Synthesis of poly-\(\varepsilon\)-caprolactone/hydroxyapatite composite materials by \textit{in situ} and mechanical mixing methods and investigation of their physico-chemical properties

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Abstract. This work is dedicated both to investigation of the influence of different conditions on the molecular weight characteristics of poly-\(\varepsilon\)-caprolactone (PCL) and to the synthesis of composites based on PCL and hydroxyapatite (HA) by \textit{in situ} and mechanical mixing methods. It was shown that \textit{in situ} synthesis is accompanied by chemical interactions between PCL and HA that were confirmed by IR spectroscopy and thermal analysis. The morphology of \textit{in situ} obtained composites has more homogeneous and regular distribution of HA particles in the polymeric matrix than composites synthesized by mechanical mixing of HA and PCL.

1. Introduction

Currently, one of the main tasks of modern materials science is the development of new functional materials for various fields, primarily for medicine. Among the huge variety of polymeric materials for medical purposes, the class of biodegradable polyesters, combining the advantages of synthetic and natural polymers, occupies a special place. One of the representatives of this class is poly-\(\varepsilon\)-caprolactone (PCL) \cite{1}. Due to its physicochemical characteristics and biocompatibility, PCL, both independently and in the presence of inorganic filler, is successfully used in various branches of medicine, especially in bone tissue engineering \cite{2}. Hydroxyapatite (HA), the most important inorganic component of bone tissue, most often acts as an inorganic filler \cite{3}. It is known that the properties of composite materials principally depend on the molecular mass characteristics of the polymer matrix and the method of their preparation. In scientific works describing the production of composite materials for orthopedics, the emphasis is placed on polymers having a molecular weight (MW) of at least 50000 g/mol. Materials with such MW have optimal mechanical properties and time of degradation.

The method of obtaining also has a significant influence on the properties of composite materials. Methods for producing composites by mechanical displacement, when inorganic filler is added to the polymer solution with constant stirring, and then a composite material is formed from the resulting mixture, the most investigated \cite{4, 5}. This method is universal; however, it has some disadvantages, among which are the low strength characteristics of the materials obtained and the irregular distribution of the filler in the polymer matrix. One of the most promising but poorly studied methods...
for producing composites is the in situ method, or synthesis in the reaction mixture. Using this approach, a large number of biodegradable polyester/inorganic filler systems were obtained [6,7]. Despite the fact that PCL is one of the representatives of the class of biodegradable polyesters, studies on the production of PCL/hydroxyapatite composites using the in situ method are currently presented only in a few publications [8, 9].

In this connection, this article is dedicated to the search for optimal conditions for the synthesis of high molecular weight PCLs, the preparation of composite materials based on PCL and HA by mechanical mixing and in situ and the investigation of some of their physicochemical properties.

2. Experimental part

2.1 Materials and methods

PCL was obtained by polymerizing of ε-caprolactone (99%) (Sigma-Aldrich, Germany) in the presence of an initiator – Sn(Oct)2 (Sigma-Aldrich, Germany) ) and co-initiators – alcohols of various functionalities: butanol C4H9OH (But), propylene glycol C3H6(OH)2 (PG) and glycerol C3H5(OH)3 (GL) (all Vecton, Russia).

All commercial reagents are previously purified, mainly from traces of water, which contributes to the deactivation of the initiator and uncontrolled breakage of the chain. Commercial ε-caprolactone was distilled under reduced pressure over 4 Å molecular sieves. To dehydrate alcohols, they were subjected to azeotropic distillation with a Dean-Stark trap.

HA was obtained by liquid-phase microwave synthesis according to the method described in [10].

Molecular mass characteristics of PCL samples and composites obtained by the in situ method were analyzed by gel permeation chromatography (GPC) on an Agilent 1200 liquid chromatograph with a refractometric detector (eluent – chloroform).

The X-ray diffraction analysis of HA powders was performed on Shimadzu XRD 6000 diffractometer at CuKα radiation. The processing of the obtained data and phase identification were carried out using a database JSPDS PDF 4+ and full-profile analysis programs POWDER CELL 2.4.

The chemical and functional composition of the composites obtained by mechanical mixing and in situ was determined by IR spectroscopy in the range of wavenumbers 4000 – 500 cm⁻¹ on IR-Fourier spectrometer Aglient Cary 630 FTIR.

Micrographs of PCL/HA composites were obtained by scanning electron microscopy (SEM) on a Hitachi TM-3000 instrument at an accelerating voltage of 15 kV under conditions of the charge-off mode (electron gun: 5·10⁻² Pa; camera for the sample: 30-50 Pa).

The thermal properties of composites based on PCL obtained by the methods of mechanical mixing and in situ were investigated by the methods of differential scanning colorimetry (DSC) and thermogravimetric analysis (TGA) on a combined NETZSCH STA 449F1 thermal analyzer in the range of 30-800 °C with a heating rate of 10 °C/min in argon atmosphere.

