

Spectrochemical Analysis of Dental Calculus by Synchrotron Radiation X-ray Fluorescence

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This work presents elemental composition studies of human dental calculus by X-ray fluorescence analysis using synchrotron radiation. The intrinsic characteristics of synchrotron light allow for a quantitative analysis of major, minor, and trace elements of very small samples in a single measurement. At present, several theories of calculus formation exist, but none of them can describe completely all the complicated mechanisms involved in the mineralization processes. For this reason, and taking into account that experimental data are long overdue, several calculi with certain degrees of formation were collected from adult patients for analysis. The ratio of calcium/phosphorus was used as an indicator of the major crystalline structure and the state of formation of the calculus. The results demonstrate a clear correlation between the concentrations of certain elements and the type of calculus (supra- or sub-gingival). In addition, the possible correlation between the elements was analyzed.

A number of works about dental calculus (tartar) structure and composition, not only in human beings but also in animals, have been published.^{1–3} From several points of view (clinical, periodontal, histochemical, and morphologic), this topic became a trigger for investigations that were aimed at giving hints for tartar control or prevention or removal of the dental calculus and optimization of the action of dentifrices and mouthwashes.

Calculus is generally classified into two types according to its position on the tooth.⁴ Supragingival calculus is found above the gingival margin, while subgingival calculus is found below the gingival margin. A single calculus is often a combination of both types, because subgingival calculus becomes supragingival as the gingiva recedes, or supragingival calculus becomes subgingival in gingival enlargement.

Human dental calculus consists of an inorganic or mineral phase and an organic phase. Spectrochemical analyses have revealed the presence of several minerals in tartar. The most

abundant elements found are calcium, phosphorus, and magnesium; sodium, potassium, and lead are often found in small amounts. Trace amounts of aluminum, iron, copper, silicon, barium, strontium, bismuth, nickel, zinc, manganese, cobalt, and gold have been reported.^{5,6}

The crystalline structure of the calculus passes through several stages during its formation in the mouth. In mature deposits (more than six months), the most abundant crystalline form is the hydroxyapatite ($\text{Ca}_{10}[\text{PO}_4]_6\cdot\text{OH}_2$), with minor amounts of octocalcium phosphate ($\text{Ca}_8[\text{HPO}_4]_4$), whitlockite (usually called tricalcium phosphate $\text{Ca}_3[\text{PO}_4]_2$), and brushite ($\text{Ca}[\text{HPO}_4]\cdot 2\text{H}_2\text{O}$). In young deposits, approximately one-half of the crystals are of brushite. A longitudinal study of the growth of calculi shows that brushite appears in the first stages, then octocalcium phosphate develops, and as they develop, whitlockite and hydroxyapatite arise.⁷ In addition, the morphology of the tartar has been studied by scanning electron microscopy.⁸

Hydroxyapatite has a considerable structural flexibility, which produces a complex dynamic process of ion exchange with its surroundings (in this case, the saliva). The calcium site may be occupied by many divalent (and some trivalent) cations, the phosphate group may be replaced by vanadate, chromate, ferrate, arsenate, silicate, etc., and the fluoride group, by any halide and several divalent anions.^{9,10} As a particular case, zinc ions can replace calcium elements. This fact seems to play an important role in the calculus formation.¹¹

Various methods were employed to make these determinations, such as radiochemical techniques,¹ atomic absorption spectrometry,¹² and X-ray diffraction analyses¹³ with a reasonable agreement among them. To obtain a good signal response, the implementation of these conventional techniques requires either a mixture of several calculi or extraction of extraordinarily big

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calculi.¹ The mixture is needed in order to have enough of the sample for analysis, because a single calculus is usually very small (typically no more than 1 mg). However, the study of an individual calculus is of significant interest because it allows establishing correlations among different aspects and characteristics of tartar.

It is well-known that the use of synchrotron radiation in trace analysis by X-ray fluorescence (SRXRF) allows the reduction of detection limits and the improvement of sensitivities.^{14,15} The intrinsic characteristics of synchrotron radiation (high intensity, polarization, natural collimation, etc.) and the construction of dedicated sources of synchrotron light make it possible to improve detection limits for trace elements by several orders of magnitude, as compared with conventional techniques.¹⁶ The use of a monochromator or wavelength-dispersive systems permits the optimization of irradiation conditions, performance of a selective analysis, and extension of the dynamic range of the technique, thus improving sensitivities and detection limits for low-Z elements. One of the most important advantages of using synchrotron radiation is the possibility of analyzing very small samples as a result of the natural brilliance and high intensity. Such samples are impossible to study with conventional techniques because of the impractical measuring times.

