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BIOINSPIRED SYNTHESIS OF CALCIUM CARBONATE COLLOID PARTICLES

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Biomimetic procedure for nucleation and growth of CaCO₃ has been presented. The wet chemical syntheses of CaCO₃ colloidal particles have been carried out. The characterization of precipitated particles of CaCO₃ has been done using the particle size distribution analysis. The effect of physical and chemical parameters on the size of precipitated particles of CaCO₃ has been investigated. This research may provide new insight into the precipitation of calcium carbonate.

key words: calcium carbonate, nanoparticles, synthesis, size distribution

INTRODUCTION

The concept of calcium carbonate synthesis inspired by natural processes has expanded greatly during three past decades (Mann, 2001). It is well known that biomineralization is achieved through the calcification process in a single cell organism. Biominerals of over 40 different types occur in organisms, ranging from bacteria to mammals. Biominerals are biocomposites of inorganic-organic hybrid materials, consisting of broad group of biological minerals (Dujardin et al., 2003).

Among biological minerals, calcium carbonate has a special place since it is the main constituent of bones and shells. In both materials, the inorganic mineral is associated with biopolymers (Hunter 1996).

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Eggshell is natural composite bioceramics containing 5% of organic and 95% of calcite components (Arias and Fernandez 2003). The eggshell biomineralization is affected by macromolecules which are produced by specialized cells. These biomineral crystals appear to be nucleated on the mammillary knobs which is proteoglycan containing oversulfated keratin sulfate. The calcite crystals growth on the knobs is affected by oveglycan. These organic components are polyanionics and polyacids, which have a high calcium affinity (Arias and Fernandez 2003).

Shells are composed of pure calcium carbonate, usually both in the form of calcite and aragonite. The precipitation of calcium carbonate can be realized inside the organism cell (the inner shell) or outside the cell.

The morphology and structural properties of calcium carbonate can be controlled by the use of specific organic additives. For instance, proteins isolated from mollusk shells shown control on the structure of calcium carbonate crystals (Feng et al., 2000). The function of proteins during biomineralization was studied by application of proteins extracted from mollusk shells (*Mytilus edulis*). The proteins from different layers of shell were used. It was shown, that proteins extracted from nacreous layer induced aragonite formation, while those extracted from prismatic layer induced calcite formation. Additionally, it was shown that the morphology of precipitated calcium carbonate crystals was controlled mainly by soluble proteins.

The effect of lysozyme on the precipitation kinetics and morphology of calcite crystals was examined (Jimenes-Lopez et al., 2003). It was found that lysozyme favored the nucleation of calcium carbonate and modified the morphology of crystals. The degree of modification was changed with the lysozyme concentration. The presence of lysozyme during the calcite precipitation process led to the formation of spherical particles. The particle size distribution depended on the lysozyme concentration (Voinescu et al., 2007).

Similar findings were reported for alcohols like ethanol, isopropanol or diethylene glycol which also were used to influence the CaCO₃ morphology (Cölfen, 2003).

The main role of biomacromolecules is an induction of calcium carbonate nucleation process. However, the experimental results showed (Sen et al., 2002) that the growth of calcium carbonate was more and more inhibited by an increasing concentration of collagen. The inhibition of calcite growth by collagen is caused because collagen is non-uniformly incorporated into the calcite crystal planes.

The cooperative influence of collagen and magnesium ions on calcium carbonate precipitation has been investigated (Jiao et al., 2006). The experimental data suggested that collagen acts in combination with magnesium ions to inhibit CaCO₃ crystal growth. Also, the influence of Mg/Ca ratio on polymorph of CaCO₃ was tested. When the ratio of Mg/Ca was in the range of 0-1, only calcite crystals were formed. When the Mg/Ca ratio was in the range 2-4 some vaterite and aragonite crystals were precipitated. However, when the ratio of Mg/Ca attains 4, most crystals were aragonite (Jiao et al., 2006).

The density and structural geometry of calcium carbonate can be easily regulated by changing the kind and the amount of the water soluble polyelectrolytes. The polyelectrolyte assembly creates a microenvironment for both the nucleation and crystal growth. It was shown (Falini, 2000), that the collagenous matrix with entrapped poly-Asp is able to control calcite and aragonite precipitation.

The results of calcium carbonate crystallization into the β -chitin scaffold has been presented (Falini et al., 2002). The three main polymorph of calcium carbonate were observed. Calcite and vaterite were precipitated in an absence of magnesium ions in solution. In the presence of Mg^{2+} ions, aragonite deposition slightly increases with crystallization time. The optimal aragonite crystallization time was 24 h.

