

## Determination of Fluorine in Fossil Bone with an Ion-Selective Electrode

By

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### Introduction

The fluorine content of fossil bone was first examined over a century ago by MIDDLETON (1844), who recognized that buried skeletal remains accumulated this element with the passing of time. As early as 1897, VAN BEMMELEN made a fluorine test on a *Stegodon* fossil from the layer that yielded Pithecanthropus Skull I to check the alleged age of the skull. There have since been occasional requirements for the fluorine assay of specimens of anthropological interest (*e.g.* OAKLEY, 1951; BERGMAN & KARSTEN, 1952; TANABE, 1966; SUZUKI, 1975; OAKLEY *et al.*, 1977; MATSU'URA, 1981).

Fluorine in fossil material has been determined by thorium nitrate titration (SHIMODA & KITAMURA, 1978), visual-colorimetric procedure (TANABE, 1962) or spectrophotometric procedure (GLOVER & PHILLIPS, 1965; YASUDA, 1968), each following the isolation of fluoride from other substances by steam distillation. Now the development of the fluoride ion-sensitive membrane electrode (FRANT & ROSS, 1966) makes it possible to measure fluoride activity directly and selectively in aqueous solutions. The fluoride electrode method eliminates the need of separation of fluoride from matrices. It, however, requires a rigorous control of ionic strength, pH, temperature and metal fluoride complex content of sample solutions. An early example of success to solve the problems involved is the use of a Total Ionic Strength Adjustment Buffer (TISAB) in determination of fluoride in water (FRANT & ROSS, 1968).

Because of its high accuracy and convenience, analyses with the specific ion-sensitive electrode would supersede alternative available methods. This paper will describe a rapid and simple technique for the determination of fluorine in fossil bone using a fluoride electrode, together with the results of an aluminum interference study on TISAB systems.

### Experimental

#### Material

The material used consisted of ten fossil bones having geologic ages of Holocene to Cretaceous. The description of the specimens is included in Table 1.

### Apparatus

The TOA Model F-125 fluoride electrode with an adaptor to the US standard jack was used in conjunction with an Corning Model M-130 digital pH/mV meter. The external reference electrode employed was of a saturated KCl calomel type, Corning Model C002.

### Reagents

All chemicals were of guaranteed reagent grade or super special grade, and glass-distilled water was used throughout.

#### *Buffer Solutions*

BUFFER A. Sodium acetate trihydrate, 36.29 g, was dissolved in 500 ml 0.2 M NaOH and diluted to 1 liter with water after standing at room temperature for 1 hr.

BUFFER B. Sodium citrate dihydrate, 98.03 g, in approximately 500 ml water was diluted to 1 liter.

BUFFER C. To 500 ml of 1 M HCl was added 161.5 g of tris (hydroxymethyl) aminomethane (TRIS) and 153.4 g of sodium tartrate dihydrate. The solution was warmed to room temperature before final dilution to 1 liter with water. This buffer is based on TISAB IV developed by Orion Research (see Orion Inc., 1977).

#### *Standard Fluoride Solutions*

Fifteen mg of hydroxyapatite was dissolved in 5 ml of 1 M HCl, then 5 ml of an appropriate standard NaF solution (prepared by dilution from TOA or DKK Fluoride Standard containing 1 mg F per ml, *i.e.*, 1000 ppm F) was added followed by 30 ml of one of the buffer solutions. The quantities may be scaled up or down as appropriate. The hydroxyapatite used has contained negligible amount of fluoride.

Stock solutions of the pure NaF standards and the buffers were stored in polyethylene containers. Standards with a buffer background were prepared each time a calibration curve was constructed.

### Procedure

Ten to 20 mg of finely ground bone sample was weighed accurately and dissolved in 5 ml 1 M HCl. After 5 ml of water was added, the total ionic strength and pH of the bone solution were fixed by addition of 30 ml of the buffer solution A, B or C. The two electrodes were placed in the solution which was stirred with a polytetrafluorethylene-covered magnetic bar. The millivolt reading was recorded after sufficient time to ensure attainment of electrode equilibrium; this requires 5 to 20 min, depending on the fluoride concentration and the buffer medium used. The electrode potential was measured again after the fluoride concentration of the sample solution was approximated to double by addition of a small but known volume of a NaF standard having the same additives as the sample.

These solution measurements were made in a 50 ml Pyrex glass beaker, not in a plastic beaker as had been usually done. No difference, however, in potential between a given solution in a Pyrex beaker and that in a polyethylene beaker was detected for the range of fluoride concentration involved in this experiment.

