Direct Titrimetric Determination of Fluoride in Natural Waters

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The fluorine content of drinking waters has become a matter of great interest to those concerned with the control and diminution of dental caries. In the U.S.A. many are of the opinion that the epidemiological studies of Dean (1938) and his co-workers (Dean, Arnold & Elvove, 1942) provide convincing evidence for the belief that, within certain limits, there is an inverse relationship between the fluorine content of drinking water and the incidence of dental caries in children. Mackenzie (1952) has given a good general account, including pertinent references, of the evidence put forward in support of this thesis. The result of such convictions has been the addition of sodium fluoride to the water supplies of a number of American communities. The relevant authorities of this country have this matter under consideration. It is essential in all these investigations to have reliable estimations of the fluorine content of the water, whether the fluorine is naturally present or artificially added.

The existing methods for the determination of small amounts of fluoride fall into two classes: (1) colorimetric methods, which use the bleaching action of the fluoride ion on a zirconium alizarin lake and require a set of fluoride standards for comparison, (2) titrimetric methods in which the fluoride is directly titrated with thorium nitrate in the presence of alizarin or a solochrome dye as indicator. The theoretical bases for these methods are discussed by Talvitie (1943); both types are sensitive to many substances found in natural waters.

The method of estimation commonly used is that of Lamar (1945), but it has the disadvantage that the alkalinity of the water needs first to be determined, and that sulphate, phosphate, etc., interfere with the colorimetry. The method applies to waters containing less than 500 parts per million (p.p.m.) SO_4^{2-} and 1000 p.p.m. Cl^- , and the suggestion is made that the errors introduced by these two ions cancel one another out.

Until recently we used a colorimetric method. Our technique resembled that of Elvove (1933), in that the bleaching was carried out in the presence of 0.4N-hydrochloric acid, which has considerable advantages: we have found that at this high acidity aluminium does not cause interference. Elvove

advised a complete analysis of each water so that the fluoride standards required for comparison could be made 'similar in composition to the unknown'. His reason for this was the interference caused by PO_4^{3-} , SO_4^{2-} and other anions. We overcame this difficulty by the addition of barium chloride. This removed most of the interfering ions and did not itself interfere with the subsequent bleaching action of fluoride on the lake. Fluoride was not precipitated or adsorbed on to the precipitate under these conditions.

Although we have had considerable success with this type of method it has the following disadvantages: it is tedious and time-consuming because of the number of standards required, it is susceptible to temperature and it takes some 6-18 hr. for the bleaching action to be complete. Moreover, the estimation may have to be repeated if the amount of fluoride present falls outside the expected range.

It is not proposed here to give a full critical consideration of the numerous titrimetric methods used by others in the past, but to describe our present method for the direct determination in natural waters. We have had long experience in the methods of estimating fluoride both in natural waters and in the distillates from natural waters, rocks, bones and teeth, and have gradually modified and simplified our technique.

METHOD

The procedure is based on the direct titration of fluoride in aqueous solution with thorium nitrate using sodium alizarinsulphonate as indicator (Talvitie, 1943; Stevens, 1948). The titration is carried out at pH 3.3 in the presence of acetic acid (Matuszak & Brown, 1945). The possibility of interference by SO_4^{2-} , PO_4^{3-} , CO_3^{2-} and HCO_3^- is eliminated by prior treatment of the water with BaCl_2 . The use of an Amberlite or Zeolite column to remove cations (Bond, Murray & Stevens, 1952) has been found unnecessary.

Reagents

(1) Thorium nitrate solution, 0.0004M, freshly made by dilution of the stock standard solution (5.52 g. $\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}/\text{l.}$); (2) NaF standard solution, 1 ml. $\equiv 5 \mu\text{g. F}$, freshly made by dilution of the stock standard NaF (2.21 g. NaF/l. 1 ml. $\equiv 1 \text{ mg. F}$); (3) approx. 0.4N-acetic acid, freshly made by dilution of 23 ml. of the glacial acid to 1 l.; (4) approx. 0.05N-NaOH; (5) approx. 0.05N-HCl; (6)

approx. 0.1 N- $\text{Na}_2\text{S}_2\text{O}_3$; (7) 0.03% (w/v) sodium alizarin sulphate solution in water, filtered through no. 30 Whatman paper; (8) 5% (w/v) BaCl_2 .

