

# Surface Study of Selected Biomaterials Using Vibrational Spectroscopy

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Vibrational spectroscopy has been extensively used for *in vitro* and *in vivo* investigations of degradation mechanism and kinetics of different biomedical materials as well as it has been used to characterize the crystalline and amorphous domains in bio-mineralization process. Infrared and Raman spectroscopy methods are valuable tools in the biomaterials engineering allowing to study processes occurring during their preparation. *In vitro* tests, where the materials are immersed in simulated body fluids and/or artificial saliva, were used to evaluate the biocompatibility of biomaterials. This kind of tests are a wide range of repeatable and reproducible methods, which are regulated by international standards for commercial use and scientific development of new materials and products. The aim of this work was to examine phase composition of materials applied in dentistry. The bioactivity of such biomaterials was studied by immersing the samples in synthetic body fluid and artificial saliva. The changes were determined by the Fourier transform infrared and Raman microspectroscopy as well as scanning electron microscopy. It was found that results obtained by vibrational spectroscopy show the differences between the studied samples. Chemical reactions occurring during incubation of cements in artificial saliva as well as in synthetic body fluid result in formation of phosphates which deposit on the cement surface.

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## 1. Introduction

Dental prevention has become a very important issue in recent years and therefore special attention has been paid to development of dentistry materials. Such a situation has created a commercial market that offers various modified dental materials. A large group of such materials consists of cements which can be classified based on their composition [1]. In order to analyze them, various techniques have been already applied. They include: scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), energy dispersive X-ray analysis (EDX), and atomic force microscopy (AFM) [2–6].

Since dental materials are exposed to action of saliva, special attention has been paid to their behavior in this medium. For example, changes of physical parameters of glass-ionomer cements upon action of artificial saliva and bacteria have been noticed [7, 8]. Also, corrosion effects of dental alloys have been observed after interaction with artificial saliva containing various concentrations of hydrogen peroxide and fluoride ions [9].

The aim of this work was to examine phase composition of biomaterials such as cements applied in dentistry. The bioactivity of this kind of materials was studied by immersing the samples in synthetic body fluid (SBF) and artificial saliva.

## 2. Materials and methods

For the present study, two different dentistry cements: carboxylic and phosphate, commonly used by dentists, were selected. The cement samples were prepared by mixing the supplied powders and liquids as recommended by the producers. In the case of both cements, the powder contained mainly zinc oxide whereas the liquids were composed of acrylic acid and phosphoric acid in carboxylic and phosphate cements, respectively. After hardening in air, the samples were formed into cubes of 27 mm<sup>3</sup> in volume. Some of them were incubated at 37°C for up to 25 days in SBF and the other ones in artificial saliva. Every day the samples were transferred into fresh solutions in order to avoid bacteria development.

Artificial saliva of pH = 5 was prepared according to the procedure described by Li and Zhou [10]. It contained 0.4 g NaCl, 0.4 g KCl, 0.795 g CaCl<sub>2</sub> · 2H<sub>2</sub>O, 0.78 g NaH<sub>2</sub>PO<sub>4</sub> · 2H<sub>2</sub>O, 0.005 g Na<sub>2</sub>S · 9H<sub>2</sub>O, 1.0 g NH<sub>2</sub>CONH<sub>2</sub> in 1 liter of distilled water.

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SBF of pH = 7.4 was composed of:  $\text{NaHCO}_3$  — 0.35 g,  $\text{NaSO}_4$  — 0.07 g,  $\text{KCl}$  — 0.29 g,  $\text{K}_2\text{HPO}_4$  — 0.17 g,  $\text{CaCl}_2$  — 0.28 g,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  — 0.30 g,  $\text{NaCl}$  — 7.99 g,  $\text{TRIS}(\text{C}_4\text{H}_{11}\text{NO}_3)$  — 6.05 g according to Kokubo [11].

All the samples were analyzed by FTIR, the Raman spectroscopy, SEM, and EDX methods.

The FTIR measurements of the samples were performed using transmission and reflection techniques. In all cases,  $4\text{ cm}^{-1}$  resolution was applied. The spectra were collected using Bio-Rad Excalibur and Bruker Equinox 55 spectrometers in the middle infrared region. The Bruker Hyperion 3000 microscope was used with  $15\times$  objective and liquid nitrogen cooled mercury cadmium telluride detector.

The Raman spectra were measured using the Renishaw spectrometer with confocal microscope Leica DMLM equipped with low level noise CCD camera. The samples were excited with argon-ion ( $514.5\text{ nm}$ ) laser.

SEM and EDX investigations were performed using a Phillips XL 30 scanning electron microscope equipped with a LINK ISIS-EDX analyzer.

### 3. Results

The study was divided into two parts. The first one was devoted to the analysis of carboxylic cement and the second one to the phosphate cement.

