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Hydroxyapatite Formation under Combined Treatment of a Gel in the Secondary Maturation Stage

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Abstract—A method was developed for combined treatment of a hydroxyapatite gel in its secondary maturation stage by reacting the gel with a dilute phosphoric acid solution to pH 7.6 and subsequently with distilled water to pH 7.0-7.2. The method allows decreasing 1.2–1.7-fold the time of the secondary maturation of hydroxyapatite gel, and 2.5-fold, the amount of the washing solution, whereby a single-phase hydroxyapatite or a hydroxyapatite containing up to 11% of α-tricalcium phosphate can be prepared.

Keywords: hydroxyapatite gel, combined treatment, tricalcium phosphate, phosphoric acid, decantation, sedimentation

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The gel form of hydroxyapatite is highly bioactive due to its nanosized state [1–5]. The preferred method of preparation of hydroxyapatite gel for medical purposes is its precipitation from aqueous solutions of calcium chloride and ammonium hydrogen phosphate in an ammonia-containing medium (pH 10–12), followed by keeping the precipitate under the mother liquor for 10–14 days. Next, the resulting hydroxyapatite precipitate is washed with distilled water to remove concomitant Cl^- ions and ammonia, whereby the gel undergoes secondary maturation with its pH stabilized to 7.0–7.2, the level satisfying the hemolysis test criteria [6]. The time of the secondary maturation stage of the gel is determined by its sedimentation time in each decantation cycle and by the time of pH stabilization to 7.0–7.2. The sedimentation of the gel in each decantation cycle takes 72–300 h, and the total time of the pH stabilization may be 105 days.

Use of dilute acid solutions decreases 2.2–2.8-fold the gel pH stabilization time, though initiates hydrolytic processes leading to the formation of up to 18% of tricalcium phosphate [7]. Therefore, for inhibition the hydrolysis, it seems expedient to treat the gel successively with an acid solution to pH 7.6 and distilled water.

Here, we developed a method for preparing hydroxyapatite by combined treatment of a gel, whereby the time of the stage of the secondary maturation of the gel can be decreased. To this end, the gel was treated with a dilute phosphoric acid solution (pH 3.7–4.1) for decreasing the sedimentation time and subsequently washed with distilled water for stabilizing pH to the hemolysis test level (7.0–7.2).

In the stage of the secondary maturation of the gel at washing with distilled water (pH 5.6) solely at a 1 : 1 gel to water volume ratio in each decantation cycle the sedimentation is a very lengthy process lasting up to 300 h; the sedimentation curve is typical for polydisperse systems (Fig. 1a). Within the first 8 hours ca. 20 wt % of hydroxyapatite particles precipitates, within 50 h, 65%, and within 150 h, 98%; after 300 h the sedimentation is complete, providing colloidal spherical particles 200–455 nm in size (Fig. 1a).

After treatment of the gel with the H_3PO_4 solution of pH 3.7 at a 1 : 1 gel to H_3PO_4 solution volume ratio in each decantation cycle, the shape of the sedimentation curve becomes typical for monodisperse