Procedure

BaCl_2 solution (10 ml.) is pipetted into 100 ml. of the water sample in a 150 ml. conical flask which is covered with a condensing bulb. This solution is just brought to the boil, cooled immediately, and then filtered.

Up to 20 ml. of the filtrate are measured accurately into a 50 ml. Nessler tube provided with a glass stirrer and 1 ml. alizarin solution is added.

The pH is adjusted with 0.05 N-HCl (and then with 0.05 N-NaOH if necessary) added dropwise so that the colour of the indicator is finally a slightly orange shade of yellow. This orange colour is discharged with three or four drops of 0.4 N-acetic acid. The colour of the solution should then be lemon-yellow. A further 1 ml. of the acetic acid is added.

To remove free chlorine one drop of 0.1 N- $\text{Na}_2\text{S}_2\text{O}_3$ is added. The mixture is allowed to stand for a few minutes and then the volume is made up to nearly 50 ml. with distilled water.

The solution is titrated with thorium nitrate solution added dropwise and with vigorous stirring from a 1 ml. burette, fitted with a fine nozzle end, until the colour of the solution matches that of the titration blank. Before the final reading is taken the volume is made up to 50 ml.

The titration blank is made as follows: to 47.5 ml. distilled water in a 50 ml. Nessler tube are added 1 ml. of alizarin solution, 1 ml. of 0.4 N-acetic acid, one drop $\text{Na}_2\text{S}_2\text{O}_3$ and a small, accurately measured volume, e.g. 0.3 ml., of thorium nitrate solution.

The titration is carried out using daylight reflected from a white surface, avoiding direct sunlight.

RESULTS

The results are computed from a graph constructed from values obtained by titrating a series of volumes of the standard sodium fluoride solution as shown in Table 1 (a). To each volume of sodium fluoride solution were added 1 ml. alizarin solution, 1 ml. 0.4 N-acetic acid and distilled water to 50 ml. When μg . fluorine are plotted against the volume of thorium nitrate used, a straight line is obtained between the convenient values 5 μg . fluorine and 25 μg . fluorine (Fig. 1). Below about 3 μg . fluorine the straight line does not continue through the origin.

Where the fluorine content of a water is very low it is advisable to add a suitable volume of the dilute standard sodium fluoride solution before titration. The results of water analyses are customarily expressed as p.p.m. which are equivalent to μg ./ml.

The limit of accuracy of the method is equivalent to ± 0.02 ml. thorium nitrate solution, that is ± 0.8 μg . fluorine in any particular titration. Where 20 ml. of water filtrate are being titrated, this amounts to a possible error of ± 0.04 p.p.m.

The sensitivity of the titration is not affected by temperature over the range of 5–40°. It is likely that the unaccountable variations in sensitivity encountered by Stevens (1948) are due to differences in the alizarin lake, the sensitivity of which is markedly affected by the paper used for its filtration. Whatman paper no. 30 has been found suitable.

Table 1 (i) gives the results of estimations carried out to show that the addition of barium chloride does not remove fluoride, and that it does not interfere with the titration (cf. Table 1 (b)). There is slight interference when the solution titrated contains the maximum concentration of barium chloride encountered, together with more than 15 μg . fluorine (see Table 1 (c)).

Fig. 1. Relation between volume of 0.0004 M-thorium nitrate and amount of fluorine titrated.

Calcium and magnesium in the concentrations expected in a very hard water do not interfere with the titration at the controlled pH. This is shown in Table 1 (f), (g) and (i).

