Hydroxypatite-polysaccharide granules for drug delivery

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The formation of hydroxyapatite by co-precipitation from sodium alginate and sodium carboxymethylcellulose aqueous solutions with the use of dibasic calcium phosphate dihydrate and calcium hydroxide as starting reagents is studied. A technique to prepare the hydroxyapatite/polysaccharide (micro)granules is developed. An introduction of an antimicrobial Biocide 1 agent in proper amount into the granules is provided, and the behavior of the granules is evaluated.

1. Introduction

Targeted and controlled in time drug delivery systems where the drugs are distributed within a biocompatible matrix are under continuous development [1–6]. For this purpose, the drug encapsulation as well as other systems, e.g. monolithic, with a biodegradable matrix, have been proposed [5–7]. The systems may contain an antimicrobial agent in polyurethane [8], gelatine [5], or other biodegradable matrix. Immobilization of cells and enzimes by entrapment into alginate and cellulose gels is widely used in biotechnology. Alginate is a typical ionic polysaccharide (PS) which can be applied in conjunction with calcium ions as a cross-linker. The very mild technique and rapid gel formation in the presence of calcium ions are the arguments for the alginate to be used as a versatile immobilization matrix. However, alginate-base gels are subjected to rapid microbial destruction. Therefore, it is important to provide a method allowing to control the matrix degradation in time.

Another aspect of the problem is to supply a bone-stimulating agent in combination with an antimicrobial agent to improve osteogenesis and the resistance of the system to the microbial destruction. Hydroxyapatite (HA) and other calcium phosphate phases in combination with a bioresorbable polymers are known to be used in bone repair [9]. Therefore, the development of a delivery system based on the PS in combination with both hydroxyapatite and antimicrobial agent seems to be a burning problem.

Recently, a method to prepare PS-base spherical granules has been developed [10, 11]. An antimicrobial agent can further be introduced into the granules. A Biocide 1 is a representative of the class of low molecular weight alkylhydroxybenzenes which reveal the protective effect from microbial destruction and antioxidative effects.

In this respect, the present work is aimed at the investigation of the formation of HA in PS solutions of sodium carboxymethylcellulose (CMC) and sodium alginate (ALG); the development of a technique to prepare HA/PS granules, and the study of a Biocide 1 effect on granules behavior.

2. Experimental details

2.1. Starting materials

Commercial samples of ALG Manicol DM (exact composition unknown) was supplied by Kelko Co. Blanose CMC was supplied by Hercules Co. Two types of CMC have been used. The former is the CMC type 7 that is characterized by the degree of substitution range of 0.65 to 0.90 and sodium content 7.0–8.0%. The second is the CMC type 12 that is of the substitution range of 1.15–1.45 and sodium content of 10.4–12.0%.

Reagents to synthesize the HA were a special grade dibasic calcium phosphate dihydrate (DCPD) $CaHPO_4 \cdot 2H_2O$ and calcium hydroxide $Ca(OH)_2$. A Biocide 1 agent was supplied by the Institute of Microbiology RAS.

2.2. Synthesis

DCPD and $Ca(OH)_2$ in amounts that corresponds to pure HA (Ca/P = 1.67) were mixed together with the use of an ultrasonic mixer for 30 min. Ultrasonic resonance frequency was about 20 kHz, ultrasonic radiation

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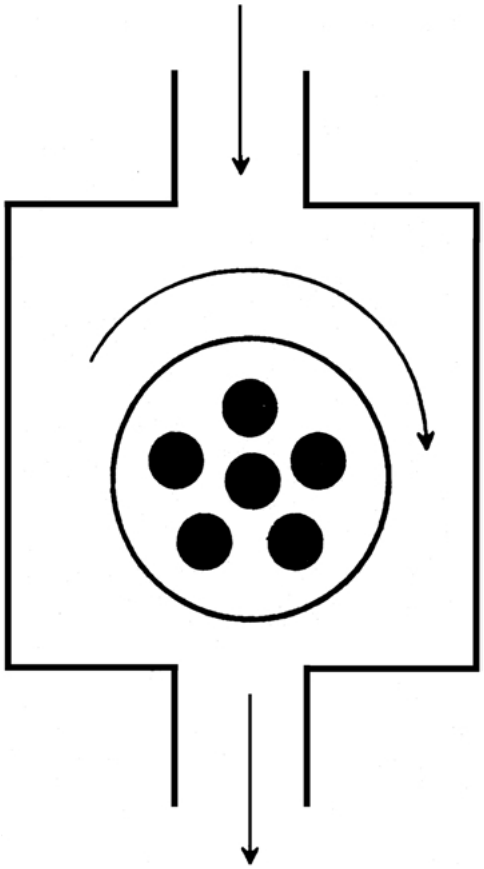