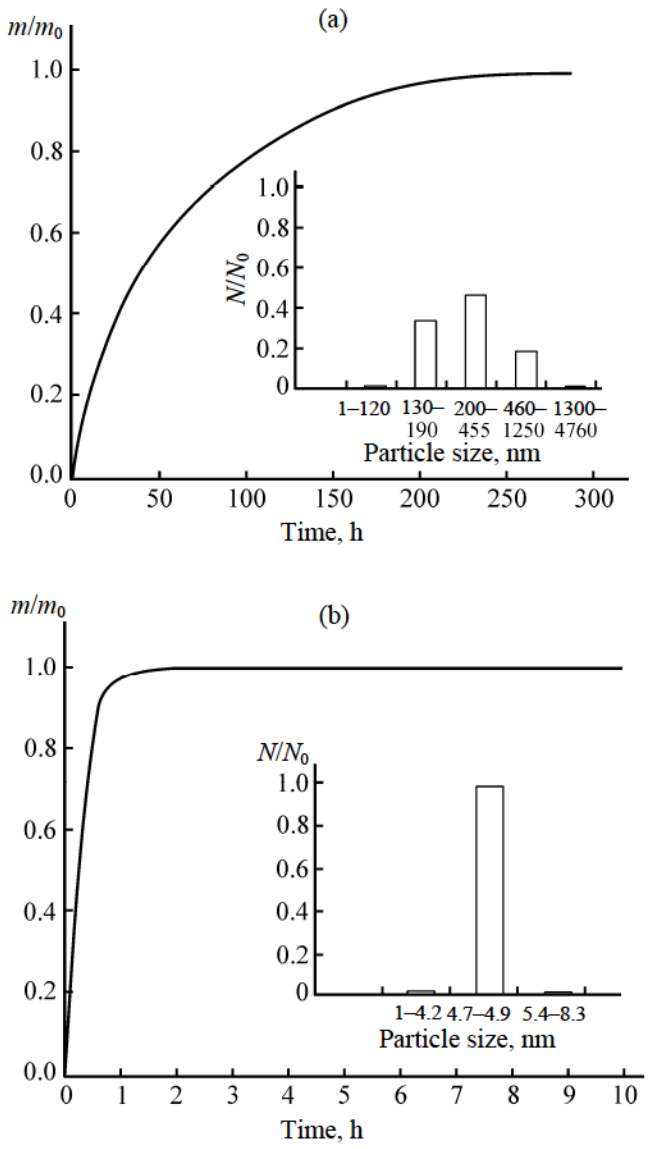

Fig. 1. Sedimentation curves of the hydroxyapatite gel after washing with (a) distilled water (pH 5.6) and (b) H_3PO_4 solution (pH 3.7) at a 1 : 1 gel to H_3PO solution volume ratio in the decantation cycle and size distribution of the spherical particles in relation to their radius.

systems (Fig. 1b). Under these conditions, within 0.5–1 h, ca. 98 wt % of hydroxyapatite particles with the predominant size of 4.7–4.9 μm settles. During the subsequent washing with distilled water, the sedimentation velocity of the gel is retained, with the sedimentation curve having a similar shape.

A decrease in the gel sedimentation time after treatment with the H_3PO_4 solution may be associated with the partial dissolution of fine hydroxyapatite crystals. Therefore the sedimentation velocity of the gel increases from 0.004–0.070 (treatment with distilled water) to 0.4–0.8 cm min^{-1} (combined treatment). At the subsequent washing of the gel with distilled water to pH 7.0 the sedimentation proceeds with a higher velocity (0.4–0.8 cm min^{-1}).

The IR spectrum of the gel (pH 10.0) after 5 cycles of decantation with distilled water (pH 5.6) contains absorption bands at 3568 and 3424 cm^{-1} corresponding to free valence O–H bond and hydration water (Fig. 2a, spectrum *1*). The broadening of the band at 3424 cm^{-1} is indicative of the presence of a hydrogen bond system. The bands at 3146, 3044, and 2811 cm^{-1} correspond to vibrations of the NH_4^+ and NH-groups [8] resulting from incomplete removal of the associated ammonia from the hydroxyapatite structure. Upon stabilization of pH of the gel to 7.0 these bands disappear (Fig. 2a, spectrum *2*), and a band appears with two maxima at 2926 and 2852 cm^{-1} corresponding to the stretching vibrations of the associated O–H bond [8–10]. After the combined or acid treatment these bands slightly increased in intensity (Fig. 2a, spectra *3* and *4*), and the peak area from the water vibrations was at a maximum (12954 $unit^2$) in the case of a slowly sedimenting gel (pH 10.0) after 5 cycles of decantation with distilled water (Fig. 2b). The peak area was at a minimum (8658 $unit^2$) when the gel was washed only with an acid solution. This presumably indicates partial destruction of hydrate hydrogen bonds.

