

A SIMPLE METHOD TO EVALUATE THE PURITY OF SYNTHETIC HYDROXYAPATITE GRANULES

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Using a method based on the spectrophotometric analysis of the liquids remaining after washing ceramic granules, the type of impurity was determined, the wavelength for measuring the impurity was established, and the type of cell (polystyrene or quartz) was analyzed. The absorbance of the samples was measured at wavelengths between 190 and 1100 nm. The results showed that both cuvette types are useful; however, the greatest absorbances were detected using quartz ones at low wavelengths around 190 nm. The comparison of absorption spectra from the samples, centrifuged and non-centrifuged, enabled us to define the impurities as small apatite particles.

Keywords: absorption spectrum, bone regeneration, hydroxyapatite, spectrophotometry.

МЕТОД ОЦЕНКИ ЧИСТОТЫ СИНТЕТИЧЕСКИХ ГИДРОКСИАПАТИТОВЫХ ГРАНУЛ

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С использованием метода, основанного на спектрофотометрическом анализе жидкостей, остающихся после промывки керамических гранул, определены тип примесей, длина волны для измерения примесей и тип ячейки (полистирол или кварц). Поглощение образцов измерено на длинах волн 190—1100 нм. Показана возможность использования кювет обоих типов, однако наибольшее поглощение обнаружено при использовании кварцевых кювет при малых длинах волн (190 нм). Сравнение спектров поглощения центрифугированных и нецентрифугированных образцов позволило идентифицировать примеси как мелкие частицы апатита.

Ключевые слова: спектр поглощения, регенерация кости, гидроксиапатит, спектрофотометрия.

Introduction. Hydroxyapatite (HAP) is the most common ceramic material used for bone tissue regeneration. The preparation of this material requires controlling its purity at different stages of the synthesis process. This study demonstrates a fast and simple method to evaluate the content of impurities in raw hydroxyapatite. HAP granules have been widely used in regenerative medicine for almost 40 years. Their physicochemical features determine the material quality and have a great influence on the biological properties such as bioactivity and biocompatibility. Regardless of the HAP synthesis method, undesirable elements may occur, such as small apatite particles (apatite dust), chemical contaminants, phase impurities, etc. Due to the biomedical application of this material, it is very important to detect these impurities and define their type. As reported previously, hydroxyapatite made of small particles such as microparticles (MPs) and nanoparticles (NPs) may have a cytotoxic effect on human bone cells [1]. Molskin et al. [2] demonstrated also the effect of HAP NPs and MPs on human monocyte–macrophages (HMM). The most common methods applied to remove impurities are washing in distilled water and filtration [3, 4]. The purity evaluation of hy-

droxyapatite and other ceramic materials dedicated for bone regeneration usually includes Fourier transform infrared spectroscopy (FTIR) [5–7], Raman spectroscopy [8, 9], X-ray crystallography [10–12], scanning electron microscopy coupled with an energy dispersive X-ray detector (SEM-EDS) [13, 14], X-ray fluorescence [15, 16], and solid state NMR [17, 18]. These advanced techniques enable one to precisely detect chemical changes and phase shifts in the prepared materials. They are mostly dedicated for solid state materials, not for gas or liquids. However, there is a need to evaluate the HAP purity during the synthesis process by the analysis of the liquids.

In this study we propose a new, simple, and fast method for the measurement of HAP impurities by the analysis of the water employed for washing the final product. This fluid was examined by absorption spectrophotometry to detect impurities, excess of the chemical reagents used for the apatite synthesis, and small particles (micro- or nanoparticles) of HAP that can separate from larger granules. In the experiment, two types of cuvettes were tested, a cheap and easily accessible polystyrene cuvette and a quartz cuvette dedicated for UV. The next aim of the study was to determine the wavelength range suitable for the detection of HAP impurities. The results presented in this paper might be helpful for laboratories and companies producing HAP and other ceramic materials for biomedical application.