Figure 1 Scheme of a rotator device to produce microgranules.

intensity was about 0.1 W cm^{-1}. HA was obtained in an aqueous medium by slow addition of the suspension to the PS solution at the continued agitation conditions. The reaction was studied at a constant concentration of DCPD and $Ca(OH)_2$ suspension (1 wt %), the content of PS in the system being varied from 0–2.0 wt %. The reaction was completed at pH value of 7.2.

2.3. Granules preparation

Spherical microgranules of about 0.5–5.0 μm diameter were prepared by co-precipitation from the solution with the use of a rotator (Fig. 1). A sphere filled with the concentrated wet precipitate was placed into a cylinder. The mechanical forces resulting from water stream inside the cylinder lead to the rotation movement and the granules formation. Finally, granules were separated and dryed at 105 °C or 150 °C.

To prepare the HA/PS beads, a solution containing 1.0–3.0 wt % of ALG or ALG/CMC, and 1.0 wt % of HA has been extruded through a syringe tip needle into a 3 wt % calcium chloride solution resulting in beads of up to 1.0 mm diameter formation. The beads were placed then into a vacuum device. The granules were further filtered and washed with deionized water, and dried at 105 °C or 150 °C.

2.4. Samples characterization

X-ray diffraction measurements were performed with powder compacts with the use of a DRON-0.5 diffractometer in CuK_α radiation. DTA was done with a MOM Derivatograph. IR spectra of the samples despersed in KBr were observed with a Specord UR-75 Spectrophotometer.

2.5. Beads stability

To simulate gastrointestinal transit *in vitro*, the granules were exposed to a gastric HCl solution (pH 1.2) for a period of 5 h followed by the immersion into $NaHCO_3$ solution (pH 7.8). A reference part of the granules was exposed to acidic pepsin (pH 1.2) followed by amylaze solution (pH 7.8).

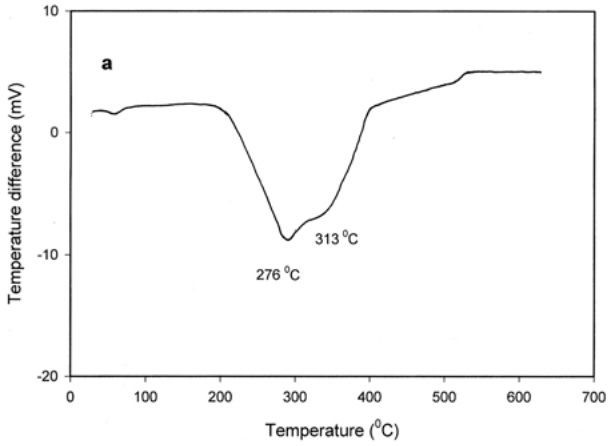
2.6. Protective effect of Biocide 1

An ethanol solution of Biocide 1 of 0; 0.0005; 0.005; 0.05 and 0.5 wt % content has been precipitated onto the surface of the granules. Cultures of test microorganisms: *Bacillus cereus*, *Escherichia coli* (bacteria), *Actinomyces* (streptomyces), *Saccharomyces sp* (yeast), and *Deuteromyces sp* (fingus), were placed on Petri dishes with corresponding agar nutrient media. After that, paper discs with gel samples containing Biocide 1 or without it (reference) have been applied onto the surface of the agar media with seeded microorganisms (5–6 replicates) under sterile conditions. The dishes were incubated for 3–7 days at 28 °C and then screened for the appearance of transparent zones around the discs in the backgrounds of microbial lawns which indicated the growth inhibition effect of Biocide 1. Diameter of these zones was measured to be used as a relative quantitative characteristic of the Biocide 1 protective effect.

3. Results and discussion

Up to 60 min reaction time is needed to obtain HA in CMC type 12 solution resulting in pH 7.2, and the duration of the synthesis was about one week for the HA in the CMC type 7 solution. A reaction time of 3 h is needed for the HA in the ALG solution. The addition of DCPD-$Ca(OH)_2$ suspension to both the ALG and CMC solutions of less than 0.75 wt % concentration results in co-precipitation reaction yielding a fine disperse precipitate. The viscous solution of higher PS contents results in a suspension of HA in the solution. The suspension was uniform for the CMC type 12 solution, but is unstable one for both the ALG and CMC type 7 solutions due to the precipitation of the HA particles.

