Chapter

# Application of Raman Spectroscopy for Dental Enamel Surface Characterization

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## Abstract

Dental enamel is the most complex and highly mineralized human body tissue, containing more than 95% of carbonated hydroxyapatite and less than 1% of organic matter. Current diagnostic methods for enamel caries detection are unable to detect incipient caries lesions. Many papers determine the re-mineralizing effect using many fluorinated compounds and different demineralizing solutions to test physical characterizations such as microhardness, roughness, wettability, among others, but there is not much information about the use of Raman Spectroscopy. Raman Spectroscopy is an efficient technique of chemical characterization to identify functional groups (phosphate-hydroxyl groups) found in the hydroxyapatite formula, which helps identify the level of mineralization on dental enamel surface. Raman spectroscopy is applicable to any state of aggregation of the material, indicated for biological samples. Given the minimum bandwidth of a laser source, as with all spectroscopic techniques that use a laser source, a small sample is sufficient, which makes it an important technique in the analysis of reactive products with very low yield. Raman spectroscopy can be used to obtain the main functional groups in order to determine the remineralization of dental enamel; these results are highly valuable as they can help us make the best decisions on dental treatments.

**Keywords:** dental enamel, remineralization, demineralization, Raman spectroscopy, hydroxyapatite

## 1. Introduction

In mineralized biological systems, it has been found in the literature that there are different ways to administer fluoride, like the application of varnishes, tablets, and different dental pastes with different concentrations of fluoride that participate in an important way in the mineralization mechanisms of the fundamental unit of enamel (hydroxyapatite prisms), modifying the chemical composition, and increasing the resistance to dissolution in an acidic environment. To inhibit the formation of

demineralized lesions and the progression to carious lesions, fluorinated compounds are currently applied to the external surface of the enamel. However, the lack of information on the different vehicles or concentrations of fluorinated compounds, as well as the extent of the effect on enamel, leads to the use of these compounds being exaggerated and at times ineffective in preventive dentistry.

Dental enamel is the outer covering of dental crowns, also known as adamantine tissue, and it is currently defined as a nanocomposite bioceramic of epithelial origin, which protects the tooth from chemical and physical aggressions, which has been considered the most mineralized and hard tissue of the organism because it is structurally constituted by millions of highly mineralized prisms that run through its entire thickness, from the amelo-dentin junction to the external or free surface in contact with the oral environment [1].

Its specific function is to form a resistant cover for the teeth that will make them suitable for chewing. In charge of covering and protecting the dentin-pulp complex from chemical and physical aggressions, it lacks vascularization and innervation, which prevents its own remodeling or repair [2].

Embryologically it is widely known that dental enamel is of ectodermal origin, and the formation of this dental structure occurs by cellular events collectively called amelogenesis and biochemical events that are called biomineralization.

The chemical composition of dental enamel is made up of 95% inorganic matter, organic matter 4%, and water 1% [3].

## 2. Raman and infrared spectroscopy

The symmetric vibrational properties of the molecules are used in systematic ways to interpret the infrared spectrum, as they can be used to predict the transitional vibrations allowed, practically only using the table of characters of the punctual group the molecule belongs to. Raman spectral analysis is often compared with the well-known infrared absorption (IR) spectroscopy. While the two techniques are similar, they work in distinct ways and measure different things. The IR technique measures light absorption by specific molecules, while the Raman technique measures Raman emission from molecules under monochromatic laser irradiation. The difference between the light signals and Raman emission corresponds to the vibrational frequencies of these molecules. The two techniques by themselves are great for obtaining important information from samples but the two can be used in combination to measure vibrational bands unique to each technique; that is why the IR and Raman techniques are often regarded to be complementary.

As mentioned earlier, IR spectroscopy is an absorption technique and the measurements are determined by changes in vibrational frequency, whereas Raman spectroscopy uses a scattering method to obtain data from changes in the polarizability tensor. These differences affect both the method of obtaining data from samples and the parameters that are necessary for calibration curves [4–6].

Raman spectroscopy can be applied to any state of aggregation: solids, liquids, or gases. In liquid dissolutions, this technique presents advantages over infrared spectroscopy, as only waves of longitude of the visible region of the spectrum are implied, so only the cells and glass optics are precise. Also, water produces very weak Raman signals, which will not tangle the spectrum. These advantages make Raman spectroscopy specially indicated for biological samples. Given the minimum bandwidth of a source laser, a small quantity of the sample is sufficient, which makes this

an important technique to analyze the reactions of products with a low yield. Other advantages are derived from the fact that as visible radiation is being employed, and overheating of the samples is notably reduced. The most notable difficulty of Raman spectroscopy is the fluorescence that the enamel emits after the application of different fluoride components such as gels, varnishes, or toothpastes.

