Secondary Ion Microanalysis of Fluorine in Apatites of Biological Interest

L. G. Petersson, G. Frostell
Department of Cariology, University of Lund, Malmö, Sweden

and

A. Lodding
Laboratoire de Physique des Materiaux, CNRS, Bellevue, France *

(Z. Naturforsch. **29 c**, 417—420 [1974] ; received February 18, 1974)

Microanalysis, Fluorine, Teeth, Apatites, Ion Microprobe

A Cameca IMS 300 ion analyzer has been used to measure fluorine concentrations in tooth material. Samples of human and shark teeth were studied. Negative oxygen primary ions produce secondary mass spectra in which positive as well as negative fluorine ion peaks are well represented. With constant measuring conditions, the reproducibility of the F/Ca ion ratios is satisfactory. Fluorine concentrations in the 10 ppm region are easily recorded. By means of samples previously measured by macroscopic F-determining methods, a calibration can be effected to obtain, from the ion current ratios, at least semiquantitative values of F-concentrations.

In a sample of human enamel, the F-concentration was found to vary from about 3000 ppm at the surface to about 100 ppm in the interior. In shark enamel the composition at the surface corresponded to nearly saturated fluorapatite, 3.6% F, but the fluorine concentration decreased distinctly at increasing depth.

Introduction

The scientific interest in biological materials, especially in teeth and bone, has been steadily increasing in recent years. Dentists and ostheopaths are particularly aware of the important clinical aspects of the presence of dilute impurities in these materials, and have in numerous instances established contacts with chemists and physicists to obtain information about the kinetics and mechanisms of such trace elements [1–3]. The most striking example is fluorine, the influence of which on the chemical resistance of tooth enamel is well recognized [3] and clinically exploited. The mechanisms of uptake and mobility of F in biological ceramics are, however, still relatively unknown and disputed, as are also the solid-state aspects of the resistance action. To clear these problems, one needs precise measurements of F concentrations in apatites down to a few ppm, preferably by means that allow simultaneous morphological definition of the details under analysis.

Most of the measurements of fluorine gradients in teeth and bone so far have been made by *macroscopic methods*, *i. e.* by stepwise grinding or etching and analysis of successive surface layers [4, 5]. These methods, sensitive down to about 50 ppm F, give average concentrations in layers a few mm^2 in area and some 10 to 100 μm in depth. The usefulness of such measurements is limited because of the heterogenous structure of these materials and because of the fact that the significant action of the fluorine may well be localized to a surface layer only a few microns thick. Of interest are particularly the concentration gradients within the mineralized pellicle and in the first micron of the enamel.

The *electron microprobe* provides another means of chemical analysis of these materials, and in addition permits a topological identification with a space resolution of a few μm [6, 7]. However, the sensitivity of the electron probe for F in apatite is low, and at concentrations normal in human teeth the detection is difficult and accuracy poor. Further, the depth resolution is well in excess of a micron, which is not sharp enough to give the vital detail information about the outermost surface layers.

The *secondary ion microanalyzer* [8–10] is an instrument based on surface sputtering and mass spectrometric analysis. The sample is bombarded by monoenergetic positive or negative ions. The secondary ions ejected from the surface are analyzed by electromagnetic mass separation; for each mass, ion currents down to about 10^{-17} A are detectable. The

---

* On leave from the Physics Department, Chalmers University of Technology, Gothenburg, Sweden.
Requests for reprints should be sent to Dr. A. Lodding, Arkitektvägen 6 B, *43080 Hovås*, Sweden.

detection limit, especially for light elements, is very low, often in the ppb range, *i. e.* some three orders of magnitude better than for the electron probe. In the Cameca version of the ion analyzer the ion image of the detail under bombardment (15 to 250 microns in diameter) can be seen magnified on a screen, and the space resolution is better than 1 micron. The depth resolution, as one penetrates by slow erosion of successive layers, is 100 Å or better, *i. e.* at least a hundred times better than for the electron probe. The fluorine peak is clearly detectable and measurable in the positive as well as negative secondary mass spectrum down to concentrations of about 1 ppm.

Experimental Scope

These features make the ion analyzer a particularly attractive means to study the concentrations and kinetics of F in biological ceramics. To our knowledge, however, no successful results of such investigations have hitherto been reported. We have undertaken to test the practical possibilities of such studies in a series of exploratory masurements, utilizing a Cameca IMS 300 ion analyzer put at our disposal by the manufacturer.