In case of carboxylic cement the results of the FTIR study are presented in Fig. 1. Spectrum (a) shows characteristic bands belonging to the main components of the carboxylic cement. They include: the intensive bands in the range of  $420\text{--}550\text{ cm}^{-1}$  characteristic of stretching Zn–O bond vibrations and the bands in the range of  $1300\text{--}1700\text{ cm}^{-1}$  originating from the bonds present in carboxylic acid.

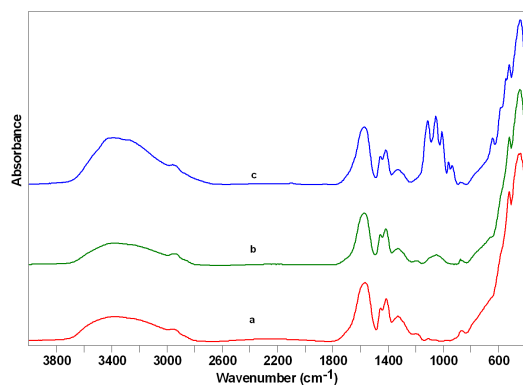


Fig. 1. FTIR spectra of carboxylic cement: (a) initial cement sample, (b) cement sample after incubation in SBF, (c) cement sample after incubation in artificial saliva.

Apart from the bands corresponding to carboxylic acid, in the spectra of cement samples after incubation additional bands in the range of  $950\text{--}1150\text{ cm}^{-1}$  appear. They are more intense in the spectrum of the cement

incubated in saliva solution (Fig. 1c). These bands can be assigned to stretching vibration of phosphate groups occurring in  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  and  $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$  [12].

In order to verify FTIR results, the Raman spectra of the same samples were recorded. Figure 2 presents the Raman spectra in the same convention as FTIR spectra presented in Fig. 1. Spectrum (a) contains a characteristic band at  $437\text{ cm}^{-1}$  which is due to Zn–O bond and the bands in the region of  $2800\text{--}3000\text{ cm}^{-1}$  which originate from C–H stretching vibrations of carboxylic acid. As one can see, spectrum (b) is very similar to spectrum (a), while spectrum (c) looks very different due to appearance of a strong band at  $1000\text{ cm}^{-1}$  which can be assigned to stretching vibrations of  $\text{PO}_4^{3-}$  group present in  $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$  and  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  [12].

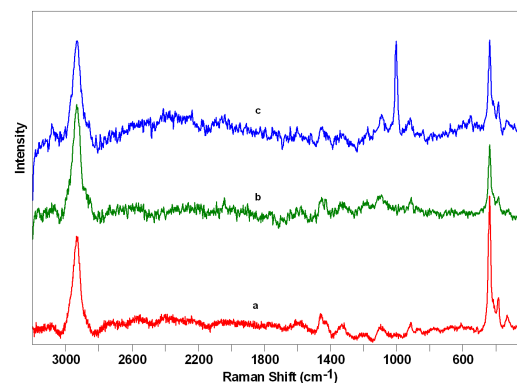


Fig. 2. Raman spectra of carboxylic cement: (a) initial cement sample, (b) cement sample after incubation in SBF, (c) cement sample after incubation in artificial saliva.

SEM imaging was performed to analyze the surface of the samples. As shown in Fig. 3, there are significant differences in the morphology of the carboxylic cement before and after incubation in the solutions studied. Moreover, EDX analysis indicated different elemental composition of these samples. In case of cement incubated in SBF and artificial saliva peaks of P and Ca, not present in the EDX spectra of the initial cement, are visible. It should be noted that Zn peaks are observed in all EDX spectra. This confirms FTIR and the Raman results which show formation of phosphate phases on the surface of the samples.

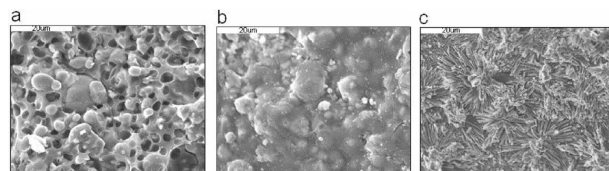


Fig. 3. SEM images of carboxylic cement: (a) initial cement sample, (b) cement sample after incubation in SBF, (c) cement sample after incubation in artificial saliva.