## 2.1 Other techniques used for characterizing dental enamel

The mineral content of dental enamel confers itself mechanical, physical, chemical, and biological properties. As it is the most exposed tissue and therefore the most susceptible to demineralization by acidic agents, different techniques have been used throughout time to better know the dental enamel. The first observations were made using scanning electronic microscopy (SEM) and semiquantitative elemental percentage analysis, and to determine the roughness of the dental enamel, atomic force microscopy, and contact and now optical profilometers have been used. To establish the superficial energy of the enamel, wettability is measured by calculating the contact angle, and this property gives us information on how hydrophobic or hydrophilic the dental enamel is. Other techniques used are the nano- and micro-hardness tests, which have been used as physical characterization to assess quantitative demineralization or remineralization of the dental enamel, and finally, Raman spectroscopy is the gold standard to determine the presence or absence of the phosphate-hydroxyl functional groups in dental enamel.

### *2.1.1 Strength*

The strength is a mechanical property of any material, consistent with the difficulty that exists to scratch or create mark on a surface by means of a penetrating point. Strength is measured using a strength tester to rehearse penetration. Depending on the type of point used and the range of loads applied, different scales exist, adequate for different ranges of strength [24].

The Vickers hardness test (HVN [hardness Vickers number]) consists in marking the testing material with a diamond indenter that has the form of a pyramid with a square base and angle of 136° in between opposing faces, put through a load of 1 to 100 kg/f.

Microhardness tests are used a lot and have an important application in dentistry. A microhardness test can evaluate the level of mineralization of a dental substrate. A specific force applied during a specific time and distance provides important data. It has the capacity of remineralizing the enamel and the dentine after different treatments, like it happens in unbalanced situations of demineralization and remineralization [7].

The roughness is a property of a material, speaking of their surface. The superficial roughness is a set of irregularities on the real surface, defined conventionally in a section where deformities and undulations have been eliminated. The appearance of the surface of a piece depends primarily on the material with what the piece was made of and its conformation process. To currently measure this property, a profilometer is used, which is an optic device that has the advantage of a no contact exploration, therefore avoiding deformities and possible harm of soft surfaces. They can also explore surfaces that are not accessible to mechanical devices, measuring through a transparent layer and measuring the roughness of a texture of a surface in contact with another [8, 9].

In dentistry, the average of roughness (Ra) has been the most used and is defined as the arrhythmic medium of all profile roughness deviations in relation to the central line.

### 2.1.2 Wettability

Wettability is an important property with many upcoming applications in various fields. It indicates the ability of a liquid to wet the surface of solid, suggesting hydrophobic or hydrophilic tendencies. In dentistry, these tendencies affect initial water absorption and the adhesion of oral bacteria to teeth surfaces.

To determine wettability, it is necessary to measure the contact angle, which depends on the surface energy of the material and the surface tension of the liquid, formed between the surface of a material and the line tangent to the curved surface of a liquid [10].

If the contact angle formed is lower than 90°, the liquid partially wets the surface of the bare solid, meaning that the surface has good wettability properties and therefore hydrophilic.

If the contact angle formed is higher than 90°, the surface has poor wettability properties and therefore hydrophobic.

This is a simple method to gauge the wettability of a sample as well as their hydrophobic/hydrophilic tendencies [11].

The measurement of the contact angle to determine the wettability of a sample is carried out with a goniometer, an instrument that allows for a precise angular position.

### 2.1.3 Determination of the quantity of the element fluorine

Of all chemical elements, fluorine is the most electronegative; therefore, it is never found on earth in elemental form. Combined chemically in forms of fluoride, fluorine occupies the 17th place in order of frequency of appearance of the elements and represents around 0.06–0.09% of the Earth crust [9].

Fluoride has scientifically shown efficacy in fighting and preventing dental caries, and is widely used in most parts of the world, through its addition to public water supplies, salt, gels, topical mouthwash solutions, fluoride varnishes, toothpastes, and restorative materials [12].

During the World Health Assembly in 2007, a resolution was approved in which universal access to fluoride for caries prevention should be a basic right to human health. There are three basic methods of administering fluoride for caries prevention:

1. Those based in communities (fluorated water, salt, and milk)

2. Those administered by professionals (fluorated gels and varnishes)

3. Those self-administered (toothpastes and mouthwash) [9]

Four mechanisms of action of fluoride have been mentioned:

1. Fluoride inhibits demineralization since fluorapatite crystals, formed by reaction with enamel apatite crystals, are more resistant to acidic attacks compared with HAP crystals.

2. Fluoride enhances remineralization by accelerating the development of fluorapatite crystals by combining calcium and phosphate ions.

3. Fluoride hinders cariogenic bacteria from producing phosphonel pyruvate (PEP), which is a key intermediate of the glycolytic pathway in acid-producing bacteria.

4. In addition, F- is retained in dental hard tissue, oral mucosa, and dental plaque to decrease demineralization and enhance remineralization [13].

Fluoride remains the gold standard for stopping caries lesions with multiple systematic reviews confirming the role of fluoride products in preventing dental caries. However, F- alone does not provide a complete solution in the remineralization process, as the formation of fluoride deposits depends on the available calcium and phosphate ions found in saliva. Therefore, to increase the potential for fluoride prevention, these necessary ions have been added to formulations to increase their retention in an oral environment [14].