SRXRF has been used in several applications to analyze metals, ceramics, pollutants, etc. A special type of application is the analysis of biological samples, which includes surveys related to the human body. The study of dental mineralized tissues by SRXRF has been dealt with in several works,^{17–20} most of them using mapping techniques. Nevertheless, dental calculus surveys are scarce and difficult to find. As mentioned before, conventional methods have been used, but to the authors' knowledge, studies of individual calculi have not been reported so far.

The purposes of this work are to determine the elemental composition of dental calculus by synchrotron radiation X-ray fluorescence, to compare subgingival and supragingival calculus morphology, and to estimate the most dominant crystalline structure by studying element ratios. Moreover, the possible ionic exchange of the measured elements will be inspected by analyzing linear regression coefficients.

EXPERIMENTAL SECTION

Sample Collection. Specimens of human dental calculi were obtained from 17 adult patients suffering from periodontal disease who requested periodontal treatment. All of the calculi had more than six months of formation. The samples were extracted in the following way: the supragingival ones were collected from the lingual surface of the lower anterior teeth (incisors and canines), and the subgingival ones were taken from the mesial surface of the upper molars. In general, the collected samples did not exceed one milligram.

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Table 1. Composition of the Standard Samples Used to Obtain the Calibration Curve

sample	concn, %	
	element	dark matrix
apatite	P, 18	H, 0.2
	Ca, 39.9	O, 41.4
standard, 193 A SO-2	Al, 9	Mg, 0.59
	Si, 25	Na, 0.3
	P, 0.13	
	K, 3.08	
	Ca, 0.69	
	Ti, 0.55	
	Mn, 0.09	
	Fe, 3.73	
Cu ₂ O	Cu, 89	O, 11
zinc	Zn, 100	

Sample Preparation. Seventeen pairs of calculus samples (supragingival and subgingival) were taken and correctly labeled and stored. The extracted calculi, in the form of irregular small stones; were powdered in a glass mortar to obtain a chalky powder. All of the apparatuses used in this process were cleaned in an ultrasonic cleaner after each step to avoid unwanted contaminations. The preparation process was carried out in a standardized manner for all of the samples. The sample holders were made of two strips of adhesive tape adhered to each other by their adhesive face. In one of these strips, a 5-mm indentation was made. The sample powders were deposited on this adhesive circular area.

The mass deposited on the adhesive surface was determined by difference of weight of the tape before and after sample deposition. Each set of measurements (with and without the sample) was performed under the same conditions and in one run to avoid drift effects of the balance.

To perform a quantitative analysis, standard samples were prepared using the same procedure as for tartar specimens. The composition of the selected standards is shown in Table 1.

Measurements. The experiments were carried out in the high-vacuum station of the XRF beamline at the Synchrotron Light National Laboratory (Campinas, Brazil).²¹ The electron energy inside the storage ring is 1.37 GeV with a dipole magnetic field of 1.65 T, which produces a critical photon energy of 2.08 keV.

The synchrotron radiation source for the XRF beamline is the DO9B (15°) bending magnet of the storage ring. The components of the line include 125- μ m beryllium windows to isolate the beamline vacuum from the ring vacuum and from the atmosphere, a channel-cut crystal monochromator, and a motorized computer-controlled set of slits to limit the beam size. Figure 1 shows the beamline and its main components.

Si(Li) and HPGe energy-dispersive detectors compose the detection systems. Both detectors are placed at 90° to the incident beam, and one of them (HPGe) is optimized for low-Z element detection (windowless).

