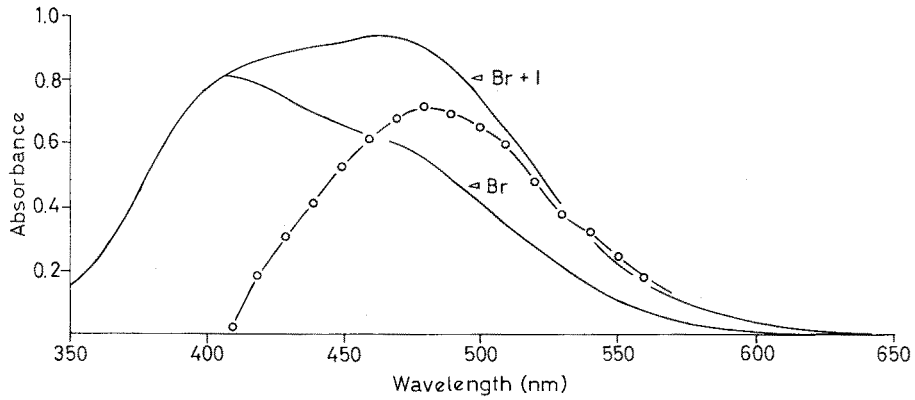

Fig. 1: Absorbance spectra of bromine in chloroform with and without iodine; o = difference spectrum of the two solutions (absorbance scale expanded ×2), showing the typical iodine maximum at 480 nm

A number of calibration solutions, containing both bromine and iodine, were prepared with concentrations ranging from 0.0—0.335 g/l and 0.0—0.382 g/l respectively. Where iodine was present, the absorbance maximum of bromine was broadened but the absorbance at 400 nm remained proportional to the bromine concentration as previously found with the KBr solutions. Thus although some iodine may be simultaneously liberated with the bromine, it does not interfere with the absorbance of the latter while measured at 400 nm.

Volatilization of bromine during ashing

Recovery of bromine after ashing known amounts of bromine (as KBr or α-bromnaphthalin) was complete, showing that the addition of 10.0 ml of 10 % Na_2CO_3 is sufficient to prevent any volatilization of bromine at the ashing temperature. Generally, 10.0 ml of Na_2CO_3 solution was added for every 3 g of algal powder to be ashed.

Bromine content of seawater and marine Rhodophyta

Knowing the volume and density of seawater used, it is a simple calculation to express bromine content in ppm (parts per million by weight). Values obtained at a number of stations are given in Table 2.

Table 2

Bromine concentrations at a number of stations in the Cape Province, South Africa

Locality	Situation	Date	ppm Br	No. of replicates
Riet River	open water	10. 12. 1970	66.5	2
	littoral rockpool	10. 12. 1970	66.1	2
	littoral rockpool	12. 3. 1970	69.6	2
Kowie River	open estuary	15. 4. 1970	67.9	2
Kleinemond	closed estuary	15. 4. 1970	45.7	2
River	above weir	15. 4. 1970	10.7	2

Similarly by using known weights of the dried algal powder for ashing and knowing the volume of the final sample solution, the percentage of bromine in the dry weight of the alga is calculated. A number of marine Rhodophyta were tested by this method and the results are given in Table 3.

Table 3

% Br/dry weight of several marine Rhodophyta from Southern Africa (collected December/January 1970/71)

Species	Locality	% Br/dry weight	No. of replicates
Calliblepharis fimbriata (C. Ag.) Kuetzing	Riet River	0.164	1
Chondrococcus hornemanni (Mert.) Schmitz	Riet River	1.45	1
Gelidium pristoides (Turn.) Kuetzing	Riet River	0.084–0.105	3
Gigartina insignis (Endl. & Dies.) Schmitz	Riet River	0.082	1
Gracilaria beckeri (J. Ag.) Papenfuss	Riet River	0.095	1
Kuetzingia natalensis J. Ag.	Inhaca Island	1.00–1.23	3
Porphyra capensis Kuetzing	Riet River	0.039	1
Pterosiphonia cloiophylla (C. Ag.) Falkenberg	Riet River	5.81–5.86	4
Vidalia fimbriata (R. Br.) J. Ag.	Inhaca Island	2.22–2.57	2

DISCUSSION

The method described is a convenient and accurate modification of that of SEGI & TAKATORI (1952) and as only the wavelength of maximum bromine absorbance is used, it increases the specificity of the determination. Other halogens do not interfere in the bromine determinations, and consistent results can be obtained for bromine over a large concentration range.

Bromine is represented in seawater in concentrations of approximately 66 ppm (HASLAM & GIBSON 1950), but has been found in much higher concentrations in the thalli of some red algae (VINOGRADOV 1953). Variation in the bromine content of seawater reported here (Table 2) is probably due to dilution and evaporation especially in the small, littoral rockpools. The closed Kleinemond Estuary is substantially diluted with freshwater (10.7 ppm Br) and the high bromine content of the Kleinemond River itself may be due to some residual methyl bromide, used as a nematocide in the pineapple plantations along its banks.

Within the Rhodophyta, the Rhodomelaceae appear to be particularly rich in bromine (AUGIER 1953), and up to 6 % Br/dry weight has been reported in *Odonthalia corymbifera* (TOKIDA 1954). In the present study, only *Chondrococcus, Kuetzingia, Pterosiphonia* and *Vidalia* contain over 1 % Br/dry weight, and of these genera only *Chondrococcus* is not a member of the Rhodomelaceae. Although present in considerable amounts, bromine does not seem to play an essential part in the metabolism of the members of the Rhodomelaceae so far studied (FRIES 1966).

SUMMARY

1. A simple spectrophotometric method is described for the determination of bromine in organic material in the presence of other halogens.
2. The absorbance at 400 nm in chloroform is proportional to the bromine concentration between 0 and 0.6 g/l, although standards should be run with each set of determinations.
3. The results of a number of bromine determinations on seawater and the ash of several marine Rhodophyta are presented and discussed.

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