## 3. Physical properties of tooth enamel

### 3.1 Hardness

The hardness of the adamantine tissue refers to the resistance of the dental enamel surface to wear, scratching, or suffering certain deformation caused by the application of external pressures. It decreases from the free surface to the dentin-enamel junction, which is directly related to the degree of mineralization [1].

### 3.2 Elasticity

The adamantine fabric has little elasticity due to the minimum percentage of water and organic matter that it has in its composition. It is a fragile tissue with a risk of macro- and microfractures. The elasticity is greater in the area of the neck and sheath of the prisms [3].

### 3.3 Color and transparency

The color of dental enamel depends directly on the underlying tissues, mainly dentin, presenting a yellowish color in areas where the thickness of the enamel is less and grayish white in areas where it is thicker. It is characterized by being translucent, which is proportional to the degree of enamel mineralization; the higher the mineralization, the higher the translucency [4].

### 3.4 Permeability

In dental enamel, it is extremely scarce. Enamel can act as a semi-permeable membrane, allowing the diffusion of water and some ions present in the oral environment [3].

### 3.5 Radiopacity

Considered tooth enamel as one of the most radiopaque elements of the human body due to its high mineralization. It appears white on dental X-rays [4].

## 4. Chemical composition

### 4.1 Inorganic matrix

It is made up of calcium mineral salts, basically phosphate and carbonate, giving rise to a crystallization process that transforms the mineral mass into hydroxyapatite crystals. Mineral salt crystals are more voluminous in dental enamel, with a length of 100–1000 nm, a width of 30–70 nm, and a height of 10–40 nm [3].

They present a hexagonal morphology when they have been sectioned perpendicularly and a rectangular morphology when they are sectioned parallel to the longitudinal axis of the crystal [3]. Within the crystal are the basic units of ionic association of mineral salts called cells or unit cells, which, being associated according to the crystal, have a chemical and crystallographic composition, with calcium ions in their vertices and in the center $OH^-$ bond [3].

### 4.2 Organic matrix

The main component of the organic matrix of the adamantine substance is of a protein nature, and these proteins are present in different stages of formation and maturation of the adamantine substance. There are three main proteins in the developing enamel stage: amelogenin, ameloblastin, and enamelin, which have therefore been called enamel proteins. Amelogenin is by far the most abundant protein component in the developing enamel layer, contributing more than 90% of its total volume, progressively decreasing as enamel maturity increases, and they are also called immature enamel proteins and are located between the crystals of mineral salts [5].

The ameloblastins represent 5% of the organic component and are located in the most superficial layers of the enamel and the periphery of the crystals, while the enamelanins represent 2–3%, located in the periphery of the crystals. Other proteins that play an important role are ruftelin, which is located in the dentin enamel junction zone at the beginning of the enamel formation process, representing 1–2% of the organic matrix finally within the most representative proteins found, the protein whose function is associated with the transport of calcium from the intracellular to the extracellular medium, which bears the name parvalbumin [5].

Both the timing of the stage and the degree of protein removal affect the composition of the organic matrix. Therefore, protein-mineral interactions change in enamel formation and regulate the structural organization, phase, and mineral composition, as well as crystal growth [6].

### 4.3 Water

It is located on the surface of the adamantine crystal, constituting to the so-called adsorbed water layer or hydration layer, the percentage of which is scarce and progressively decreases with age [5]. The unique physicochemical properties of enamel are due to its high hydroxyapatite content, the parallel arrangement of individual elongated apatite crystals in enamel prisms, and the interwoven alignment of perpendicular prisms. Together, these characteristics result in a biomaterial of great hardness and physical resilience [6].

## 5. Influence of the environment on the structure of enamel

Dental caries is an infectious disease that causes local destruction of the hard tissues of the tooth and is associated with diet, the accumulation of microorganisms, and the conditions of the saliva. The development of a clinically visible caries lesion is a consequence of the interaction of various factors in the oral cavity and dental tissues [7]. Carbohydrate fermentation by dental plaque bacteria leads to the formation of various inorganic acids, causing a decrease in pH. When the pH of the oral cavity reaches a critical value of 5.5, an undersaturation of $Ca + ^{2}$ and $PO_4^{-3}$ occur, and ions are produced. The tendency is, therefore, the loss of ions from the teeth with the environment, which is called demineralization. This can lead to carious lesions. When the pH becomes higher than 5.5 through the buffering action of saliva, a supersaturation of $Ca^{+2}$ and $PO_4^{-3}$ occurs in the medium. In this situation, the tendency is to incorporate the ions into the tooth, and this phenomenon is known as remineralization [8].

There is a constant ion exchange between dental tissues and the environment, always seeking balance. Studies have shown that the use of fluorides causes a decrease in caries. A series of investigations have shown the importance of fluorides in demineralization and remineralization, and in controlling the appearance of caries, when fluoride is constantly present in the oral environment [9].