Materials and methods. HAP was prepared by the sol-gel method, sintered at 800°C for 2 h, and manually ground in a mortar to obtain granules of 0.2–0.3 mm. Ten grams of the resulting granules was soaked in a beaker with 1 L of MiliQ water, gently stirred, and separated from the mixture by decantation. A 10-mL amount of the post-incubation liquid was saved for spectrophotometric analysis. The washing procedure in MiliQ water was then repeated 14 times. After that, the absorbance of each sample was measured at a wavelength between 190 and 1100 nm using a Genesys 10S spectrophotometer (Thermo scientific, Madison, WI, United States). The step between the measurement wavelengths was set to 1 nm. Pure MiliQ water was used as a blank. Absorption spectra were collected for all samples with and without centrifuging in two types of cuvettes: disposable polystyrene cuvettes (Sarstedt, Numbrecht, Germany) and quartz UV-cuvettes (HELLMA, Optik GmbH Jena, Germany).

Results and discussion. The results of absorption spectrum analysis of non-centrifuged samples in quartz cuvettes were presented in the Fig. 1a. The highest absorption values in all samples were at 190 nm (up to 2.77 in sample 1). Rapid decrease in absorbance (from 2.77 to 2.18 in sample 1) was observed in a wavelength between 190 and 200 nm. Farther, in the range 200–1100 nm was observed constant decrease in absorbance. In a wavelength between 1000 and 1100 nm absorption values were insignificantly unstable.

The absorption spectra of samples centrifuged before measurement by spectrophotometry in quartz cuvettes were different and shown in the Fig. 1b. Generally, the absorption values were low for all samples and close to the zero in the range 300–1100 nm. The highest absorbance were observed between 190–195 nm; however, there was no dependence between the absorbance and washing repeats.

The results obtained for the same samples taken in polystyrene cuvettes were different. The absorption spectra of non-centrifuged samples were presented in the Fig. 2a. In a wavelength between 190 and 290 nm, the absorbance was unstable. Between 290 and 1100 nm the spectra had taken different shapes in different samples. The absorbance in sample 1 and 2 was high around 290 nm and then was observed constant decrease. In samples 3–15 spectrum could be divided into four parts: 190–290 nm, unstable absorbance; 290–420 nm, increase in absorbance; 420–450 nm, constant absorption rate; 450–1100 nm, decrease in absorbance.

The absorption spectra of samples centrifuged before measurement by spectrophotometry in polystyrene cuvettes shown in the Fig. 2b, were similar to those obtained in quartz cuvettes. Some absorbance was detected in the range 190–300 nm, however the results were unstable. In the range 300–1100 nm the absorption values were similar in all samples and close to the zero.

The exemplary absorbance values taken at 400 nm were presented in the Table 1. The absorbances measured after centrifuging were close to the zero in all 15 samples regardless of cuvette type. In samples non-centrifuged the absorbance is similar in plastic and quartz cuvettes. In the first sample the values are relatively high, and suddenly drop during next 2–3 washing. Starting from sample 6 the values do not exceed 0.1 (with one exception in sample 10). The comparison of absorbance from first five samples taken at 300, 350, and 400 nm was presented in the Table 2. It shows that the highest absorbance values were observed in quartz cuvette at 300 nm. It was also noticed that the highest differences between two types of cuvette among every sample were observed at 300 nm. For example, the absorbance in sample 1 were 1.602 in quartz and 1.337 in polystyrene cuvette.