The material analyzed comprised samples of human and shark teeth, some of which had previously been tested by macroscopic means[4] as to their fluorine concentrations. Our first objective was to investigate the reproducibility of the obtained F/Ca secondary ion ratios and to devise, if possible, a method of quantitative calibration, *i. e.* of establishing, from the *ion* ratios, the F/Ca *atom* ratios. These efforts have yielded promising preliminary results, and are reported in a separate paper[11].

The second objective was to try out several practicable methods to obtain F/Ca ratios at different concentrations, and to demonstrate acceptable agreement with results obtained macroscopically. For these purposes the following series of measurements were undertaken: a. Concentration profile of F across the enamel of a human tooth, from the surface to the dentin boundary; b. concentration profile of F in the outermost layers of a shark tooth.

The moment a. was to be effected with a small (approx. 20 µm diam.) primary ion beam at probing spots on a line across the enamel of a tooth; the precise position of the various points in relation to the surface could be determined by successive microscope scrutiny. For moment b. a somewhat larger primary beam was used, about 100 µm, which was scanned with an amplitude of about 150 µm about the line of impact, to ensure a flat crater bottom and good depth resolution. The primary ion species chosen was O^-, as it had been empirically found[12] that negative primaries lead to considerably less serious surface and bulk charge effects in insulators than do positive ions. To further eliminate surface charge-up the samples were steeped in a low-melting Bi-Sn alloy and coated with a 200 µm thick layer of pure gold, in electric contact with the sample-holder. For the major part of the experimental procedures, the standard analyzing conditions of the Cameca IMS 300 instrument[10] were utilized, with the AS 200 multiscaler[13] as an important accessory. Throughout, relative measurements of the F and Ca ion currents were recorded. By way of further reference to the matrix, a comparison was usually made with the P ion emission.

Typical positive and negative secondary ion mass spectra between mass numbers 10 and 55 are shown in Fig. 1. It is seen that for both polarities the ^{19}F peak is large and well resolved. Although the sensitivity of F is particularly great in the negative spectrum, for the present studies it was chosen to work with positive secondaries, which permits a better comparison with Ca and P. The peaks of relevance in this study were $^{19}F^+$, $^{20}Ca^{2+}$, $^{31}P^+$ and $^{43}Ca^+$; in the multiscaler unit AS 200 three of these could be seen at a time. It may also be quoted here that the ppm-impurities of Sr, Mn, Si, Ti, Mg, Al and Pb, some of them of clinical interest[1], are readily detected in the positive spectrum, and that carbon gives a large peak in the negative spectrum.

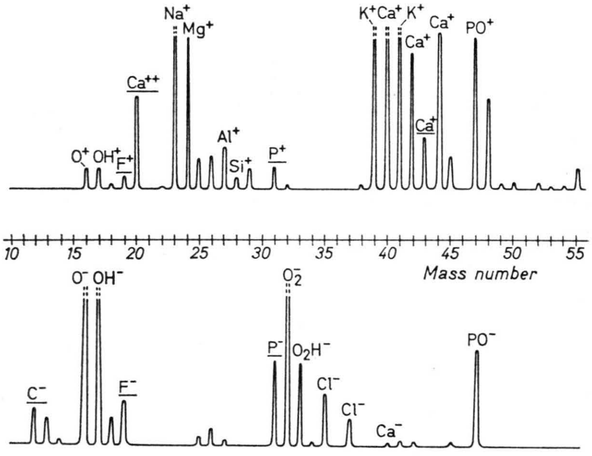

Fig. 1. Positive and negative secondary ion mass spectrum of human enamel bombarded by O^- primary ions. The symbols of peaks of particular interest in this investigation are underlined.

Results

a. The measured variation of the $^{19}F^+/^{20}Ca^{2+}$ ratio across a section of human enamel is shown in Fig. 2 (upper curve). Also drawn is a curve representing the F concentration in ppm, calibrated according to the macroscopically determined [4] value of concentration in the interior of the same tooth, 100 ± 15 ppm, and corrected for variable spot conditions according to ref. [11].