All of the measurements were performed in conventional geometry (45° + 45°), exciting with a white beam, and during

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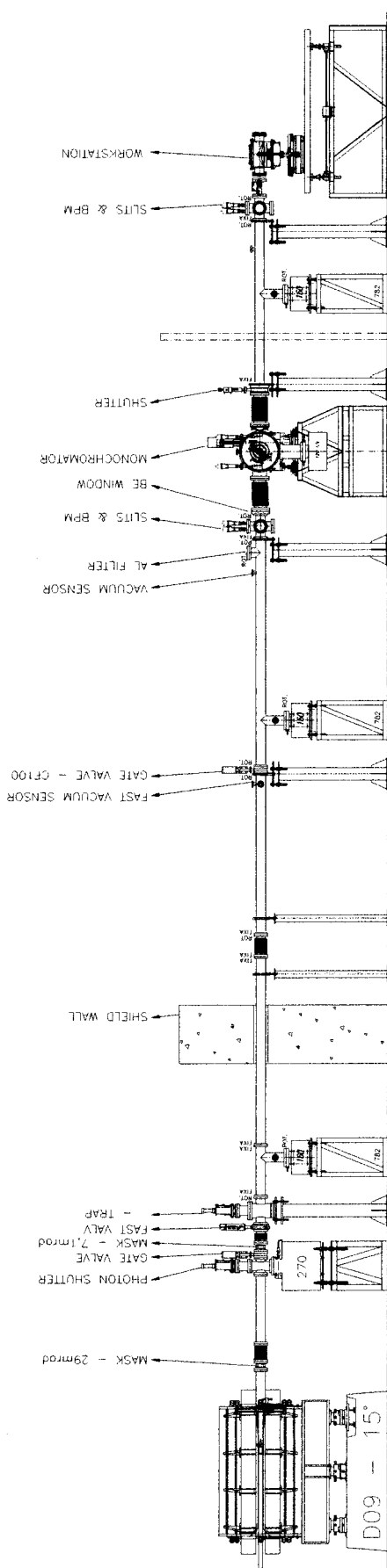


Figure 1. Schematic view of the XRF beamline and its main components.

Table 2. Average Elemental Concentrations of Supragingival and Subgingival Calculi Considering All the Samples

element	supragingival	subgingival	bias; sub/sup [%]
Si [%]	5 ± 1	5.9 ± 0.1	64.7
P [%]	22 ± 3	24 ± 5	24.0
S [%]	0.43 ± 0.08	0.62 ± 0.09	82.3
Cl [%]	0.24 ± 0.03	0.40 ± 0.06	82.3
K [%]	0.85 ± 0.09	1.0 ± 0.2	70.6
Ca [%]	37 ± 5	44 ± 11	64.7
Ti [ppm]	230 ± 100	360 ± 160	52.9
Mn [ppm]	60 ± 20	90 ± 80	41.1
Fe [ppm]	20 ± 3	50 ± 30	82.3
Co [ppm]	20 ± 5	25 ± 3	52.9
Cu [ppm]	7 ± 2	12 ± 4	82.4
Zn [ppm]	55 ± 58	280 ± 230	82.3

200 s of live time for each sample. The experiments were performed in an air atmosphere using the Si(Li) solid-state detector.

Data Analysis. Figure 2 shows a typical spectrum of a supragingival sample. All of the spectra were analyzed using a conventional program for spectrum analysis (AXIL²²), considering a background model of a 9° orthogonal polynomial, escape peaks, and peak-shape corrections.

The fluorescence intensities of the standard samples were used to obtain a calibration curve in the range of the elements of interest (Figure 3). The elemental sensitivities method,^{22,23} which comes with the AXIL package, was used to calculate elemental compositions.

RESULTS AND DISCUSSION

The average values of elemental concentrations obtained are presented in Table 2. These values are in good agreement with the findings reported by other methods, such as radiochemical analysis, atomic absorption, and X-ray diffraction.^{1,12,13} The major elements observed are Ca and P, as expected for calcium phosphates. Their concentrations have an acceptable deviation in supragingival tartar and present higher deviations on subgingival calculi. Other elements, such as Si, S, Cl, and K, have deviations of approximately 15%. On the other hand, minor and trace elements, such as Ti, Mn, Zn, and Fe, show a broad Gaussian shape. This could indicate that these last elements depend not only on the buccal environment but also on the action of external agents. In the case of Mn, it has been suggested that its concentrations may depend on the local concentration of this metal in the tap water supply,¹² which would explain the high deviation that is observed (88%).¹

The last entry of Table 2, named bias, represents the percentage of cases in which the concentration of a particular element in a subgingival calculus was greater than the corresponding supragingival one (of the same patient), taking into account all of the 17 pairs under analysis. This value helps to characterize each kind of calculus according to the amount of a given element.