The maximum permissible concentration of chloride has been given a different value for every modification of the original thorium titration method. Under the conditions of this particular method, in the presence of barium chloride, fluoride in a water containing 5500 p.p.m. chloride as sodium chloride may be estimated satisfactorily, i.e. the titration may be carried out in the presence of 2000 p.p.m. chloride as sodium chloride (cf. Table 1 (e)).

In the presence of aluminium, which is detected by failure to obtain the pure yellow colour of the indicator on addition of 0.05 N-hydrochloric acid to the filtrate, the method cannot be carried out. Aluminium also forms a lake with alizarin at the pH of the titration (see Table 1 (h)).

A series of determinations was carried out, using the tap water supplied to this laboratory, in order to demonstrate that, under the conditions

Table 1. *The titration of fluoride in the presence of barium, calcium, magnesium and aluminium ions*

Composition of solution titrated (before adjustment of pH)	Comments	F added ($\mu\text{g.}$)	Titre minus titration blank: thorium nitrate (ml.)	F found* ($\mu\text{g.}$)
(a) NaF in distilled water	—	5 10 20 25	0.12 0.26 0.54 0.68	— — — —
(b) NaF in distilled water + 2 ml. 5% (w/v) BaCl_2	—	5 10 20 25	0.11 0.26 0.54 0.67	5.0 10.0 20.0 24.6
(c) NaF in distilled water + 4 ml. 5% BaCl_2	This corresponds to the maximum concentration of BaCl_2 encountered in this method	5 10 20 25	0.11 0.26 0.52 0.64	5.0 10.0 19.4 23.5
(d) NaF in distilled water + 10 mg. NaCl	10 mg. NaCl is equivalent to 2000 p.p.m. Cl^-	5 10 20 25	0.12 0.25 0.55 0.73	5.0 9.9 20.2 26.0
(e) NaF in distilled water + 10 mg. NaCl + 2 ml. 5% BaCl_2	—	5 10 20 25	0.12 0.25 0.54 0.68	5.0 9.9 20.0 25.0
(f) NaF in distilled water + 2 ml. 5% BaCl_2 + 10 mg. CaCl_2	Ca^{2+} concentration is equivalent to twice that encountered in 20 ml. filtrate of a 'hard' water contain- ing 24 degrees of hardness, i.e. equivalent to 24 mg. CaCO_3 /100 ml.	5 10 20 25	0.11 0.25 0.54 0.67	5.0 9.9 20.0 24.6
(g) NaF in distilled water + 2 ml. 5% BaCl_2 + 10 mg. CaCl_2 + 1 drop 0.1 N- $\text{Na}_2\text{S}_2\text{O}_8$	—	5 10 20	0.12 0.25 0.53	5.0 10.0 19.6
(h) NaF in distilled water + 2 ml. 5% BaCl_2 + 0.0025 mg. AlCl_3	0.0025 mg. AlCl_3 is equivalent to 0.1 p.p.m. Al	5 10 20	End point could not be obtained	— — —
(i) A solution (100 ml.) containing 48 mg. $\text{Ca}(\text{HCO}_3)_2$ (dissolved with the aid of CO_2), 1.5 mg. MgSO_4 and varying amounts of NaF, given in the next column (p.p.m.), in distilled water was treated with 10 ml. 5% BaCl_2 . The filtrate (40 ml.) + one drop 0.1 N- $\text{Na}_2\text{S}_2\text{O}_8$ + distilled water was titrated. The original mixture without fluoride corresponds with a 'hard' water having 30 degrees of hardness, i.e. resembles Luton water		5 10 20	0.10 0.24 0.50	5.0† 10.0† 20.2†

* Values in Exps. (b)–(i) are read from Fig. 1.

† F found (p.p.m.) calculated to the concentration in the original 'hard water'.