In the stage of the secondary maturation of the gel at washing with distilled water (pH 5.6) solely at a 1 : 1 gel to water volume ratio in each decantation cycle, pH exhibits a slow decrease from 11 to 8.6 (Fig. 3a, curve *1*). The stabilization of pH of the gel to 7.0–7.2 proceeds slowly (over 42 days), with 41 L of water consumed per a gel liter (see table).

When the gel was subjected to the combined washing treatment with the H_3PO_4 solution (pH 4.1) at a 1 : 1 gel to H_3PO_4 solution volume ratio in each decantation cycle, pH of the gel decreased from 11 to 7.3–7.6 within 18 days, with 15 L of the washings consumed per a gel liter (Fig. 3a, curve *2*). Further stabilization of pH of the gel to 7.0 at washing with distilled water (pH 5.6) under identical conditions takes 36 days, with 33 L of the washings consumed per a hydroxyapatite gel liter (see table). Use of the H_3PO_4 solution (pH 3.7) and distilled water (pH 5.6) for the combined washing treatment of the gel under identical conditions allows decreasing the time of the secondary maturation stage of the gel to 30 days, with 26 L of the washing solution consumed per a gel liter (Fig. 3a, curve *3*; see table).

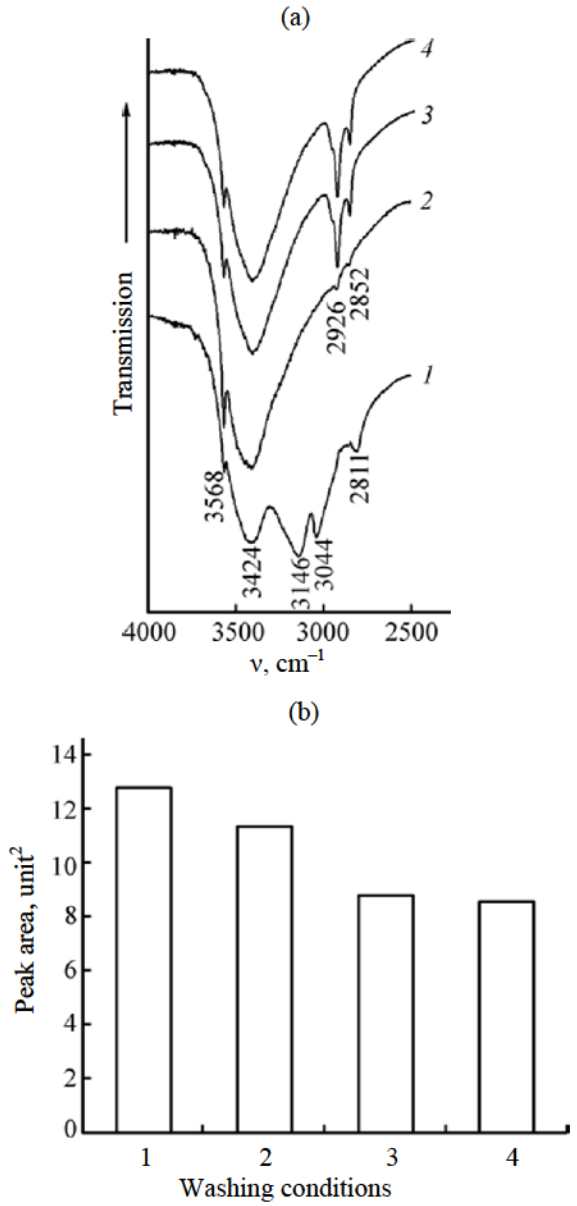

Fig. 2. (a) IR spectra of hydroxyapatite treated with different washing solutions at a 1 : 1 hydroxyapatite gel to H_3PO_4 solution volume ratio in the decantation cycle and (b) dependence of the absorption peak areas on the washing conditions. (*1*) Distilled water, pH 5.6 (5 decantation cycles, pH_{gel} 10.0); (*2*) distilled water, pH 5.6 (22 decantation cycles, pH_{gel} 7.0); (*3*) H_3PO_4 solution (pH 3.7) and distilled water (pH 5.6) (17 decantation cycles, pH_{gel} 7.0); and (*4*) H_3PO_4 solution (pH 3.7) (12 decantation cycles, pH_{gel} 7.0).