As can be observed in Table 2, all of the analyzed elements seem to have higher concentrations in subgingival calculus, which

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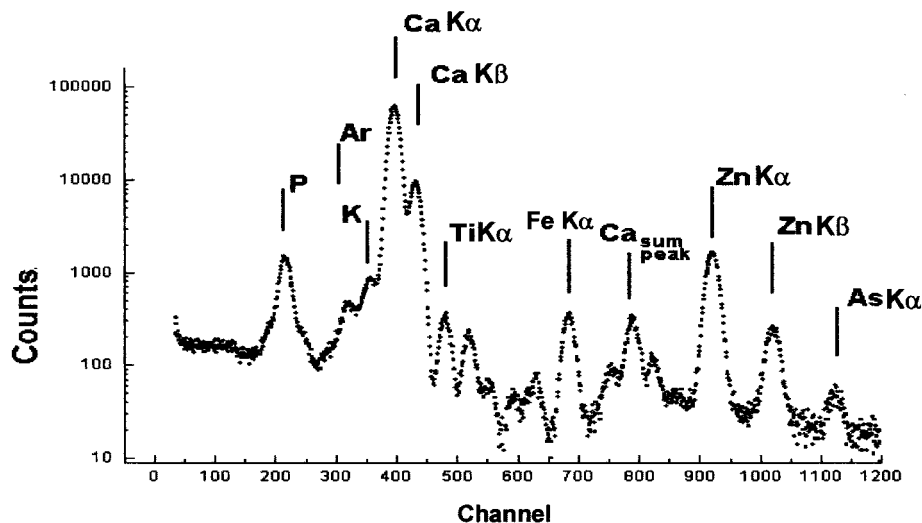


Figure 2. Typical spectrum of a supragingival calculus.

Table 3. Average Molar Ratios of Ca/P for Supra- and Subgingival Calculi Considering Individual Patients

Ca/P		of compds
subgingival	supragingival	
1.41 ± 0.09	1.41 ± 0.07	DCPD, dicalcium phosphate dihydrate, 1.0 OCP, octocalcium phosphate, 1.33 β -TCMP, tricalcium phosphate, 1.50 hydroxyapatite, 1.7–2.3

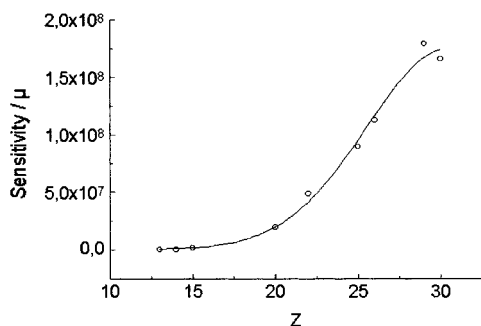


Figure 3. Sensitivity curve obtained from the standard samples.

is in agreement with the fact that the interstitial gingival fluid present is richer in these elements than is saliva. As mentioned before, Mn exhibits an exception to this behavior.¹ As an example, Figure 4 shows histograms of concentrations for some elements. In despite of Mn, which exhibits scattered values, the rest of the elements present Gaussian-like distributions.

As mentioned earlier, the inorganic or mineral phase of tartar usually consists of a mixture of different calcium phosphates. Hence, the molar ratio Ca/P can be used to estimate the more preponderant crystalline structure by comparing it with the one obtained by stoichiometric calculations. Table 3 shows these results.