### Direct measurement

The potential ( $E$ ) of the fluoride ion-selective electrode constructed from a single-crystal section of  $\text{LaF}_3$  is given by the Nernst equation:

$$E = \text{constant} - 2.303 \frac{RT}{F} \log \gamma C_{\text{free}} \quad (\text{i})$$

where

$\gamma$  = activity coefficient

and

$C_{\text{free}}$  = free fluoride ion concentration

The value of the constant depends on the fluoride activity in the internal electrode solution and the choice of reference electrode while  $2.303RT/F$  is the Nernstian factor, viz, 59.16 mV at 25°C. The activity coefficient,  $\gamma$ , is variable and depends on the total ionic strength of the solution (MOODY & THOMAS, 1971), which can be overcome by providing a constant background ionic strength by addition of a buffer of high ionic strength.

Plots of the measured electrode potentials on the abscissa (linear axis) of semilog graph paper against the fluoride concentrations of standard solutions on the ordinate provide calibration curves for direct measurements. The fluoride in any standard solution used for calibration is supposed to be present only in the ionic (free) form, i.e.,  $C_{\text{total}} = C_{\text{free}}$ ; since the solution contains no complexing agents such as aluminum or iron, and buffered in a pH range where neither undissociated hydrofluoric acid nor  $\text{HF}_2^-$  ions should exist (see FRANT & ROSS, 1966; SRINIVASAN & RECHNITZ, 1968). The presence of polyvalent metal ions, of which  $\text{Al}^{3+}$  is of the utmost respect, frequently causes a decrease in free fluoride ions in solution through the formation of metal fluoride complexes (DUFF & STUART, 1972; DKK, 1976; Orion Inc., 1977; and others). Two of the three buffer media employed in this study respectively comprise a metal complexant, citrate or tartrate, which preferentially complexes metal cations and releases fluoride ions as sensed by the electrode.

Since electrode potentials are related to temperature (Equation i) and are also to drift slightly over an extended period, the fluoride concentrations of sample solutions should be calculated by reference to a calibration curve prepared on the same day using fluoride standards with nearly identical temperature ( $\pm 0.2^\circ\text{C}$ ) as the samples.

### Known Addition Measurement

Let  $E_1$  be the measured electrode potential of a sample and  $E_2$  the potential after addition of a fluoride standard having the same additives as the sample. The activity coefficient being practically constant in the solutions under consideration, the change in potential,  $E_2 - E_1$ , can be expressed as

$$E_2 - E_1 = \Delta E = -S \log \left( \frac{\alpha_2}{\alpha_1} \cdot \frac{V_1 C_1 + V_0 C_0}{(V_1 + V_0) C_1} \right)$$

where

$S$  = electrode slope, i.e., theoretically,  $2.303RT/F$

$\alpha_1$  = fraction of free fluoride ions before addition

$\alpha_2$  = fraction of free fluoride ions after addition

$C_1$  = total F concentration of sample

$C_0$  = total F concentration of added standard

$V_1$  = volume of sample

$V_0$  = volume of standard added to sample

When the complex formation with cations in the solutions is neglected or at least  $\alpha_1 = \alpha_2$ , the total F concentration of the unknown is obtained from the equation

$$C_1 = \frac{\rho C_0}{(1 + \rho) 10^{-\Delta E/S} - 1}$$

where

$$\rho = V_0/V_1$$

Where interfering cations are active in the formation of undissociated fluoride complexes, known addition result is to be larger than direct measurement.

## Results

Fig. 1 illustrates calibration curves for the electrode response to fluoride levels in buffered sample solutions. In either 0.125 M NaCl—0.05 M HOAc—0.15 M NaOAc (pH 5.1) or 0.125 M HCl—0.25 M NaCitrate (pH 5.4) medium, the fluoride electrode gives a straight line from well above 20 ppm to below 0.5 ppm having a slope of 59.8 mV and 59.7 mV respectively, which is close to the theoretical slope of 59.75 at 28°C. While the linear detection limit falls short of 0.5 ppm and there is a gradual curvature below 0.8 ppm in 0.5 M HCl—1 M TRIS—0.5 M NaTartrate medium (pH 8.3), the slope remains 59.6 mV per decade. The fluoride levels in the sample solutions measured here fell within the regions of linear electrode response.

Table 1 summarizes the analytical results of fluorine in fossilized specimens spanning the age from Early Holocene to Late Cretaceous. As Table 1 shows, the use of Buffer B or C yields a good agreement between the F values obtained by direct measurement and by known addition procedure. In using Buffer A, the direct measurement gave lower readings of fluorine than the known addition procedure for some specimens.