It is worth mentioning that saliva and its mucous components keep teeth moist and coated to help preserve them under the presence of calcium and phosphorus ions, thus protecting enamel from dissolution by acids. Saliva has organic and inorganic constituents. One litter of human saliva consists of 994 g of water, 1 g of suspended solids, and 5 g of dissolved substances, of which 2 g are organic matter and 3 g is inorganic matter. Sodium and potassium ions are the most abundant inorganic constituents in saliva. Sodium-ion and chloride ion concentrations increase with salivary flow rate. Among the inorganic constituents of saliva are the following: [10].

- Sodium: 14.8 mg/L
- Potassium: 22.1 mg/L
- Calcium: 3.1 mg/L
- Magnesium: 0.6 mg/L
- Chloride: 10.0 mg/L
- Phosphorus: 193.0 mg/L
- Sulfur: 149.0 mg/L

Among the organic components of saliva are glucose (200 mg/L), cholesterol (80 mg/L), creatine (10 mg/L), urea (200 mg/L), uric acid (15 mg/L). L), and other components of the parotid gland [10].

Remineralization is the natural process of carious lesion repair. Remineralization has been known for many years now. However, it is only recently that the importance of the remineralization process has been accepted as a valid therapeutic option for

caries treatment. Topically administering fluoride in various forms and vehicles has shown to reduce notoriously the prevalence and incidence of dental caries. The remineralization process can be difficult if the bacterial load is high or if the salivary components are low, which is why there is a need to improve this process and apply this knowledge in a clinical setting [11].

## 6. Demineralization and remineralization

Demineralization and remineralization are dental caries processes that are often described as the only physical-chemical event. Although this allows for an easier understanding, description, and mechanism of the process of this disease, dental caries is much more complex. Dental caries is a multifactorial disease that includes factors such as pathogenic bacteria, salivary proteins, enzymes, ions, and fermentation of food sources (carbohydrates). This leads to the formation of biofilm, which can compromise enamel integrity, and the caries process occurs along the interface between the dental biofilm and the enamel surface [12].

## 7. μ-Raman

μ-Raman spectroscopy is a technique used to identify and characterize in a non-destructive and non-contact way, the chemical composition of organic and inorganic compounds by identifying functional groups without destroying the samples and without carrying out a special preparation. This technique can be used in any state of aggregation of matter [13]. With the help of the optical microscope associated with the equipment, it is possible to identify the isolated particles of the order of the micron. There are reports of the application of this technique to identify the crystallinity of human dental enamel [14].

Obtaining the spectrum is carried out using a laser that generates the beam of light incident on the sample, which is focused on a conventional optical objective in the area of interest. The same target absorbs Rayleigh (elastic) and Raman (inelastic) scattered photons. The radiation is broken down by a monochromator, analyzed using a photomultiplier, and recorded with a recording module. When a polyatomic structure is illuminated by a laser beam (monochromatic radiation of the visible spectrum), several phenomena are observed: the reflection of light, absorption, transmission, and scattering of photons. In the case of inelastic scattering, the scattered photons obtain an additive energy thanks to the energy exchange between the incident photons and the quantized energy levels of the polyatomic structure. The mechanism of this phenomenon is as follows: As a result of the action of the incident photons, which have higher energy than that of the vibrating state of the polyatomic structure, the irradiated material temporarily attains an unstable level and then returns to one of the allowed states, emitting a photon of higher energy than the initial photons [13]; the same wavelength that strikes the molecule will be the same in Rayleigh scattering (elastic), but not in Raman scattering (inelastic) as shown in **Figure 1a** and **b**.

μ-Raman spectroscopy is widely used for chemical analysis of biological and synthetic materials, in the same way it has innovated within medicine since recent studies have obtained the spectra of different human tissues, DNA, white blood cells