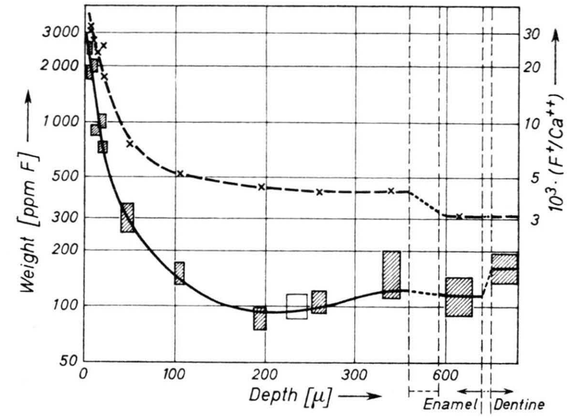

Fig. 2. Human tooth; variation of the fluorine to calcium ion ratio and of the fluorine concentration as function of depth under frontal surface. Open square: calibration by macroscopic analysis.

b. The variation with time of the observed $^{19}F^+/^{10}Ca^{2+}$ ion current ratio during ion bombardment perpendicular to the front surface of a shark tooth is shown in Fig. 3. Successive layers are eroded with a penetration speed of the order of 5 Å/sec; the exact sputtering speed is not known, but the order-of-magnitude assessment is based on interferometric observation of craters sputtered on apatite crystals under similar conditions as in the present study. Under the assumption of 5 Å/sec, and with calibration by comparison with fluorapatite according to ref. [11], the concentration values listed in Table I are obtained. For comparison, the Table also shows the result of the first F profile determination in shark enamel, obtained by one of us (LGP) by a macroscopic method [4].

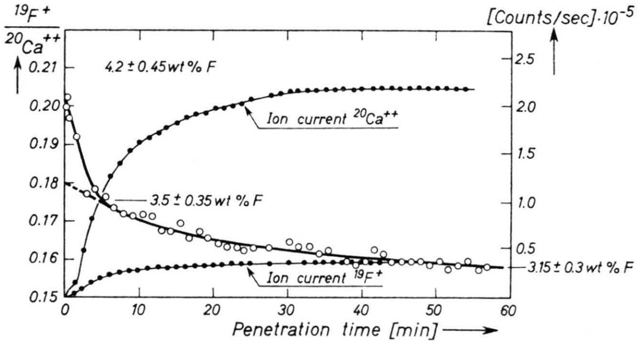

Fig. 3. Shark tooth; variation of the F to Ca ion ratio in the outermost layers of enamel.

Table I. Fluorine concentration at different depths of shark tooth enamel. Points 1–4 obtained by ion microanalysis; their concentration values are calibrated by comparison with fluorapatite crystal, with a systematic uncertainty of about 15%; the depth scale z of these points is uncertain within ±50%. Points 5–7 are obtained by chemical analysis according to ref. [4]. Systematic error in c_F within about 10%, in z about 5%. The logarithmic decrement of c_F is defined by $\lambda = -(\Delta \ln c_F)/\Delta z$.

Point	z [μm]	c_F [weight %]	λ [μm^{-1}]
1	0.03	3.85	1.2
2	0.10	3.55	0.18
3	0.50	3.30	
4	1.80	3.15	0.036
5	5.2	3.8	0.023
6	16.9	2.9	0.022
7	32.7	2.05	

Discussion

The primary results as shown by the upper (thin-drawn) curve of Fig. 2 are a set of ion current ratios, $^{19}F^+/^{20}Ca^{2+}$, obtained at fairly constant primary beam conditions, bombarding at points along a line on a *human tooth* section perpendicular to the frontal surface. Although the curve can be seen to be very smooth, and the concentration in the interior is known by previous chemical analysis [4] with fair accuracy (100 ± 15 ppm), the conversion of the ion ratios to atomic concentrations does not follow immediately. As described in ref. [11], even minor variation in beam and surface conditions may influence the conversion factor. A calibration is available by looking at the ion ratios $^{31}P^+/^{20}Ca^{2+}$ at the same time as at the fluorine to calcium ratios. At any given chemical composition the observed ion current ratios of the matrix elements may exhibit variations, and these changes indicate the simultaneous changes of the ionization sensitivity of F vs Ca. This correcting method, described in more detail in ref. [11], has been applied to the primary points in Fig. 2. The result is the lower curve, expressing the variation of F concentration in weight ppm. The investigated tooth exhibits a fairly normal fluorine profile, corresponding to adult enamel in an environment with ca 3 ppm fluoride in the drinking water [1]. The minimum of c_F and the slow increase towards the inner portion of enamel, as well as the somewhat higher c_F value in the dentine, are also in accordance with normal observation [1]. The corrected curve is seen to exhibit good reproducibility and resolution even in the initial steep decrease region (from 100% to about 40% within the first 10 μm).