The electrode potentials were measured on buffered solutions containing various amounts of fluoride and of aluminum, and the meter readings of solutions with aluminum were compared with those of non-aluminum ones in order to determine the error due to the formation of aluminum fluoride complex. Data so obtained are plotted in graphical form in Figs. 2 and 3.

In NaCl—HOAc—NaOAc medium, the fraction of fluoride in the ionic form is 87% for 5-ppm F solution with 0.37 ppm Al present (*i.e.*, 2% F and ca. 0.3%  $\text{Al}_2\text{O}_3$  in sample, calculated for a 10 mg sample powder), showing a rapid decrease with increasing amount of Al (Fig. 2). Without metal complexant in the buffer employed,

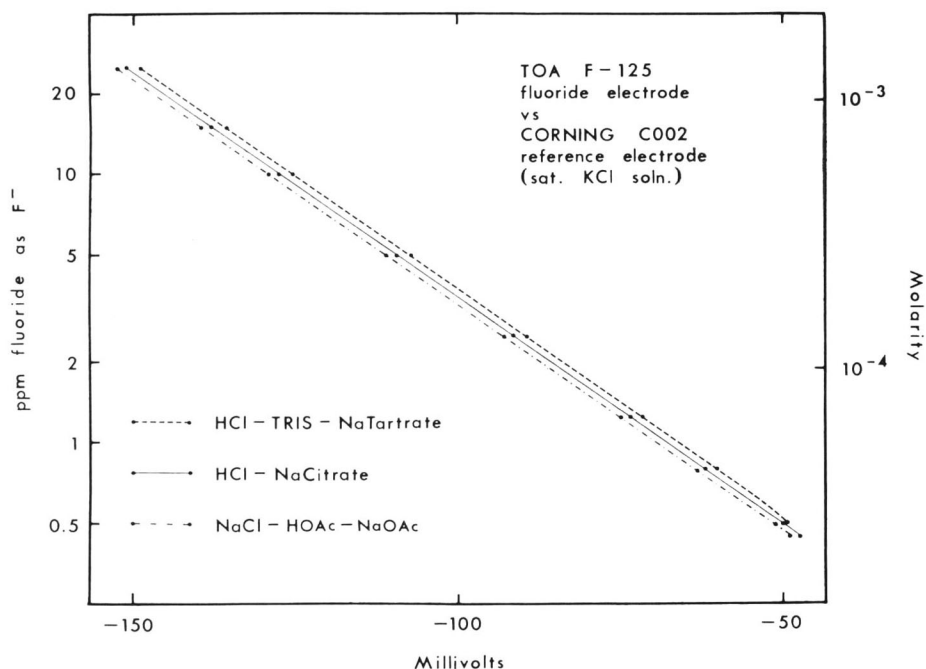


Fig. 1. Electrode response in buffered solutions of sodium fluoride at 28°C. The concentration on the ordinate represents that of fluoride in buffered systems. For description of buffer media, see note to Table I.

Table 1. Comparison of fluorine determinations in fossil bones obtained by direct measurement and known addition procedures with use of three TISAB media.

| Specimen   |            |           | Age               | Ratio of F found, direct measurement/known addition |           |           | wt. %F in sample** |
|------------|------------|-----------|-------------------|-----------------------------------------------------|-----------|-----------|--------------------|
|            |            |           |                   | Buffer A*                                           | Buffer B* | Buffer C* |                    |
| deer       | metapodial | compacta  | Early Holocene    | 0.98                                                | 1.00      | 1.00      | 0.613              |
| deer       | metacarpus | compacta  | Late Pleistocene  | 0.98                                                | 1.01      | 0.99      | 0.628              |
| boar       | mandible   | compacta  | Late Pleistocene  | 0.96                                                | 1.00      | 1.01      | 0.742              |
| deer       | humerus    | compacta  | Late Pleistocene  | 1.00                                                | 1.01      | 0.99      | 1.30               |
| mammoth    |            | compacta  | Late Pleistocene  | 0.99                                                | 1.00      | 1.01      | 2.31               |
| elephant   |            | dentine   | M./L. Pleistocene | 1.01                                                | 1.00      | 1.02      | 1.52               |
| vertebrate | rib        | spongiosa | Early Pleistocene | 1.01                                                | 0.99      | 0.99      | 0.352***           |
| vertebrate | long bone  | spongiosa | Early Pleistocene | 0.95                                                | 0.98      | 1.01      | 1.42               |
| whale?     |            | compacta  | Pliocene          | 0.93                                                | 1.01      | 1.01      | 2.64               |
| dinosaur   |            | compacta  | Late Cetaceous    | 0.95                                                | 1.01      | 0.99      | 3.09               |

\* Final concentrations in buffered systems:

A—0.125 M NaCl, 0.05 M HOAc, 0.15 M NaOAc,

B—0.125 M HCl, 0.25 M NaCitrate,

C—0.5 M HCl, 1 M TRIS, 0.5 M NaTartrate.