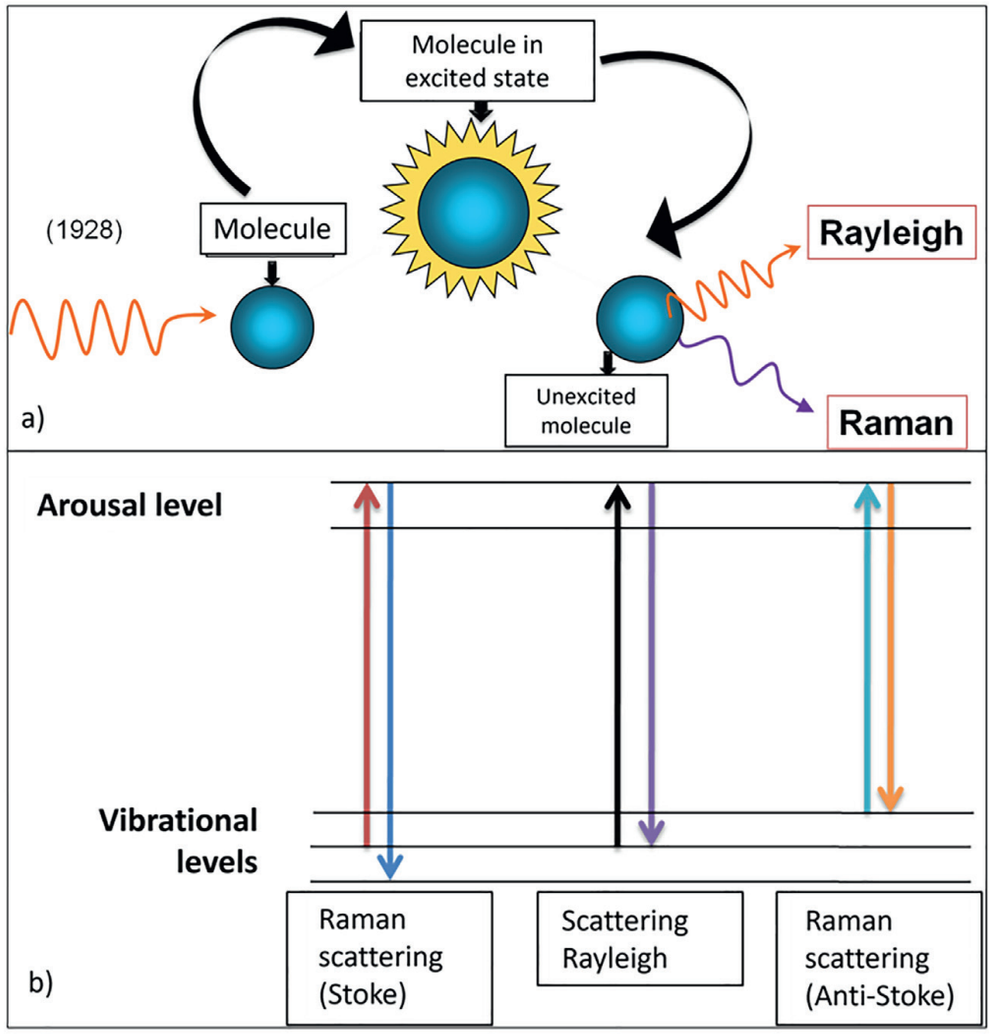


**Figure 1.**
*(a) Representative diagram of the biphotonic effect on a molecule and the two resulting dispersions (elastic and inelastic), and (b) diagram of the energy levels. Direct source.*

(leukocytes), this *in vitro* and more recently *in vivo* in studies of premalignant lesions in the chest and atherosclerotic plaques [15, 16].

### 7.1 Raman spectroscopic investigations

Malignant or diseased tissue is known to provoke cellular and biochemical changes in an organism, also known as tumor markers, and these changes also alter vibrational spectra. The spectrum of healthy and diseased tissue can be compared using Raman spectroscopy, which is highly specific and sensitive compared with other spectroscopic analysis in the biomedical field. This is not limited to diagnosing external tumors, but by employing miniature fiber optical probes, the technique can be used to diagnose pathologies in the oral cavity, gastrointestinal tract, and brain and ocular tissue. Other advantages of Raman spectroscopy are that the technique is non-invasive, non-destructive, uncomplicated, and the results obtained can be replicated and require minimal or no sample preparation. Applications are not only limited to soft tissues as extended research has been carried out to characterize and diagnose hard tissue pathologies such as normal and diseased bones and teeth tissues.

## 8. Applications of Raman spectroscopy

In the beginning, the major drawbacks of Raman spectroscopy were the fluorescence from biological tissues and the lack of sensitive instruments. Recent advances have made this technique popular due to its advantages over IR spectroscopy, such as its minimal water interference and sample preparation. IR spectroscopy requires the sectioning of samples into a specific size, which can lead to spectral changes in the chemical characterization, and this is not necessary in Raman spectroscopy where the samples can be tested without modifying their state. Modifications in Raman spectroscopy imaging have been made to characterize specific areas of interest in the medical and dental field [3]; all aimed to determined specific peaks in the spectrum, which are used as fingerprints to distinguish diseased tissue from healthy tissue.

Raman spectroscopy can also be applied to the study of milk composition as an easy, non-destructive, and fast method to determine the quality of the nutritional components found in dairy products, such as proteins, fats, fatty acids (Fas), and lactose. This makes it easy to provide consumers reliable nutritional information. [16].

Raman spectroscopy is a vibrational spectroscopy with a number of useful properties (nondestructive, no-contact, high molecular-specificity, and robustness) that make it particularly suited for PAT applications in which molecular information (composition and variance) is required. There are important applications of Raman analysis in the production of bio-pharmaceuticals, such as the characterization of raw materials, cell culture media preparation, real-time bioprocess monitoring, and the analysis of the macromolecule product,, the manufacturing and analysis of biopharmaceuticals, for example, in the biopharmaceutical production process such as Raw Materials, Cell Culture Media, Bioprocess, Protein Product, formulated medicine [17].