The thin-drawn curves of Fig. 3 show the variation of the two observed ion current intensities ($^{19}F^+$ and $^{20}Ca^{2+}$) as functions of time after exposure of the frontal surface of a *shark tooth* to the primary beam. Both curves are very smooth; after the first half-hour pairs of successive readings on each curve differ by less than 0.5%. The decreasing curve shows the time variation of the ratio of the two ion currents. At the conclusion of the sputtering run (after one hour's penetration) the fluorine-calcium ratio was read simultaneously with the phosphorus-calcium ratio for a calibration according to ref. [11]. This indicated an F concentration of 3.15 ± 0.4 wt.% at the bottom of the crater. As the sputtering conditions were practically constant throughout the run, apart from the first penetrating stage, one may assume that after the first four minutes the c_F concentration was proportional to the observed ion current ratio. Extrapolating, one obtains a fluorine concentration of 3.6 ± 0.4 wt.% at the surface. As the F concentration of pure fluorapatite is 3.77%, the surface of the shark tooth appears very nearly saturated with fluorine. This agrees with the data obtained earlier by one of us (LGP) using a macroscopic etching technique [4]; the two sets of results are seen in Table I. The absolute values of the two series are mutually consistent within their margins of error. One may note that also the logarithmic decrements of c_F *vs* depth are similar and steady after the first micron's penetration. The log-decrement of 0.022 corresponds to a doubling in about 30 μm, which extrapolates to a surface value of about 4.1% for the macroscopically determined series; with the quoted margin (10%) of systematic error, one just overlaps the F-saturation value of apatite. It has been commonly assumed that shark enamel consists throughout of nearly pure fluorapatite; our values listed in Table I are, to our knowledge, the first measurements of a systematic decrease of the F/Ca ratio with penetration below the surface.

These two series of measurements demonstrate that the ion microanalyzer is a useful instrument for fluorine studies in biological apatites. The sensitivity is quite sufficient to give quantitative results down to the ppm concentration region. The method is quick and the sample preparation not too complicated. This exploratory investigation, carried out with an instrument borrowed from the factory during a limited time period, indicates that the technique will find many-sided applications and yield accurate results in this field.

This work has been supported by the Patentmedelsfonden för Odontologisk Forskning. We thank M. J.-M. Gourgout for help with the analyses. The kindness of the Cameca company in putting an IMS 300 instrument at our disposal is hereby gratefully acknowledged. We are indebted for the encouraging interest of Prof. J. Philibert, Bellevue, and of the French-Swedish Research Association.

---

[1] F. Brudevold and R. Söremark, Organization and Structure of Teeth (ed. A. Miles), Academic Press, New York and London 1967.

[2] See *e. g.* Tooth Enamel (ed. M. V. Stack and R. W. Fearnhead), John Wright & Sons, Bristol 1965 and 1971.

[3] P. Torell and Y. Ericsson, Int. Dental Journ. **17**, 564 [1967].

[4] G. Koch and L. G. Petersson, Odont. Revy **23**, 437 [1972].

[5] H. R. Mühlemann, A. Schait, and K. G. König, Helv. Odont. Acta **8**, 147 [1964].

[6] C. A. Andersen, An introduction to the electron probe microanalyzer and its application to biochemistry, **Vol. V** (ed. Glick), Wiley, New York 1967.

[7] C. A. Baud and S. Bang, Caries Res. **4**, 1 [1970].

[8] R. Castaing and G. Slodzian, J. Microscopie **1**, 395 [1962].

[9] H. J. Liebl and R. F. K. Herzog, J. Appl. Phys. **34**, 2893 [1963].

[10] J. M. Rouberol, J. Guernet, P. Deschamps, J. P. Dagnot, and J. M. Guon de la Berge, Proc. 5th Intern. Congress on X-Ray Optics and Microanalysis, Tübingen 1968.

[11] A. Lodding, J.-M. Gourgout, L. G. Petersson, and G. Frostell, Z. Naturforsch. **29 a**, in press.

[12] A. Lodding, L. Lundkvist, G. A. Sullivan, and J. M. Hinthorne, in preparation.

[13] Cameca, Courbevoie, technical note AS 200.