Hydrogels of polyacrylamide modified by copolymerization with acrylic acid were used as growth medium for $CaCO_3$ precipitation. This procedure gives an organic microstructure similar to natural biomineralization process (Grassman and Löbman, 2004). The introduction of $-COOH$ group by poly-organic acid into hydrogel altered both the morphology and stability of calcium carbonate.

Polyacrylamide was also used as an organic substrate to induce the nucleation and growth of calcium carbonate. The calcium carbonate precipitation was realized by bubbling of CO_2/N_2 gas mixture through the water solution of calcium hydroxide with polyacrylamide. It was shown (Wang et al., 2006), that the concentration of organic substrate and temperature influence the nucleation, growth and morphology of calcium carbonate particles.

Recently, dendrimers were discovered as active additive for the controlled $CaCO_3$ precipitation (Cölfen, 2003).

SYNTHESIS OF NANO-SIZED PARTICLES OF METAL CARBONATES

Recently calcium inorganic particles in micro- and nano-size are prepared by various methods for the various applications. Several synthetic procedures have been designed for control of the formation of nanoparticles of metal carbonates. Two routes seem important: precipitation from homogeneous solution and synthesis in microemulsion (reversed micelles).

The nanosize calcium carbonate was synthesized by an *in situ* deposition technique (Mishra et al., 2005). Using this procedure, calcium chloride solution was mixed with PEG solution. This mixture was blended with K_2CO_3 water solution. Four different molar ratios (1:4; 1:8; 1:12 and 1:16) were used. Calcium carbonate precipitate was filtered off, washed with water, and dried in vacuum.

Microemulsions are used as special microreactors to limit the size of nano-particles (Karagiozov and Momchilova, 2005). Nano-size particles of $CaCO_3$ and $BaCO_3$ were obtained using chemical reaction in water-in-oil microemulsion. Nano-particles synthesized in this way have a spherical shape and almost equal size varying from 20

to 30 nm. Chemical interaction between CO_2 and the corresponding drops of microemulsions takes place in the reactor equipped with cooling jacket. The process of metal carbonate precipitation is endothermic. The microemulsion used in these experiments was aqueous alkali solution in oil (w/o) where the water drops contained dissolved calcium or barium hydroxide, and organic phase was n-hexane. The synthesis was carried out at temperature 20-23°C at constant flow rate for 60 min.

The growth of calcium carbonate in a reverse macroemulsion system based on n-stearoyl acid surfactant has been described (Menahem and Mastai, 2008). X-ray diffraction data showed that crystallization of CaCO_3 in macroemulsion led to the formation of vaterite and calcite nanocrystals.

The formation of nanoparticles using the microemulsion method is completely governed by the droplet exchange, which provides new reactants in small amounts of few molecules from other droplets.

A new method for preparation of nanoparticles was based on using two types of foams. Foam one was formed by the aqueous solution of CaCl_2 with anionic surfactant. The second foam was created by aqueous solution of Na_2CO_3 and cationic surfactant. Two types of foam were contacted each other in a specially designed apparatus (Guo et al., 2007). The size of nanoparticles can be controlled by changing the solution concentration,

Many nanoparticles have been studied for the use in drug delivery systems (DDS). The study of incorporation of bioactive proteins into nano- CaCO_3 has been carried out (Uano et al., 2005).

Among the various method employed to the drug delivery, CaCO_3 microcapsules seem be useful in numerous clinical applications (Fischer et al., 2000).

The preparation of CaCO_3 microcapsules encapsulating some biomacromolecules has been reported (Fujiwara et al., 2008). Proteins, such as bovine serum albumin was added to the $(\text{NH}_4)_2\text{CO}_3$ water solution. No change of the solution occurred after the protein addition into the $(\text{NH}_4)_2\text{CO}_3$ solution. It was shown that the contents of protein in CaCO_3 microcapsules strongly depended on their molecular weight.

The purpose of this study was to synthesize precipitated calcium carbonate of various sizes using different methods. The data are presented as particle size distribution curves.

MATERIALS AND METHODS

All chemicals used in these syntheses were commercial available (POCh, Poland) and were used without further purification. The poly(ethylene glycol) PEG of molecular weight 1000, 6000, 20000, 300000, and 5000000 was used.

Some bacteria cultures were isolated from soil samples. From isolated bacteria cultures two bacterial strains: *Bacillus circulans* and *Streptomyces* sp. were separated

for further investigations. The biosurfactant production was determined by the measurements of the surface tension. Biosurfactants produced by *Bacillus circulans* reduced the surface tension from 74 to 28.66 mN/m after 20h period of bacteria growth. Biosurfactants produced by *Streptomyces* sp. reduced the surface tension to 29.30 mN/m. The filtrates of microorganisms culture were marked B4 and B6, respectively.