Shown in Fig. 2 are the DTA data for HA/CMC granules. Two endothermic effects with a maximum at 276 °C and 313 °C for HA/CMC type 7 (curve 1), and at 291 °C and 325 °C for HA/CMC type 12 (curve 2) are seen, respectively. For the system HA/ALG the peaks are found to be at 320 °C and 520 °C. The endothermic effects are caused by the decomposition of the organic constituent of the system. The weight losses at a temperature higher than 600 °C equal to about 10.0% for the HA/CMC system and to 14.0% for the HA/ALG system. This amount is related to the whole escape of the organic component from the granules. The endothermic effects were not revealed for amorphous HA. These data demonstrate that the HA/CMC granules are thermally stable up to about 270 °C, whereas the HA/ALG ones are



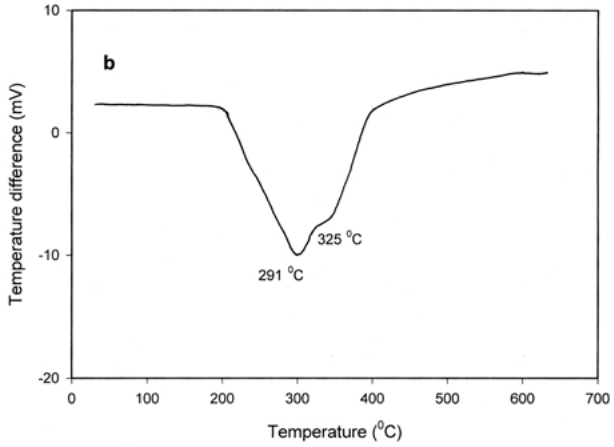


Figure 2 DTA of HA/CMC type 7 (a) and HA/CMC type 12 (b) granules.

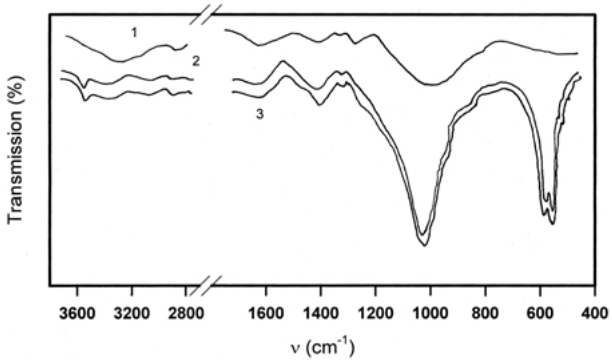


Figure 3 IR spectra of CMC type 12 (1), HA/CMC type 7 (2), and HA/CMC type 12 (3) granules.

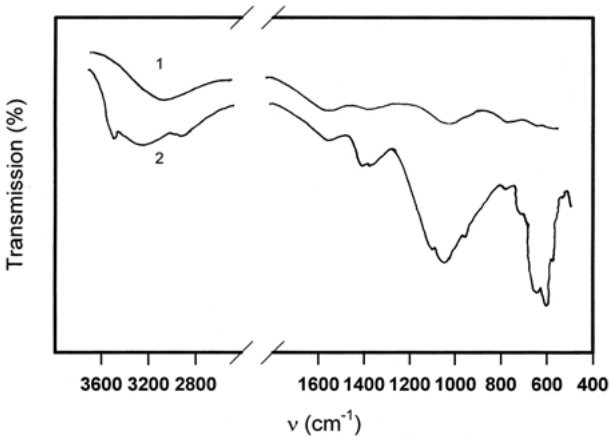


Figure 4 IR spectra of ALG (1) and HA/ALG (2) granules.

stable up to 320 °C. The organic phase content in the granules is as high as 10–14 wt %.

XRD analysis and IR spectra of the precipitates after being dried at 105 °C or 150 °C indicate that they are X-ray amorphous HA. IR spectra of HA contain bands which are typical of phosphate PO_4^{3-} groups: symmetric stretching vibrations at 900–1100 cm^{-1}, and deformation mode at 550–650 cm^{-1}. Narrow band at 3570 cm^{-1} indicates a symmetric stretching vibrations mode of free OH^- groups. Additionally, the IR spectrum contains bands of stretching vibrations at 3000–3500 cm^{-1}, and deformation mode at 1650 cm^{-1} associated with the water of hydratation. The IR spectrum of the product with Ca/P ratio < 1.667 calcined at 900 °C contains bands which are typical of tricalcium phosphate. The narrow band at 3570 cm^{-1} of OH^- groups as well as PO_4^{3-} deformation vibrations band at 630 cm^{-1} are not revealed.