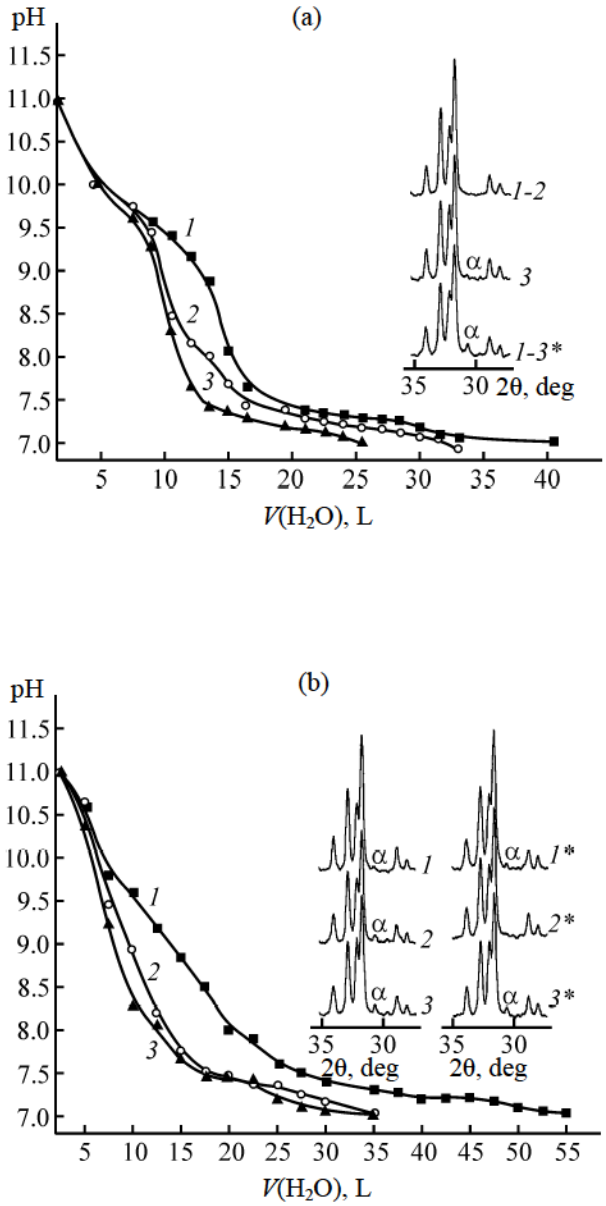

Fig. 3. Dependence of pH of the hydroxyapatite gel on the washing solution volume at (a) 1 : 1 and (b) 1 : 1.7 gel to H_3PO_4 solution, or water, volume ratio in the decantation cycle. (*1*) Distilled water (pH 5.6), (*2*) H_3PO_4 solution (pH 4.1) and distilled water, and (*3*) H_3PO_4 solution (pH 3.7) and distilled water. The inset shows the X-ray diffraction patterns of the gel samples (pH 7.6) and end product (pH 7.0). (*) End product and (α) 100% α-tricalcium phosphate peak.

From the X-ray diffraction patterns of the gel samples, pH 7.6, taken after washing with the H_3PO_4 solution (pH 4.1) or only with distilled water it follows that they are 100% hydroxyapatite (analysis accuracy 1–2%). The sample taken after washing with the H_3PO_4 solution (pH 3.7) contains 5% of α-tricalcium phosphate impurity (Fig. 3a; see table), whose formation may be associated with a decrease in the Ca to P stoichiometric ratio from 1.67 to 1.5 due to the introduction of extra PO_4^{3-} ions.