\*\* Calculated from the direct measurement results with use of Buffer C.

\*\*\* Heavily contaminated with adventitious mineral matter. P<sub>2</sub>O<sub>5</sub> in sample=11.9 wt. %.

the presence of aluminum results in a significant error in direct measurement of fluoride by the electrode, and known addition procedure also fails to yield an accurate measurement since the extent of complexation is contingent on the total F concentration in the solution even if the Al concentration is held constant (Fig. 3). Under the conditions as shown in Fig. 3, a known addition result is still to be smaller than the true value of total F content.

The aluminum complex in a 5-ppm F solution containing 7.5 ppm Al or more is not entirely dissociated by 0.25 M citrate at pH 5.4 (Fig. 2). An additional study of aluminum interference for higher F concentrations indicated that the amount of recovery (percentage) of fluoride ions in this buffer medium with a given Al concentration is reduced as the F concentration of the solution increases.

In 0.5 M tartrate at pH 8.3, there is no interference for a 5-ppm F solution containing up to 62.5 ppm Al (Fig. 2), and even for a concentrated solution of 62.5 ppm F with 50 ppm Al present.

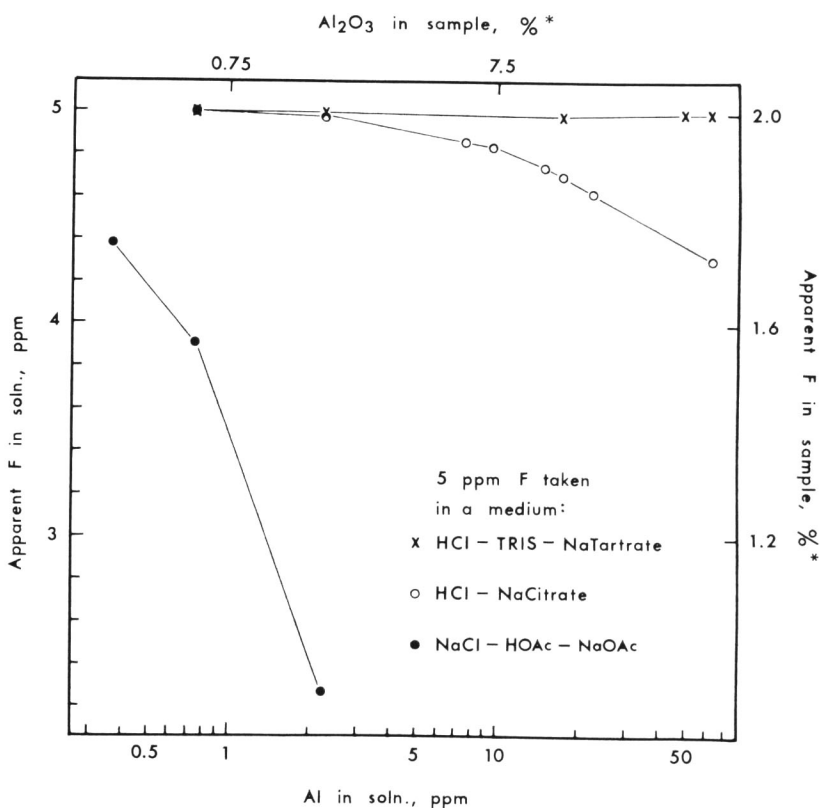


Fig. 2. Apparent fluoride content of solutions with varying amounts of aluminum present, given by direct measurement procedure. See note to Table 1 in regard to buffer media.

\* Based on sample weight of 10 mg.

### Discussion and Conclusion

Hydroxide ion is the only reported interferant to the response of the lanthanum fluoride electrode to fluoride ions in solution (FRANT & ROSS, 1966; EVANS *et al.*, 1971). The curvature in the calibration curve for a HCl—TRIS—NaTartrate medium (Fig. 1) may result partly from hydroxide interference because of the higher pH of this medium, and also possibly from fluoride contamination in the reagents which are used at high concentrations to prepare the buffer. This non-linear region is not to concern fluoride determination provided that the F content of the specimen (assuming a 10-mg sample used) exceeds 0.3% as is usually the case with fossil bones of Pleistocene or older ages.