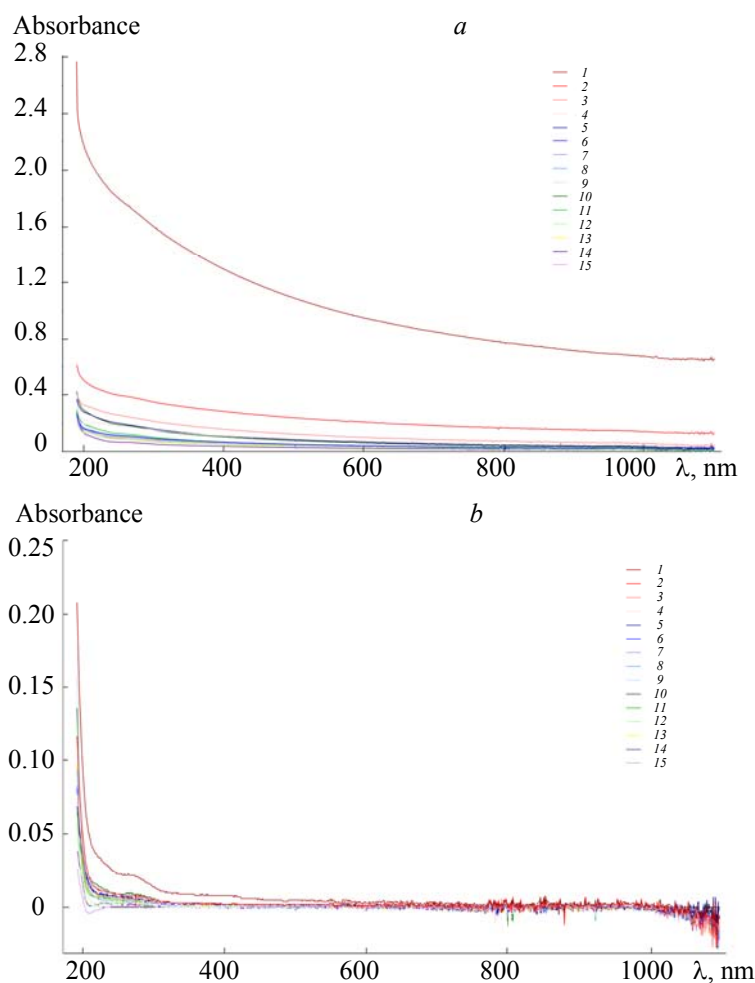


Fig. 1. Absorption spectra of 15 non-centrifuged (a) and centrifuged (b) samples measured spectrophotometrically in quartz cuvettes.

TABLE 1. The Absorbance of 15 Samples Measured Spectrophotometrically at 400 nm

Cuvette type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Samples non-centrifuged before measurement														
Quartz	1.298	0.283	0.158	0.112	0.111	0.066	0.059	0.066	0.061	0.103	0.067	0.047	0.052	0.036	0.048
Polystyrene	1.157	0.298	0.140	0.118	0.098	0.066	0.040	0.045	0.075	0.030	0.059	0.004	0.044	0.044	0.041
	Samples centrifuged before measurement														
Quartz	0.008	0.002	0.002	0.001	0.002	0.002	0.001	0.001	0.001	0.002	0.001	0.000	0.000	0.000	0.000
Polystyrene	-0.005	-0.005	-0.007	-0.007	-0.006	-0.005	-0.003	-0.005	-0.003	-0.004	-0.005	-0.005	-0.004	-0.005	-0.002

TABLE 2. The Absorbance of Samples 1–5 Measured Spectrophotometrically at 300, 350, and 400 nm

Cuvette type	λ , nm	1	2	3	4	5
Quartz	300	1.602	0.35	0.213	0.151	0.16
Polystyrene		1.337	0.303	0.118	0.092	0.067
Quartz	350	1.435	0.312	0.182	0.129	0.131
Polystyrene		1.246	0.306	0.136	0.111	0.089
Quartz	400	1.298	0.283	0.158	0.112	0.111
Polystyrene		1.157	0.298	0.14	0.118	0.098