Characterization of the treatment conditions for hydroxyapatite gel in the secondary maturation stage and composition of the products

Gel to washing solution volume ratio in the decantation cycle	pH of an H_3PO_4 solution (H_2O_{dist})	Washing solution volume consumed per a liter of hydroxyapatite gel, L	Number of decantation cycles	Time of the secondary maturation stage, days	Composition of the gel sample (pH 7.6), %		Composition of the end product (pH 7.0), %	
					hydroxyapatite	tricalcium phosphate	hydroxyapatite	tricalcium phosphate
1 : 1	– (5.6)	41	27	42	100	0	91	9
	4.1 (5.6)	33	22	36	100	0	91	9
	3.7 (5.6)	26	17	30	95	5	89	11
1 : 1.7	– (5.6)	55	22	34	95	5	95	5
	4.1 (5.6)	35	15	20	93	7	100	0
	3.7 (5.6)	35	15	20	90	10	93	7

The end products (pH 7.0) resulting from washing the gel only with distilled water or from the combined washing treatment with the H_3PO_4 solution having pH 4.1 are identical in composition and contain 9% of α-tricalcium phosphate each. However, combined washing treatment of the gel with the H_3PO_4 solution having pH 3.7 led to the formation of 11% α-tricalcium phosphate (Fig. 3a).

Thus, combined washing with the H_3PO_4 solutions (pH 3.7–4.1) at a 1 : 1 gel to H_3PO_4 solution volume ratio in each decantation cycle decreases 1.2–1.4-fold the time of the secondary stage of the gel maturation as compared to washing only with distilled water (pH 5.6). The amounts of α-tricalcium phosphate in the end product are nearly identical, 9–11% (see table).

To decrease the time of the stage of the gel secondary maturation when subjected to the combined washing treatment, the gel to H_3PO_4 solution volume ratio in the decantation cycle was increased to 1 : 1.7. Like in the previous case, 15 L of the H_3PO_4 solution (pH 3.7–4.1) was consumed per a gel liter in the combined washing treatment of the gel for 12 days, and this was accompanied by a sharp decrease in pH of the gel from 11 to pH 7.4–7.5 (Fig. 3b, curves *2* and *3*). When the gel was washed solely with distilled water (15 L) at the identical volume ratio, pH of the gel decreased from 11 to 8.85, after which it slowly decreased further to 7.0 (Fig. 3b, curve *1*).

An increase in the gel to water volume ratio in the decantation cycle from 1 : 1 to 1 : 1.7 causes a 1.2-fold decrease in the time of the stage of the secondary maturation of the gel in the zero comparison experiment, though leads to the formation of 5% α-tricalcium phosphate after gel reaches pH 7.6 (Fig. 3b, curve *1*). In the end product, this amount of α-tricalcium phosphate formed is preserved (Fig. 3b, curve *1**, see table).

The combined washing treatment with H_3PO_4 solutions (pH 3.7–4.1) at a 1 : 1.7 gel to H_3PO_4 solution volume ratio in the decantation cycle leads to a decrease in the time of the stage of the secondary maturation of the gel to 20 days (by a factor of 1.7 against that in the zero comparison experiments), with 35 L of the washing solution consumed per a gel liter (Fig. 3b, curves *2* and *3*; see table). For the gel washed only with distilled water at the same volume ratio, the time of the secondary maturation stage is 34 days, and 55 L of distilled water is consumed per a gel liter (Fig. 3b, curve *1*; see table).