2.2 Synthesis of PCL as polymeric component of composites and studying the effect of various factors on its molecular weight characteristics

Polymerization of ε-caprolactone with a ring opening in the presence of Sn(Oct)2 and alcohols of various functionalities was performed according to the scheme shown in Figure 1.

![Figure 1. Ring opening polymerization of ε-caprolactone.](image)

The synthesis of PCL was carried out in test tubes per 5 g of distilled ε-caprolactone. The amount of initiator was calculated from the ratio [monomer]: [initiator] 1:500 and all components were added into the tube with
monomer. For the selection of optimal polymerization conditions for $\varepsilon$-caprolactone, co-initiators with different functionalities were used and the ratio [initiator]:[co-initiator] was varied. The polymerization reaction system was prepared in a glove box under a nitrogen atmosphere. After all the components were added, the tube with the monomer, the initiator and the co-initiator was placed in a Heidolph Hei-VAP Advantage rotary evaporator bath with constant stirring of the reaction mixture. The synthesis was carried out at 140-170 °C for 5 hours.

2.3 Preparation of composite materials based on PCL and HA

2.3.1 Mechanical mixing method. Composite materials of composition PCL/HA 90/10, 80/20 and 70/30 were obtained by mixing a solution of PCL in chloroform ($c = 0.1$ g/ml) and HA powder with constant stirring on a magnetic stirrer (IKA C - MAG HS 4). The resulting suspension was treated with ultrasound in an ultrasonic bath (JEIOTECH) for 20 minutes at a frequency of 40 kHz. The obtained samples were cast in the form of films on a Petri dish.

2.3.2 In situ method. When producing composites by the in situ method, reaction systems were prepared in a glove box. In four flasks were placed on 20 g of $\varepsilon$-caprolactone and Sn(Oct)$_2$, the amount of which was calculated from the ratio [monomer]:[initiator] 2000:1, and added 5 mass.% HA (composite 1 - C1), 10 mass.% HA (composite 2 - C2), 20 mass.% HA (composite 3 - C3) and 30 mass.% HA (composite 4 - C4). Suspensions of the monomer with different amounts of HA were pre-ground for 10 minutes to homogenize before adding to the flasks. Then the flasks were placed in a Heidolph Hei-VAP Advantage rotary evaporator bath at $T = 170$ °C with constant stirring. Synthesis was carried out for 5 hours. At the end of the synthesis, the selected samples of the composites were cast in the form of films on a Petri dish. To determine the MW of the composites obtained in situ, HA was removed by filtration on a Schott filter with a pore size of 1.6 μm.

3. Results and discussion

3.1 Studying the effect of various factors on the molecular weight characteristics of PCL

During the experiments, the effect of temperature, functionality of a co-initiator, and the ratio [initiator]: [co-initiator] on the molecular weight of PCL as a polymer component of composites synthesized by mechanical mixing was evaluated. It can be seen from the Figure 2 that for the ratio [Sn(Oct)$_2$]:[alcohol] = 1:1 the curves have the same profile for all three alcohols.

It should be noted that at 140 °C there is a maximum of MW value of about 100 000 g/mol for the system with But. A further increase of temperature leads to a decrease in MW. The appearance of the maxima on the curves at 160 °C is associated with the boiling point of co-initiators (But – 117.7 °C, PG –187.4 °C, GL – 290 °C), which increases from But to GL and leads to a decrease in the amount of co-initiator, involved in the polymerization. An increase in the amount of co-initiator leads to a decrease in MW and to the broadening of the molecular weight distribution (MWD), which indicates that alcohol is not only a co-initiator of polymerization, but also acts as a regulator of MW, i.e.
involved in chain transfer reactions. For PG and GL the tendency to broadening of MWD can also be associated with the possibility of the formation of branched macromolecules due to the presence of two and three hydroxyl groups, respectively [11]. An increase in the amount of initiator (ratio [initiator]: [coordinator] = 2:1 and 3:1) leads to a decrease in MM at elevated temperatures due to adverse reactions of intra- and intermolecular transesterification occurring due to the active centers Sn(Oct)₂, which is an Lewis acid.

Thus, to obtain PCL with MW close to 100000 g/mol and with narrow MWD, polymerization should be carried out at 140 °C in the presence of But and the ratio [initiator]:[co-initiator] = 1:1.

3.2 Composite materials based on PCL and HA

The phase composition of the synthesized HA was determined by XRD analysis. Its diffraction pattern is presented in Figure 3, and the general phase characteristics are shown in Table 1. The diffraction pattern contains both characteristic reflections of HA at the angles 2θ equal to 25.8, 32, 39.8, 46.7 and 49.5°, as well as low-intensity reflections of Ca₃(PO₄)₂ at 31.5, 34, 40.2, 46.7 and 49.5°.