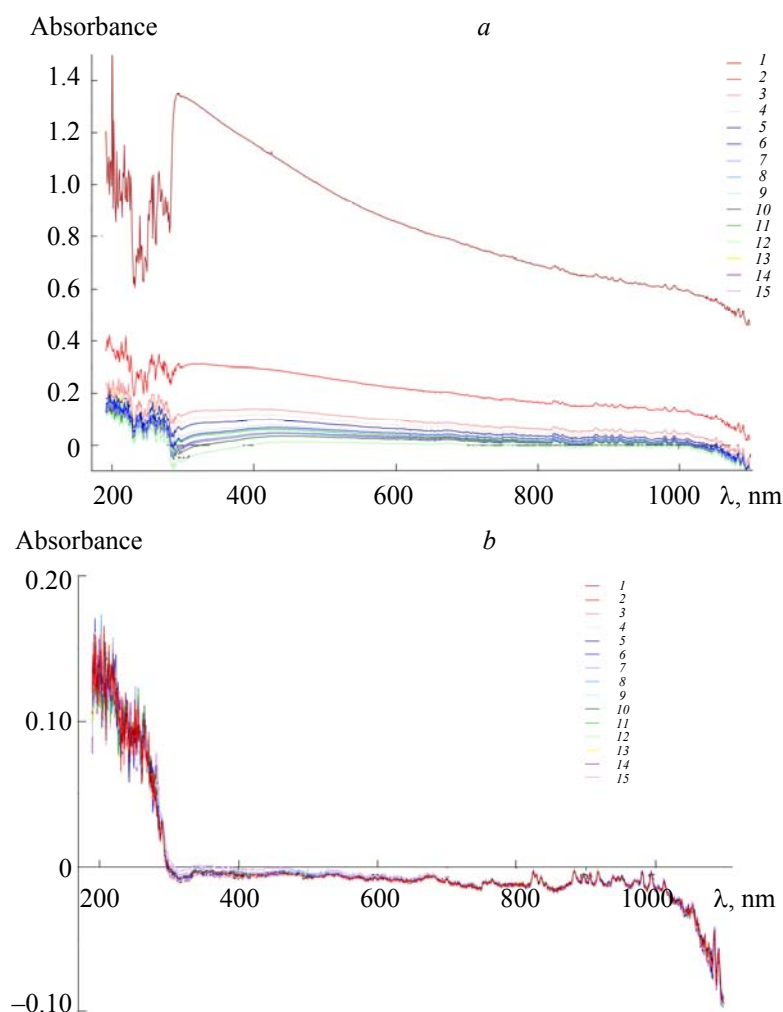


Fig. 2. Absorption spectra of non-centrifuged (a) and centrifuged (b) samples measured spectrophotometrically in polystyrene cuvettes.

Synthetic hydroxyapatite is a ceramic material intended for various biomedical applications, especially for bone tissue regeneration. Several methods of preparing HAP crystals have been utilized, including chemical precipitation, sol-gel, hydrothermal technique, electrodeposition, and biomimetic deposition [19, 20]. Regardless of the method, the preparation of HAP has to follow specific criteria, such as the composition, reproducibility, and purity of the material [21]. As reported before, undesirable effects may occur during the synthesis of HAP, such as chemical contamination and phase impurity, which could be harmful to the biocompatibility and bioactivity of HAP [3]. Therefore, it is essential to control the quality and purity of the fabricated ceramic material. Our study analyzed the potential of the spectrophotometric method for the purity evaluation of synthetic HAP granules.

Based on the results, it can be concluded that the best absorption spectrum was obtained for non-centrifuged samples measured in quartz cuvettes, where the difference between the samples was observed within the entire analyzed wavelength range (Fig. 1). This means that every wavelength between 190 and 1100 nm may be used to assess the amount of impurities. The highest absorbance was registered for samples 1–5. The graph shows that the washing of HAP granules significantly reduced the absorption rates. Similar results were obtained when the samples were measured using plastic cuvettes. However, polystyrene is not useful at wavelengths below 290 nm in the UV range (Fig. 2a).

The second factor studied was the influence of centrifuging on the absorption spectra of the samples. The results collected from the centrifuged samples and presented in Figs. 1b and 2b show that the absorption values were low for all samples and close to zero. This suggests that only pure water was left. A comparison of the centrifuged and non-centrifuged samples (Table 1) indicates that the absorption derives from small

HAP particles rather than from chemical impurities or excessive chemical reagents used for the HAP synthesis, such as calcium hydroxide or phosphoric acid. No significant difference was noticed between the absorption spectra of the centrifuged samples measured in plastic and quartz cuvettes. The results presented in Table 2 suggest that the best separations were at 300 nm in the quartz cuvette due to the highest absorbance difference between the samples.

Conclusion. In this study we demonstrated the potential application of the spectrophotometric method in the purity evaluation of synthetic HAP granules. The results showed that both cuvette types (polystyrene and quartz) are useful in the absorption measurement of the fluids left after the HAP washing. The applicable wavelength range was 290–1000 nm for plastic cuvettes and 190–1100 nm for quartz cuvettes. The highest absorbance is registered for the samples measured at 190 nm in the quartz cuvette. The use of centrifuged and non-centrifuged samples enabled us to draw the conclusion that the opacity came from small hydroxyapatite crystals. Finally, the results indicated that the first five rinsings were the most important in the HAP washing. The study showed that the amount of hydroxyapatite powder may be quickly and easily assessed using quartz cuvettes with a UV-Vis spectrophotometer or cheap polystyrene cuvettes with a simple Vis-spectrophotometer. The method presented in this paper might be helpful for scientists and manufacturers of biomedical materials in the purity evaluation of different ceramics.

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