Raman analysis has proven to be a useful tool for the examination of synthetic and biological materials, in the medical field to study hard tissue, DNA, and white blood cells. The technique has been commonly used as a result of the development of lasers and CCD detectors, which make spectrum analysis even faster.. More recently, MRS has also been used *in vivo* to study pre-malignant lesions in breast and atherosclerotic plaques [18, 19].

There are physical characterizations of dental enamel such as microhardness, roughness, wettability, zeta potential, compressive, and flexural strength, which provide important data, but chemically, Raman is the test that complements this chemical characterization by identifying the functional groups.

## 9. Raman on dental enamel

In 1971, the Raman technique began to be used to identify the functional groups in mineral compounds and it was not until 1993 that it began to be used in dental research, informing about the fluorescence problems of biological materials. Tsuda [6] mentions that $CaF_2$ can be identified in a shift of 322 $cm^{-1}$ in dental enamel when it has a loss of mineralization and different concentrations of fluorides are applied to the sample to reduce mineral loss; however in this investigation, the spectra were acquired in an interval of 400–4000 $cm^{-1}$, having this as a limitation for the detection of $CaF_2$. Tsuda [2] in another article mentions the use of pure hydroxyapatite crystals and dental enamel to determine the orientation of the prisms longitudinally and transversally [20].

In dentistry, natural apatites are studied, with calcium phosphates, which by adding an $OH^-$ group form hydroxyapatite (HA), an F- form fluorapatite (FA), a $Cl^-$ and form chlorapatite (CA); the first two are found in bone, dentin, and enamel and thanks to this characterization technique, we can observe each of these groups expressed in the bands. [17, 18].

When talking about functional groups of dental enamel, the hydroxyl groups should be located:

## 9.1 Hydroxyl group on Raman spectroscopy

When the concentration is increased the intensity of the band decreases, this is due to the hydroxyl stretching vibration. Additional broader bands appear at lower frequencies 3580–3200 cm $^{-1.}$ The appearance of these bands is due to intermolecular bonding, which also increases when the concentrations rise. The precision of the O-H band is dependent on the strength of the hydrogen bond. In some samples, intramolecular hydrogen bonding may occur, the resulting hydroxyl group band which appears at 3590–3400 cm $^{-1}$ being sharp and unaffected by concentration changes [21].

For solids, liquids, and concentrated solutions, a broad band is normally observed at about 3300 $cm^{-1}$. Overtone bands of carbonyl stretching vibrations also occur in the region 3600–3200 cm –1 but are, of course, of weak intensity. Bands due to N-H stretching vibrations may also cause confusion. However, these bands are normally sharper than those due to intermolecular hydrogen-bonded o-H groups. Particularly on dental enamel, the hydroxyl group in Raman spectra appears in the vibration range 3510–3650 $cm^{-1}$, when speaking of it in a clinical context [22]. When the band is observed in this vibration range, it can be translated that when the tooth is in the oral cavity it is healthy, and when the intensity of the band of this group is observed diminished, we can infer that there was an acid attack on the dental enamel that What is clinically weakening it is known as incipient caries lesion, and if that enamel is not remineralized, dental caries can appear.

## 9.2 Organic phosphate compounds on Raman spectroscopy

### *9.2.1 P-O-C vibrations*

For aliphatic compound, the asymmetric stretching vibration of the P-O-C group gives a very strong broad, normally found in the region 1050–970 $cm^{-1}$. In the case of pentavalent and trivalent methoxy compounds, this band is sharp and strong, occurring at 1090–1010 $cm^{-1}$. In general, the band occurs at lower frequencies than that for the trivalent compound due to the asymmetric stretching vibration of the P-O-C group of pentavalent phosphate.

### *9.2.2 P=O vibrations*

The band is strong due to the stretching vibration of the P=O group and in the region of 1350–1150 $cm^{-1}$. Taking into account the size of the phosphate atom, the frequency of the P=O stretching vibration is almost independent from the type of compound in which the group occurs and from the size of the substituents. However, it is governed by the number of electronegative substituents directly bonded to it, as well as its sensitivity to the association effects [22].

Particularly in dental enamel, the spectra of the Raman phosphate group appear in the vibration range 1100–900 $cm^{-1}$, where we can locate them.

Clinically, the presence of the high intensity of this band speaks of a good saturation of the dental enamel. In contrast, a weak band, slightly outside the range, speaks of a loss in the mineralization of the dental enamel, also known as incipient caries lesion or loss of continuity of dental enamel and as consequence of cavitation, that is, caries.

**Figure 2** shows the characteristic bands of synthetic HA where in the vibration range 1100–900 $cm^{-1}$ we can identify the group $PO_4^{3-}$ (955.9 $cm^{-1}$) in its "strong" expression and in the vibration range 3510–3650 $cm^{-1}$ is the $OH^-$ group (3567.1 $cm^{-1}$).

