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Tracking trace chemical alterations in biogenic apatite – improvements in tooth sample preparation for experimental approach

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Abstract

Studying the properties of hard tissues, such as bones or teeth, often requires an experimental approach that enables the mechanisms observed in clinical settings to be explained or supports the safe planning of clinical trials. This paper compiles some methodological insights on the proper preparation of biogenic apatite found in human teeth for *in vitro* studies. These insights were gathered through experimental work and a review of some literature related to *in vitro* studies on the impact of metal orthodontic appliances on the chemical and crystallographic properties of dental enamel.

Keywords: experimental mineralogy, *in vitro* study, enamel, apatite supergroup minerals, dental

The unique properties of the minerals within the apatite supergroup (Pasero, Kampf, Ferraris, Pekov, Rakovan, & White, 2010) make them components of substantial economic and scientific significance, as well as fundamental, evolutionarily selected building blocks of hard tissues such as bones and teeth (Ptáček, 2016). The structure of these minerals, represented by the general formula $X_{10}(YO_4)_6Z_2$, where *X* may include elements such as Ca, Pb, or Sr; *Y* may represent P, V, or As; and *Z* may correspond to OH, Cl, F, or I, permits extensive elemental substitution at both cationic and anionic sites (White & ZhiLi, 2003). These substitutions directly impact the physicochemical characteristics of the supergroup members, thereby influencing their stability and extending their applications across fields such as geology, palaeontology, environmental engineering, and medical sciences (Drouet, 2015, 2019).

Bioapatite constituting human tooth enamel is located in the oral cavity, an environment that exhibits considerable variability in elemental composition due to the diversity of dissolved substances (Rosier, Marsh, & Mira, 2018). Consequently, chemical modifications in this biogenic mineral occur continuously throughout human life and may be associated with various diseases, the etiology of which is not yet fully understood (Curzon & Crocker, 1978; Fischer, Wiechula, & Przybyla-Misztela, 2013; Zantner, Martus, & Kielbassa, 2006, Kidd & Fejerskov, 2004)). However, both the complicated structure of dental tissues and the current limitations of analytical techniques restrict the ability to perform *in situ*, *in vivo* studies on changes in the trace elemental composition of dental enamel. Examination typically would require the removal of the tooth, thus impeding a full understanding of certain processes over time.

Therefore, while clinical studies are foundational to medical research, experimental methodologies remain essential in specific contexts (Hellak, Riepe, Seubert, & Korbmacher-Steiner, 2015; Ten Cate, Timmer, Shariati,

& Featherstone, 1988; Topolska et al., 2024; Vitkov et al., 2008). In some instances, hybrid approaches have been proposed to tackle challenges specific to the enamel layer of teeth. In a hybrid approach, enamel samples from extracted teeth are temporarily placed in the oral cavity of selected participants in a clinical trial or experiment (Hannig, 1999). Both *in vitro* and mixed approaches present particular challenges, not only in accurately simulating the chemical environment but also in the meticulous preparation of tissue samples—a task demanding advanced expertise in crystallography and experimental mineralogy. Presented below are several key considerations that have emerged from recent studies on the chemical alteration of human enamel (Topolska et al., 2024), offering valuable insights for future research in this area.

Firstly, it is essential to recognize that a tooth consists of several biological layers, each of which is characterized by a different structure, composition, and therefore different chemical reactivity or ability to sorption and diffusion of ions (e.g.: LeGeros, 2008). The enamel, predominantly composed of biohydroxylapatite (>90 wt%), borders directly on dentine, whose composition is notably more complex. The latter includes considerable amount of carbonate, substitutions in the hydroxylapatite and significant organic matter content (Angker, Nockolds, Swain, & Kilpatrick, 2004; Guede et al., 2017; Shellis, 1996). In contrast, the root and its surrounding cementum exhibit distinct characteristics. Furthermore, the thickness and percentage of each of the biological layers of the tooth varies depending on the type of tooth and the individual characteristics of the donor (Sarna-Boś et al., 2023). Therefore, prior to initiating *in vitro* studies, it is essential to carefully select the specific layer to be examined, as the effectiveness of experimental and analytical preparation methods may vary depending on the part of tooth. Additionally, a detailed description of the layer isolation procedure should be incorporated into the experimental methodology, as this critical step is often overlooked in research protocols (e.g.: Ghadimi et al., 2013).

Secondly, the crystallographic analyses of apatite in dental enamel have demonstrated that unit cell parameters, elemental composition, crystal size, and the spatial arrangement of crystals within the enamel matrix vary significantly depending on the tooth region and kind (Aljawad et al., 2007; Beniash et al., 2019). This variability implies that enamel samples obtained as randomly excised fragments, as used in certain experimental setup, may exhibit inconsistent chemical reactivity even when of identical dimensions (Hannig, 1999; Hellak et al., 2015). Thus, it is advisable to employ as enamel samples, whole teeth of a similar type, or at a minimum, use hemisected specimens formed by sectioning in the buccal-lingual plane for enhanced experimental consistency.

Furthermore, careful consideration must be given to the preparation of enamel (but also other teeth layers) for chemical composition analysis via methods such as Inductively Coupled Plasma (ICP) or Atomic Absorption Spectroscopy (AAS). Both methods necessitate the mineralization of a pre-powdered solid sample. Given the previously mentioned heterogeneity in enamel structure, it is crucial to pulverize the entire enamel layer of the analysed tooth, homogenize it thoroughly, and proceed with the mineralization process only afterward. Merely powdering the outer enamel layer using small drilling tools, such as high-speed drills with diamond burs commonly used in mineral samples preparation (Branscombe, Lee-Thorp, Schulting, & Leng, 2022), may yield results that do not accurately reflect the overall composition.

Lastly, the material composition of tools employed to powder enamel samples deserves attention. Although it may seem elementary, certain tools marketed as “diamond” are actually composed of metal alloys (Topolska, & Kozub - Budzyń, 2024) which may introduce contamination into the sample, potentially skewing analytical results. Based on these considerations, it seems reasonable to remove parts of not analysed tooth layer entirely by pulverizing them with a durable bur and the intact layer designated for chemical analysis rinse with concentrated nitric acid and pulverize in an agate mortar to prevent contamination (Topolska et al., 2024).

Although the structure and the composition of teeth have been studied for several decades and the general characteristics of this tissue are well-established, advancements in analytical and medical methods, alongside a deeper understanding of properties of minerals from apatite supergroup and biomineralization processes, continue to uncover new areas for exploration. Based on the considerations outlined in this paper, it can be concluded that future experimental setups should: (i) focus on a specific tooth biolayer for experimentation, as these layers exhibit significant differences in structure and properties, and (ii) aim to comprehensively examine the entirety of the selected layer, given its anisotropic nature with respect to both structure and properties. Furthermore, due to the hardness of apatite supergroup minerals, their sorption characteristics, and crystallographic structure that is prone to substitutions, potential contamination from solutions and from tools used in powdering or grinding must be carefully considered during experimental design. Ideally, this should involve assessing the chemical reactivity and composition of the tools to mitigate such risks.

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Statement about the conflicts of interest

There are no conflicts of interest.

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