The dependence of MW of in situ obtained composites is shown in Figure 4.

| Sample | Phase                  | Phase content (wt.%) | Lattice parameters (Å)       | Average crystallite size (nm) |
|--------|------------------------|----------------------|-------------------------------|-------------------------------|
| HA     | Ca₅(PO₄)₃(OH)          | 88                   | a = 9.4065; c = 6.8732        | 69                            |
|        | Ca₃(PO₄)₂              | 12                   | a = 10.4593; c = 37.5029      | 18                            |

**Table 1.** Phase composition of HA and its crystal structure parameters.

It was noted that during the preparation of composites by the in situ method, HA acts not only as an inorganic filler, but also as a co-initiator. Moreover, with an increase in the amount of HA, a decrease in MW is observed. So, addition of 5 wt. %, 10 wt. %, 20 wt. %, 30 wt. % of HA allows to obtain a composites with MW 120000 g/mol, 109000 g/mol, 50000 g/mol and 10000 g/mol, respectively. Such regularity is associated with the structure of HA itself, containing hydroxyl groups, which, by analogy with alcohols, with increasing mass fraction in the reaction mixture, contribute to chain transfer reactions and, as a consequence, decrease in MW and broadening of MWD.

Along with the phase and molecular mass characteristics of the composites synthesized by in situ method, other physicochemical properties of the composites obtained by both methods were compared. The functional composition of the composites of both types was determined by IR spectroscopy. Figure 5 shows the IR spectra of both individual PCL, HA, and composite materials based on them: PCL / HA 90/10 and PCL / HA 80/20 (mechanical mixing); C2 and C3 (in situ).
The IR spectra of the composites obtained by both methods contain the absorption bands of HA and PCL. Moreover, in the case of composites synthesized by \textit{in situ} method, there is a low-intensity band at 1624 cm\(^{-1}\), related to the carboxylate anion, which indicates the chemical interaction between HA and PCL.

Another method confirming the chemical interaction between HA and PCL in composites obtained by \textit{in situ} method is thermal analysis. So, DSC and TG curves of PCL/HA 90/10, PCL/HA 80/20, C2 and C3 composites are shown in Figures 6 and 7, respectively. From the presented dependences it can be seen that, despite the similarity in melting points of composites obtained by mechanical mixing and \textit{in situ} method (~ 60 °C), the nature of their decomposition differs. This is characterized, on the one hand, by the appearance in the case of composites synthesized by \textit{in situ} method of additional steps on the TG curves at a temperature of 280-360 °C, on the other hand, by displacement of heat efficiency on the DSC curves at almost 100 °C towards of higher temperatures.

Comparison of the morphology of the composites obtained by the two methods was carried out on the basis of SEM micrographs, which are presented in Figure 8.

![Figure 5. IR spectra of HA, PCL, PCL/HA 90/10, PCL/HA 80/20, C2 and C3.](image1)

![Figure 6. DSC curves of PCL/HA 90/10, PCL/HA 80/20, C2 and C3.](image2)

![Figure 7. TG curves of PCL/HA 90/10, PCL/HA 80/20, C2 and C3.](image3)

![Figure 8. SEM images of the surface of composites: a) PCL/HA 90/10, b) PCL/HA 80/20, c) C2 and d) C3(1000 times magnification)](image4)
Micrographs show that the composites obtained by mechanical mixing of the PCL solution and ground HA, the aggregation of HA particles and their irregular distribution in the polymer are observed (Figure 8, a and b). In composites obtained by in situ method, on the contrary, the distribution of HA particles in the polymer is homogeneous and regular, and the formation of agglomerates is not observed (Figure 8, c and d). At the same time, HA is well retained in the polymer matrix, which indicates a good adhesion of the filler particles to the matrix, probably, due to chemical interaction between HA and PCL.

4. Conclusions
It is shown that PCL with MW of about 100000 g/mol can be obtained during a ring opening polymerization at 140 °C in the presence of Sn(Oct)₂ and But with the ratio 1:1. Obtained under such conditions PCL can be used in the synthesis of composites by mechanical mixing method.

The in situ method should be considered as the most promising method for the synthesis of composite materials based on HA and PCL. It allows not only to obtain composites with a high MW of polymer matrix, but also with a homogeneous and regular distribution of HA particles, which cannot be achieved by mechanical mixing of the components. In the case of in situ polymerization of ε-caprolactone and HA, the latter acts simultaneously as a co-initiator and regulator of the polymer chains length. It is also noted that during the synthesis, the chemical interaction of the components occurs, which is confirmed by the results of IR spectroscopy and thermal analysis.

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