A cluster of minerals containing phosphate is known as apatite, and Ha is known as calcium (Ca) apatite in mineral form and is the phosphate that most resembles the bone phosphate complex. Different salts of calcium phosphate (CaP) are summarized in **Table 1** [23].

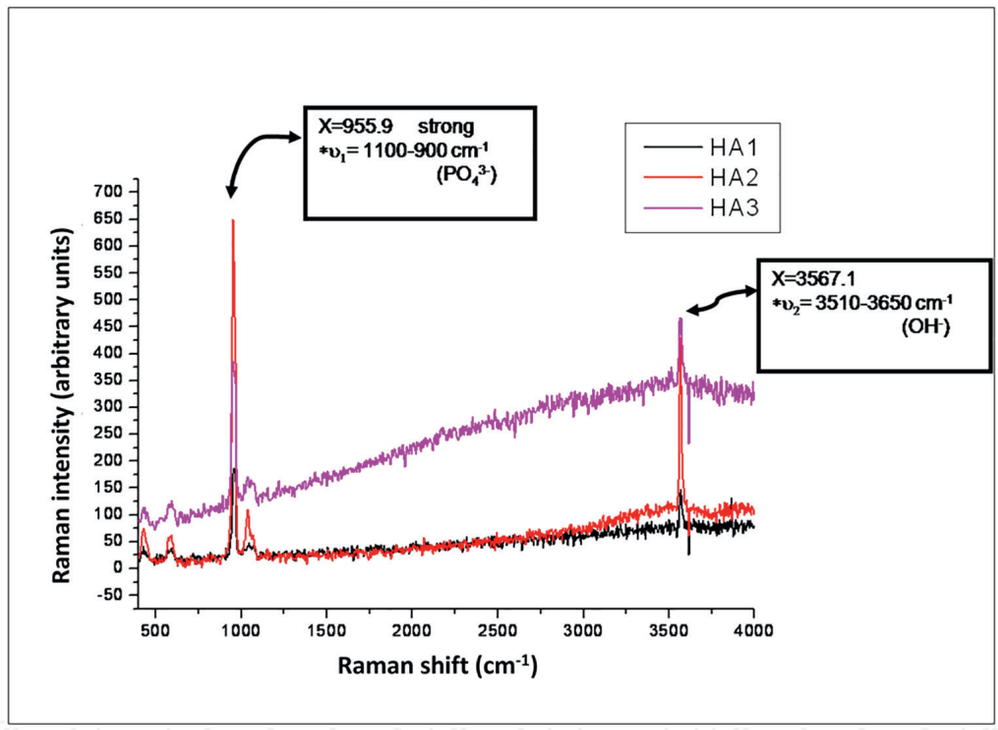


**Figure 2.**
*Spectra of synthetic hydroxyapatite (HA) with the characteristic bands of the $PO_4^{3-}$ group and the $OH^-$ group. Direct source.*

| Name | Symbols | Chemical Formula | Ca/P ratio |
|---|---|---|---|
| Tetracalcium phosphate | TTCP | $Ca_4 (PO_4)_2O$ | 2.0 |
| Hydroxyapatite | HA or HAp | $Ca_4 (PO_4)_6 (OH)_2$ | 1.67 |
| Fluoroapatite | FA or FAp | $Ca_4 (PO_4)_6F_2$ | 1.67 |
| Oxyapatite | OA or OAp | $Ca_4 (PO_4)_6O$ | 1.67 |
| b Tricalcium phosphate | b-TCP | $Ca_3 (PO_4)_2$ | 1.5 |
| a Tricalcium phosphate | a-TCP | $Ca_3 (PO_4)_2$ | 1.5 |
| Octacalcium phosphate | OCP | $Ca_8 (HPO_4)_2 (PO_4)_4 5H_20$ | |

**Table 1.**
*Different salts of calcium phosphate.*

## 10. Conclusions

Currently, in the implementation of different biomaterials in tissue engineering, the characterization with μ-Raman has been very useful since it does not structurally modify the sample, whether of synthetic or biological origin, and it can also be measured in any state of aggregation.

In Dentistry, it has become an excellent chemical characterization of enamel and dentin with various treatments used for mineral recovery, which is clinically known as remineralization.

Hydroxyapatite is a biological material of great interest within the apatite family, as it is a component of bones and teeth, by using m-Raman it is possible to identify the characteristic vibrational modes of the $PO_4^{3-}$ group and of the $OH^-$ group since the sample is not modified in its structure.

In studies where tooth enamel is demineralized or re-mineralized, characterized by means of m-Raman spectroscopy it can be concluded whether fluorinated compounds are useful or not.

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## Conflict of interest

The author declares no conflict of interest.