As can be observed, the average ratios correspond to the tricalcium phosphate structure, which is in agreement with the fact that the analyzed calculi were more than six months old. As mentioned before, these crystals and apatite crystals dominate mature calculi.²⁴

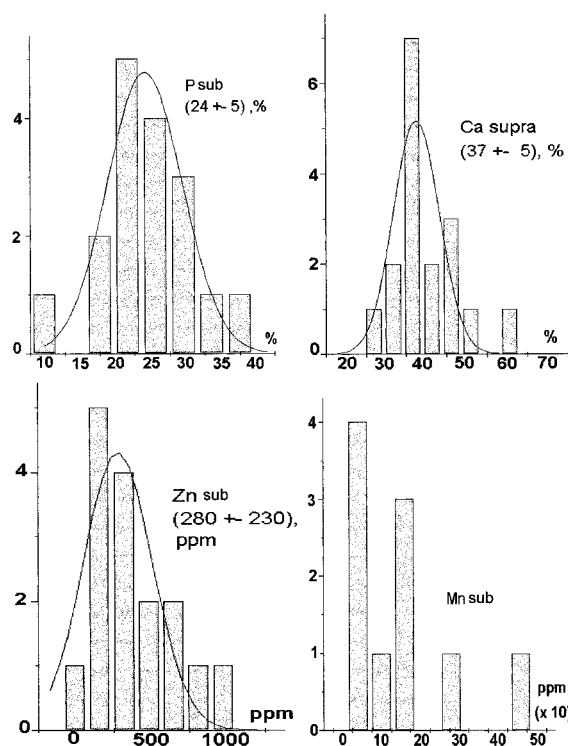


Figure 4. Histograms of concentrations for some measured elements.

Apatite can host a variety of ionic substituents, both anionic and cationic. For example, F^- and Cl^- can replace OH^- ions. In a simple model, if a certain degree of substitution among ions exists, a linear correspondence between their concentrations can be expected. This would give an idea of the probability that one unoccupied site in the lattice could be filled by a different ion. Many such correlations were found (see Figure 5a–e). Correlation coefficients are presented in Table 4. This table shows correlations between Ca and Fe and between Co and Ni; these correlations can arise because in hydroxyapatite, the sites of calcium cations can be replaced by others divalent cations,^{25,26} such as Fe^{2+} , Co^{2+} , Ni^{2+} , Mg^{2+} , etc., and in some cases, by trivalent cations. Besides

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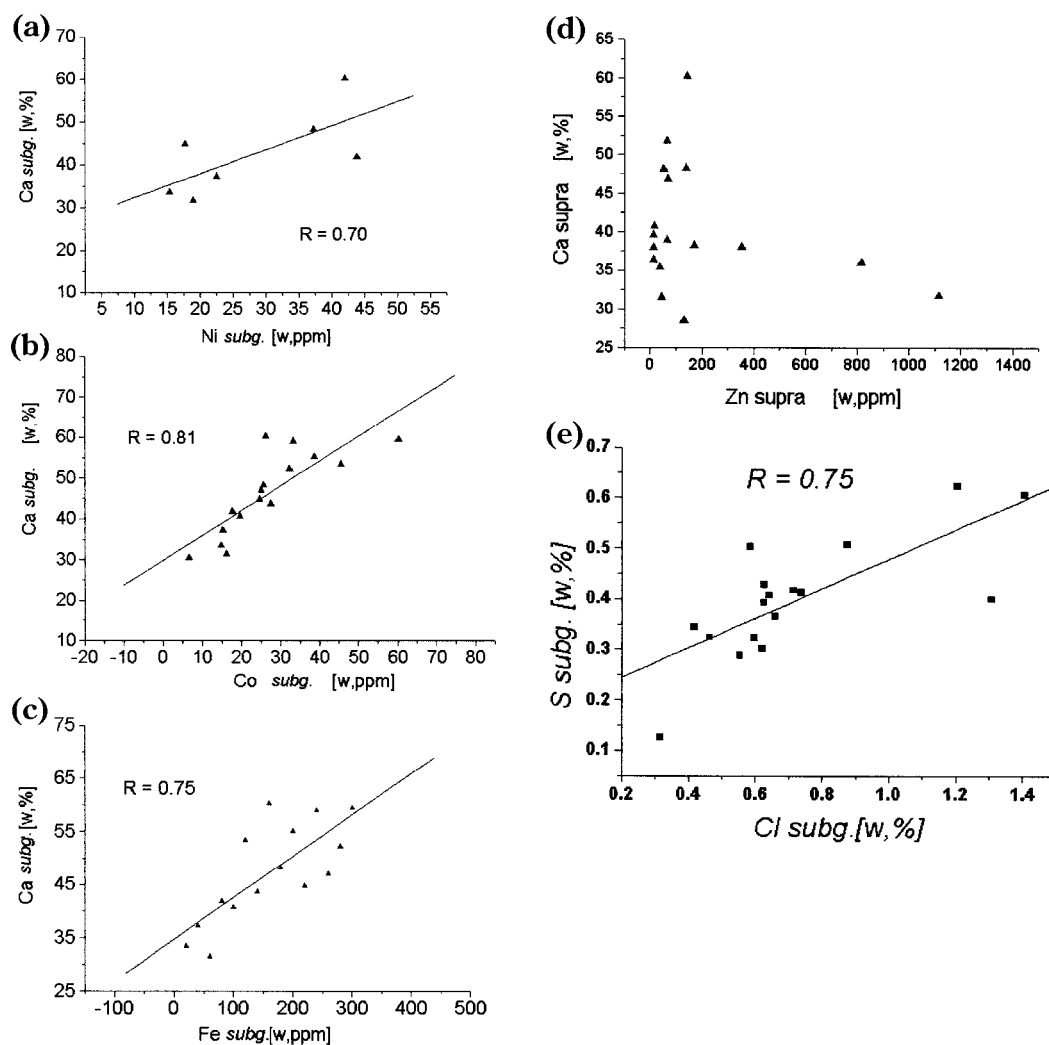