Like in the case of washing only with distilled water, an increase in the gel to H_3PO_4 solution volume ratio to 1 : 1.7 leads to the appearance of 7–10% of α-tricalcium phosphate impurity in the samples taken after washing with H_3PO_4 solutions (pH 3.7–4.1) (Fig. 3b, curves *2* and *3*). The end product resulting from the combined washing treatment of the gel with

the H_3PO_4 solution having pH 3.7 contains 7% of α-tricalcium phosphate (Fig. 3b, curve *3**), and in the case of the combined washing treatment with the H_3PO_4 solution having pH 4.1, α-tricalcium phosphate reflections were not detected (Fig. 3b, curve *2**).

Our data show that the combined washing treatment of the gel at a 1 : 1.7 gel to H_3PO_4 solution volume ratio in each decantation cycle leads to a 1.5-1.8-fold decrease in the time of the secondary maturation stage compared to the combined washing treatment at a 1 : 1 gel to H_3PO_4 solution volume ratio. Washing with a phosphoric acid solution not only increases the sedimentation velocity but also promotes more rapid stabilization of pH of the gel to 7.0–7.2. Subsequent washing with distilled water is necessary for shifting the equilibrium towards hydroxylapatite formation.

EXPERIMENTAL

Hydroxyapatite gel (2.7 wt %) was prepared by reacting aqueous solutions of 1.3 M $CaCl_2$ and 0.8 M $(NH_4)_2HPO_4$ in an ammonia-containing medium (pH 10–12), followed by washing the resulting precipitate to pH 7.0–7.2. The washing procedure included a number of successive cycles; the time of each cycle was determined by the sedimentation process. The sedimentation radius of the particles was calculated in the spherical particle approximation by formula (1) [11]:

$$r_i = \sqrt{9\mu \cdot u_i / 2(\rho - \rho_0) \cdot g}, \quad (1)$$

where μ is the dynamic viscosity of the gel; ρ and ρ_0, particle and water densities, respectively; g, acceleration of gravity (9.80665 m s^{-2}); and u_i, particle settling velocity for each fraction.

The particle sedimentation velocity for each fraction of the gel was calculated by formula (2) [12]:

$$u_i = h/\tau_i, \quad (2)$$

where h is the sedimentation height of the gel particles, and τ_i, sedimentation time of the fraction of the hydroxylapatite particles.

To decrease the time of the secondary maturation stage, the 2.7 wt % gel was separated from the mother liquor, divided into three equal portions, and washed for 18 days with dilute phosphoric acid solutions (pH 3.7–4.1) and subsequently with distilled water (pH 5.6) at a 1 : (1–1.7) gel to H_3PO_4 solution volume ratio in each decantation cycle. The choice of pH of the phosphoric acid solutions was made on the basis of the previously obtained data [7]. The gel washing procedure was performed daily. Upon completion of the washing of the gel with a phosphoric acid solution to pH 7.6, samples for analysis were taken, after which the gel was washed with distilled water, also at a 1 : (1–1.7) gel to water volume ratio in the decantation cycle. For zero comparison experiment, one portion of the gel was washed with distilled water (pH 5.6) solely. The change in pH was monitored with an pH-meter (HI 221, Romania).

The IR-spectroscopic analysis was performed on a MIDAC 2000 (USA) FTIR spectrometer in the high-frequency region of 2500–4000 cm^{-1} in which absorption bands of the stretching vibrations of OH-groups, hydration water, and N–H bonds are manifested. The samples for analysis were KBr pellets prepared with the use of a hydroxyapatite powder dried at 60°C (1 mg of hydroxylapatite per 800 mg of KBr).

The samples taken upon washing the gel with a phosphoric acid solution and the end products were dried at 60°C, maintained at 800°C for 5 h, and identified on an Advanced D8 (Bruker, Germany) X-ray diffractometer using CuK_α radiation (λ = 0.154 nm). In the X-ray diffraction patterns, the 2θ region of 27.5° to 35° was analyzed in which the main peaks of hydroxyapatite and tricalcium phosphate are manifested.

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