SINGER & ARMSTRONG (1968) have applied the electrometric technique to the analysis of fluoride in recent bone with success by using a NaCl—HOAc—NaOAc medium (pH 4.7). Fossil bone and tooth, however, as well as the surface layer of recent tooth enamel, embody appreciable quantities of metal ions sometimes amounting to several percent (see BRUDEVOLD & SÖREMARK, 1967; PARKER & TOOTS, 1970; SEITZ & TAYLOR, 1974; KIMURA, 1975, 1977; KEELEY *et al.*, 1977; LAMBERT *et al.*, 1979; and others), which by complexing fluoride in bone solution could interfere with the fluoride determination using the ion-selective electrode. This is supposed to have

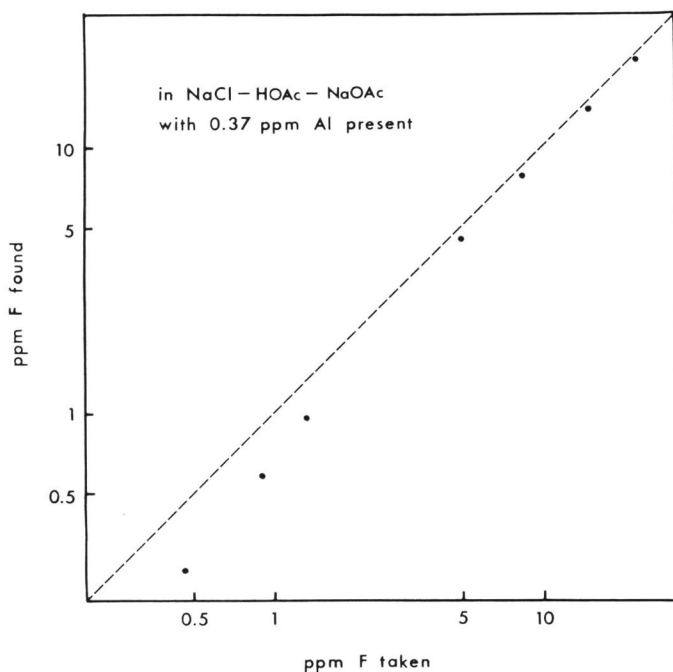


Fig. 3. Plot of F found from direct measurement in the presence of Al against total F taken in a 0.125 M NaCl—0.05 M HOAc—0.15 M NaOAc medium. The broken line represents the exact agreement between the two concentrations of F.

generated the bias observed for the fluorine result in using Buffer A which comprises no metal complexant.

PARKER *et al.* (1974) determined fluorine in fossilized bone and tooth electrometrically by treating sample solutions with citric acid and triethanolamine following the method described by DUFF & STUART (1972), with the measurements verified by standard addition of fluoride. The use of triethanolamine in the presence of citric acid as complexing ligands has proved to minimize the majority of metal ion interferences except a serious one from aluminum; however a rating of the buffer is not practicable because no concentrations regarding citric acid and triethanolamine were quoted in the table in DUFF & STUART (1972) which presented the interference data.

The citrate buffer used in this study is less operative in controlling aluminum interference than the 0.5 M tartate buffer (Fig. 2); still it may be effective for aluminum to permit the analyses of most fossil bones, judging from reported aluminum content of fossilized specimens (see *e.g.* KIMURA, 1975, 1977) and considering that aluminum is likely to occur partly as dilute HCl-insoluble minerals in voids in bone. This buffer is to be particularly useful for samples with lower levels of F since it becomes increasingly easy to overcome aluminum interference with decreasing concentrations of F in the solution (INGRAM, 1970; KANISAWA, 1978; and this work). Yet one can elect with reason to use the HCl—TRIS—NaTatrate medium where information on bone to be analysed or lack of it warrants its use. Although this medium is not readily applicable for very low-level measurements, some 3-mg amount of sample is sufficient for an accurate determination of fluorine in fossil bone containing more than 1% F.

### Acknowledgements

The fossils used in this work were obtained through the courtesy of Professor Naotune WATANABE, Professor Yoshikazu HASEGAWA, Dr. Satoshi MATSUBARA and Mr. Keiichi ONO, to whom the author expresses his sincerest gratitude. The author benefited from reviews of the manuscript by Dr. Hajime SAKURA and Dr. Bin YAMAGUCHI. Acknowledgement is also made to Miss Akiko NAKATSUKA for drafting help.

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