In Fig. 3, the IR spectra for the CMC type 12 (1), HA/CMC type 7 (2) and HA/CMC type 12 (3) microgranules are shown. No significant difference between the spectra of CMC type 7 and CMC type 12 can be pointed out. For the HA/CMC granules, additional bands of CMC are present as compared to the individual HA and CMC spectra. The band at 1640 cm^{-1} which is characteristic of carboxyl ions in CMC is changed in the presence of HA. This effect seems to be related to the interaction between Ca ions from HA with carboxyl groups of CMC. Additionally, a gel network is probably formed by hydrogen bonds of various types.

Comparing the IR spectra of HA/ALG (Fig. 4) and HA/CMC (Fig. 3), it can be supposed that the mechanism of the interaction between the organic and inorganic components is the same for both the ALG and the CMC.

The beads which have been prepared by the extrusion through a syringe needle were tested in gastrointenstinal transit simulating media. Behavior of the beads is evaluated by weight loss and size changes after exposure in testing media. It is revealed that no shrinkage of beads occurs after the exposure in hydrochloric acid (pH 1.2), but swelling up to 25% of initial weight is found when the beads were further immersed into $NaHCO_3$ solution of pH 7 for 3 h. Maximum 3 h is needed for the beads to be destructed in an acidic pepsin (pH 1.2) followed by soaking into amylaze silution (pH 7.8). To enhance the beads stability, magnesium ions have been added to the matrix forming solution. The addition increases the stability noticeably, and the beads become stable to acidic pepsin/amylaze treatment at the same conditions.

Shown in Fig. 5 are the data on the protective effect of the Biocide 1 agent. It should be noted that the effect increases with an increase of the Biocide 1 content. If the Biocide 1 has been introduced directly into the granules, so it was found that an optimum Biocide 1 content to inhibit the growth of the tested microorganisms is in the range from 0.1–0.5 wt % for both the HA/ALG and the

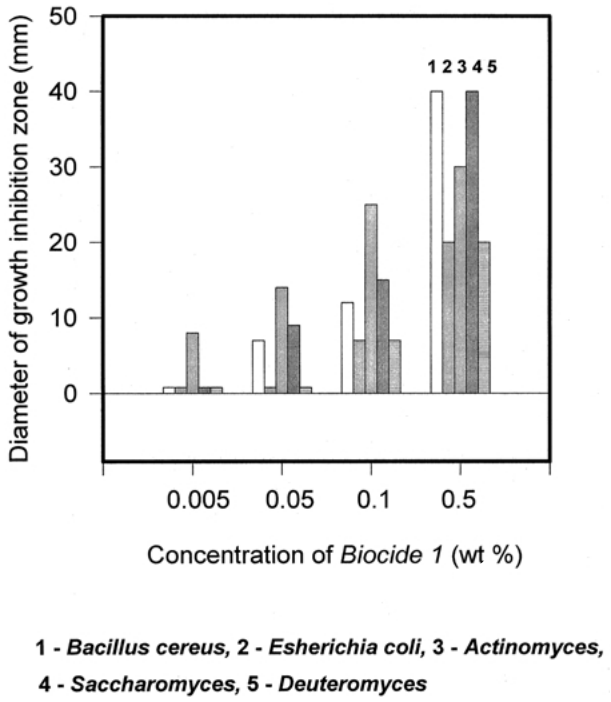


Figure 5 Protective effect of Biocide 1.

HA/CMC systems. Further increase of the Biocide 1 content can result in possible deterioration of the gel structure and, therefore, seems to be undesirable.

4. Conclusions

1. The formation of HA in aqueous solutions of sodium ALG and CMC by reaction of dibasic calcium phosphate dihydrate and calcium hydroxide is proved.

2. DTA/TG analysis, XRD and IR spectra of the reaction products indicate that HA is mainly in X-ray amorphous state, and possible interaction between HA and carboxy groups occurs.

3. Techniques to prepare (micro)granules of HA/PS system are developed, the granules being of 10–14% PS content.

4. The HA/PS granules protected from microbial destruction by the addition of a Biocide 1 antimicrobial agent are prepared. The granules can be used as a stable drug delivery system resistant to the gastrointestinal conditions.

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