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## References

[1] Sa Y, Feng X, Lei C, Yu Y, Jiang T, Wang Y. Evaluation of the effectiveness of micro-Raman spectroscopy in monitoring the mineral contents change of human enamel in vitro. Lasers in Medical Science. 2017;**32**(5):985-991

[2] Tsuda H, Arends J. Orientational Micro-Raman Spectroscopy on Hydroxyapatite Single Crystals and Human Enamel Crystallities. Journal of Dental Research. 1994;**73**(11):1703-1710

[3] Barrera-Ortega CC, Vázquez-Olmos AR, Sato-Berrú RY, Araiza-Téllez MA. Study of demineralized dental enamel treated with different fluorinated compounds by Raman spectroscopy. Journal of Biomedical Physics and Engineering. 2020;**10**(5):635-644

[4] Shin WS, Li XF, Schwartz B, Wunder V, Baran GR. Determination of the degree of cure of dental resins using Raman and FT-Raman spectroscopy. Dental Materials. 1993;**9**:317-324

[5] Requena A, Zúniga J. Espectroscopia. Madrid: Pearson Educación; 2005

[6] Tsuda H, Arends J. Raman spectroscopy IN dental research: A short review of recent studies. Advances in Dental Research. 1997;**11**(4):539-547

[7] Gutiérrez M, Reyes J. Microhardness and chemical composition of human tooth. Materials Research. 2003;**6**:367-373

[8] Visscher M, Struik KG. Optical profilornetry and its application to mechanically inaccessible surfaces part I: Principles of focus error detection. Precision Engineering. 1994;**3**:16

[9] Mullane O, Baez R, Jones S, Lennon M, Petersen P, Rugg A. Fluoride and Oral health. Community Dental Health. 2016;**33**:69-99

[10] Tiznado-Orozco GE, Reyes-Gasga J, Elefterie F, Beyens C, Maschke U, Bres EF. Wettability modification of human tooth surface by water and UV and electron-beam radiation. Materials Science and Engineering: C. 2015;**57**:133-146

[11] Navarro M, Planell JA. Nanotechnology in regenerative medicine. In: MaazouZ Y, Aparicio DYC, editors. Measuring Wettability of Biosurfaces at the Microsurfaces. s.l. ed. USA, New Jersey: Springer Science+Business Media; 2012. pp. 163-172

[12] Carvalho RB, Medeiros UV, dos Santos KT, Pacheco Filho AC. Influência de diferentes concentrações de flúor na água em indicadores epidemiológicos de saúde/doença bucal. Ciência & Saúde Coletiva. 2011;**16**:3509-3518

[13] Arifa MK, Ephraim R, Rajamani T. Recent advances in dental hard tissue remineralization: A review of literature. International Journal of Clinical Pediatric Dentistry. 2019;**12**:2

[14] Gonçalves FMC, Delbem ACB, Gomes LF, Emerenciano NG, Pessan JP, Romero GDA, et al. Effect of fluoride, casein phosphopeptide-amorphous calcium phosphate and sodium trimetaphosphate combination treatment on the remineralization of caries lesions. An in vitro study. Archives of Oral Biology. 2021;**122**:10

[15] Barrera-Ortega CC, Araiza-Tellez MA, García-Perez A. Assessment of enamel surface microhardness with different fluorinated compounds under pH cycling

conditions: An in vitro study. Journal of Clinical and Diagnostic Research. 2019;**13**(8):ZC05-ZC10

[16] He H, Sun D-W, Pu H, Chen L, Lin L. Applications of Raman spectroscopic techniques for quality and safety evaluation of milk: A review of recent developments. Critical Reviews in Food Science and Nutrition. 2019;**59**:770-793

[17] Buckley K, Ryder A. Applications of Raman spectroscopy in biopharmaceutical manufacturing: A short review. Applied Spectroscopy. 2017;**71**(6):1085-1116

[18] Gilchrist F, Santini A, Harley K, Deery C. he use of micro-Raman spectroscopy to differentiate between sound and eroded primary enamel. Journal of Paediatric Dentistry. 2007;**17**:274-280

[19] Penel G, Leroy G, Sombret B, Huvenne J, Bres E. Infrared and Raman microspectrometry study of fluor-fluor-hydroxy and hydroxy-apatite powders. Journal of Dental Matrials. 1997;**8**:271-276

[20] Kirchner M, Edwards H. Ancient and modern specimens of human teeth o Fourier transform Raman spectroscopic study. Journal of Raman Spectroscopy. 1997;**28**:171-178

[21] Chen J, Yu Z, Zhu P, Wang J, Gan Z, Wei J, et al. Effects of fluorine on the structure of fluorohydroxyapatite: A study by XRD, solid-state NMR and Raman spectroscopy. Journal of Materials Chemistry B. 2015;**3**(1):34-38

[22] Socrates G. Infrared and Raman Characteristics Group Frequencies. Tables and Charts. 3TH ed. London, United Kingdom: John Wiley & Sons, Ltd;

[23] Munir MU, Salman S, Ihsan A, Elsaman T. Synthesis, characterization, functionalization and bio-applications of hydroxyapatite nanomaterials an overview. Journal of Nanomedicine. 2022;**17**:1903-1925

[24] Bona GA, Bidlack F. Tooth enamel and its dynamic protein matrix. International Journal of Molecular Sciences. 2020;**21**:12