Figure 5. Correlations between different elements. Solid lines show linear fitting.

Table 4. Correlation Coefficients for Several Pairs of Elements in Supragingival and Subgingival Calculus

	Elements												
	Ca, Co	Ca, P	Zn, Cu	Ca, Fe	Cl, S	Cl, P	Ca, Ni	Ca, Zn	K, S	Zn, Ti	P, S	Fe, Zn	Ca, K
	Correlation Coefficient												
supra	0.80	0.88	0.46	0.32	0.73	-0.17	0.73	-0.29	0.62	0.49	-0.13	0.61	0.84
sub	0.80	0.80	0.71	0.75	0.75	-0.23	0.70	-0.06	0.56	0.30	-0.52	0.19	0.67

the calcium correlation, other correlations were observed, for example, K-S, Cl-S (see Figure 5e), Zn-Cu, etc. Previous experiments of scanning microscope spectrometry in a sample of dental calculus also showed a correlation between Zn and Cu.²⁷

Ingram et al.²⁸ reported that zinc would tend to substitute calcium atoms in some calcium phosphates; this result was obtained in an in vitro assay and using synthetic apatite. According to the results obtained in the present work, it could be inferred

(see Figure 5d) that in real conditions, Zn would not mediate in the concentration of Ca up to certain amount of Zn (about 200 ppm). Beyond this value, the slope shows a slight negative correlation between Ca and Zn concentrations. Possibly, a change in the pH of saliva promotes the ionic exchange between these two elements, facilitating the allocation of Zn atoms into the crystalline lattice.²⁹ At present, it is assumed that zinc may be absorbed on the surface of the apatite crystallites or in the amorphous phases strongly inhibiting the crystallization of the hydroxyapatite, which may be considered as a unique crystalline phase up to a determined concentration of zinc.³⁰

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CONCLUSIONS

After a simple procedure of sample preparation, several specimens of dental calculus were studied by synchrotron radiation X-ray fluorescence. Each sample was analyzed independently, taking into account the type of calculus (supra- and subgingival) and the correlation between them for a given patient. The properties of synchrotron radiation (brilliance, high intensity, and polarization) played an important role because of the small amount of sample under analysis. The use of a conventional X-ray source would be impractical and would demand unacceptable measuring times.

The differences between the composition of supragingival and subgingival tartar were studied. The role of the interstitial gingival fluid and saliva was analyzed, showing the importance of the crevicular media. Finally, by means of inspecting molar ratios between elements, the most dominant crystalline structure was determined. For the first time, it was established by considering the samples independently by type and by individual patients.

The relevance of the results obtained in this work demonstrates that SRXRF can be used to establish a systematic program to study morphologic and dynamic characteristics of calculus. A detailed analysis of calculus composition and its structure can contribute to the determination of the influence of certain elements in the mineralization process of calculus^{13,28} and to the relation between calculus composition and identified risk factors associated with periodontal disease.³¹

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