In case of phosphate cement the results of the FTIR study are presented in Fig. 4. Spectrum (a) shows characteristic bands belonging to the main components of the phosphate cement. They include: the intensive bands in the range of  $420\text{--}550\text{ cm}^{-1}$  characteristic of stretching Zn–O bond vibrations (the same as in case of carboxylic cement) and the bands in the range of  $800\text{--}1500\text{ cm}^{-1}$  originating from the bonds present in phosphate group. Spectrum (b) originates from phosphate cement after incubation in SBF solution and it does not show any difference from spectrum (a). The following spectrum (c) corresponds to phosphate cement after incubation in artificial saliva solution. In comparison to spectrum (a) and (b) the shape of the most intense band at around  $1000\text{ cm}^{-1}$  is more complex. It indicates the appearance of additional phosphate (apart from  $\text{Zn}_3(\text{PO}_4)_2$ ). This complex band can be assigned to stretching vibration of phosphate groups occurring in  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  [12].

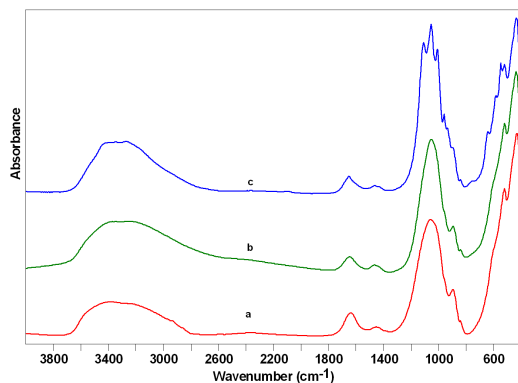


Fig. 4. FTIR spectra of phosphate cement: (a) initial cement sample, (b) cement sample after incubation in SBF, (c) cement sample after incubation in artificial saliva.

Also in the case of phosphate cement the Raman technique has been applied. The spectra of the same samples were measured. Figure 5 presents the Raman spectra in the same convention as FTIR spectra presented in Fig. 4. Spectrum (a) contains characteristic bands in the region of  $200\text{--}600\text{ cm}^{-1}$  which can be ascribed to ZnO and MgO bonds. As is seen, the spectrum of the sample incubated in SBF (spectrum (b)) is very similar to the spectrum corresponding to the initial cement (spectrum (a)). In contrast, the spectrum of the sample incubated in artificial saliva (spectrum (c)) contains the intense band at  $1000\text{ cm}^{-1}$  which can be assigned to stretching vibrations of  $\text{PO}_4^{3-}$  group in  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  and  $\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ .

Similarly to the case of carboxylic cement, SEM imaging was performed to analyze the surface of the phosphate cement. As shown in Fig. 6, there are significant differences between the initial and modified phosphate cement. Moreover, EDX analysis indicated different elemental compositions of these samples. In case of cement incubated in artificial saliva significant change of both, surface morphology and elemental composition occurred.

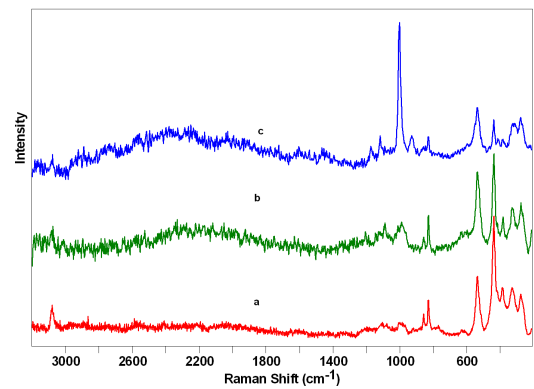


Fig. 5. Raman spectra of phosphate cement: (a) initial cement sample, (b) cement sample after incubation in SBF, (c) cement sample after incubation in artificial saliva.

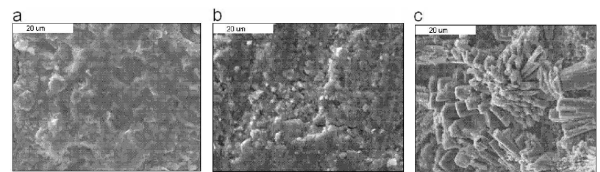


Fig. 6. SEM images of phosphate cement: (a) initial cement sample, (b) cement sample after incubation in SBF, (c) cement sample after incubation in artificial saliva.

Strong peaks of Zn and P in all EDX spectra were detected while Ca strong peak appeared only in case of incubation in artificial saliva. This confirms FTIR and Raman results which show formation of new kind of phosphate phases on the surface of the samples.

#### 4. Conclusions

FTIR and Raman spectroscopies applied to study dentistry cements are very sensitive techniques that deliver information about chemical composition of the analyzed biomaterials.

The results obtained show differences between the analyzed samples of dentistry cements. Incubation of cements in artificial saliva as well as in synthetic body fluid results in the deposition of phosphates on the cement surface induced by chemical reactions taking place in the solutions. Formation of phosphates is advantageous for biocompatibility of these materials.

EDX analysis presented in this study confirm the results obtained by FTIR and Raman spectroscopy methods. SEM images of microstructure show significant differences of cement surfaces after incubation which indicates the cement solution interaction.

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