Table 2. *Titration of the fluoride added to tap water as sodium fluoride*

Water sample	Volume titrated of filtrate from BaCl_2 separation (ml.)	F added to filtrate ($\mu\text{g.}$)	Titre minus titration blank: thorium nitrate (ml.)	Total F found in solution titrated (from Fig. 1) ($\mu\text{g.}$)	F found in tap water (*p.p.m. or $\mu\text{g./ml.}$)
Tap water	20	—	0.10	4.4	0.25
	20	5	0.26	10.0	0.30
	20	10	0.40	15.0	0.30
Tap water	40	—	0.29	11.2	0.30
	40	5	0.32	12.0	0.20
	40	10	0.52	19.2	0.25
Tap water and 1.0 $\mu\text{g./ml.}$ F added as NaF	5	—	0.13	5.6	0.20
	10	—	0.27	10.6	0.20
	20	—	0.65	24.0	0.30
Tap water and 2.0 $\mu\text{g./ml.}$ F added as NaF	5	—	0.27	10.5	0.30
	10	—	0.60	22.0	0.40

* P.p.m. fluorine in water sample = $\frac{(\text{graph reading} - \mu\text{g. F added})}{\text{vol. of } \text{BaCl}_2 \text{ filtrate titrated}} \times \frac{110}{100}$.

mentioned above, the titre of thorium nitrate varies in accordance with the amount of fluoride present.

After much experience with the method described we have encountered only one natural water in which the estimation could not be satisfactorily carried out. This sample contained a relatively high concentration of aluminium, sufficient to give a visible precipitate of aluminium hydroxide on the addition of alkali. This sample was, however, satisfactorily analysed and showed good recovery of added fluoride by our modification of the colorimetric method of Elvove (1933).

Out of 295 samples of domestic water in Great Britain only seven were found to contain more than 1 p.p.m. of fluorine.

SUMMARY

1. A method for the estimation of fluoride in natural waters by titration with thorium nitrate at pH 3.3, using sodium alizarinsulphonate as indicator, is described.

2. Interfering anions are first removed by addition of barium chloride and filtration.

3. Calcium and magnesium in concentrations found in hard waters do not interfere.

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3:5:3'-Triiodothyronine

1. ISOLATION FROM THYROID GLAND AND SYNTHESIS

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In the course of an investigation on thyroid metabolism with the aid of radioactive iodine (Gross, Leblond, Franklin & Quastel, 1950; Gross & Leblond, 1951*a, b*), an unknown radioactive compound (unknown 1) was detected in extracts of thyroid gland, plasma, tissues and faeces of animals which had been injected with radioactive iodide or with thyroxine labelled with radioactive iodine. This compound was later found in human plasma after the administration to patients of therapeutic doses of radioactive iodide (Gross & Pitt-Rivers, 1951). Evidence will now be presented identifying unknown 1 as 3:5:3'-triiodothyronine, and its isolation from thyroid gland and synthesis will be described.

Unknown 1 was originally detected by autoradiographs of two-dimensional chromatograms of butanol extracts of tissues without previous hydro-

lysis; the distribution of the radioactive spots was the same as that shown in Fig. 1*a*; this demonstrates the presence of unknown 1 in butanol extracts of a tryptic hydrolysate of radioactive rat thyroid; the existence of this compound in thyroid tissue itself suggested that it might be possible to isolate it from the thyroid gland. Accordingly, large amounts (5 kg. batches) of ox thyroid gland were digested with trypsin and the acidified hydrolysate was extracted with chloroform repeatedly, to remove fat. Butanol extracts were then made and their contents analysed by paper chromatography using butanol-acetic acid and butanol-dioxan-ammonia as the solvents and developing the spots with diazotized sulphanilic acid (Gross & Leblond, 1951*b*). In the first crude preparations, minute amounts of unknown 1 were detectable, and a considerable degree of purification was achieved by elution from a large number of papers; this laborious method was, however, unsatisfactory, in that it still yielded relatively impure fractions. It was nevertheless possible to demonstrate that the substance was

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