The preparation of CaCO₃ colloid particles was carried out in the Erlenmeyer flasks at the speed of the magnetic stirrer 300 rpm, using three reaction systems:

- i. Na₂CO₃ 0.01M in H₂O (100 ml) + CaCl₂ 0.01M in H₂O (100 ml);
- ii. Na₂CO₃ 0.01M in PEG 0.1 or 0.7% aqueous solution (100 ml) + CaCl₂ 0.01M in PEG 0.1 or 0.7% aqueous solution (100 ml);
- iii. Na₂CO₃ 0.01M in filtrate 10% aqueous solution (100 ml) + CaCl₂ 0.01M in filtrate 10% aqueous solution (100 ml).

The solutions of sodium carbonate and calcium chloride with PEG and filtrate were prepared one day before the calcium carbonate synthesis and have been stirred overnight. The precipitation experiments were carried out at 25°C. The pH of solutions in which precipitation has occurred was 11.2 (without any additives), and 12.6-13 with PEG or broth. The particle size distribution of the colloidal CaCO₃ particles was measured using a laser diffraction technique (Mastersizer 2000, Malvern).

RESULTS AND DISCUSSION

The objective of this work was to synthesize calcium carbonate of various sizes using the precipitation technique. The synthesis of CaCO₃ was followed by basic synthetic route. The aqueous solutions of Na₂CO₃ and CaCl₂ were combined. Additionally, the precipitated calcium carbonate suspensions were homogenized for the time period of 5 and 10 min. The effect of time and homogenization on the size of precipitated calcium carbonate was compared. Figure 1. presents the changes of distribution curves of precipitated CaCO₃ in time and after the homogenization of the precipitate. After one day the particles become larger due to recrystallization process (Rodriguez-Navarro et al., 2007). On the other hand, the mechanical homogenization of formed particles decreased the size of precipitated calcium carbonate, but not considerably.

The second experimental part has been realized using the way described by Mishra et al. (2005). Calcite precipitation experiments were conducted in the presence of poly(ethylene glycol) PEG. The experimental procedure was as follows: 100 ml of 0.01M CaCl₂ (and Na₂CO₃, simultaneously) solution was mixed with appropriate amount of PEG. After one day two mixtures were combined while mixing on the magnetic stirrer.

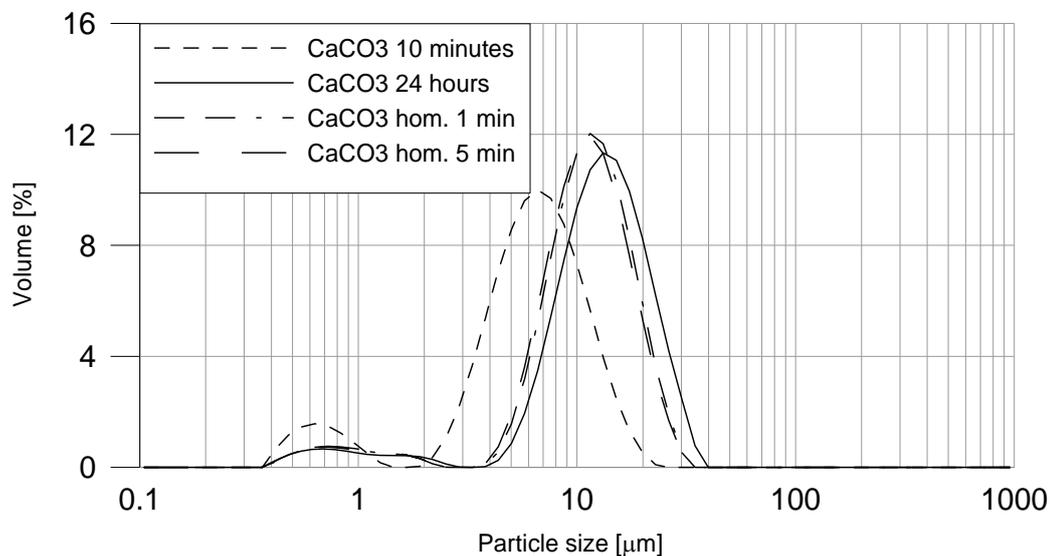


Fig. 1. Changes of size distribution curves for precipitated CaCO_3 in time and after homogenization at 24 000 rpm

Two concentrations (0.1 and 0.7%) of PEG 1000 were applied. The results obtained after 24 hours of synthesis (Fig. 2) were compared with the size distribution of precipitated CaCO_3 without polymer.

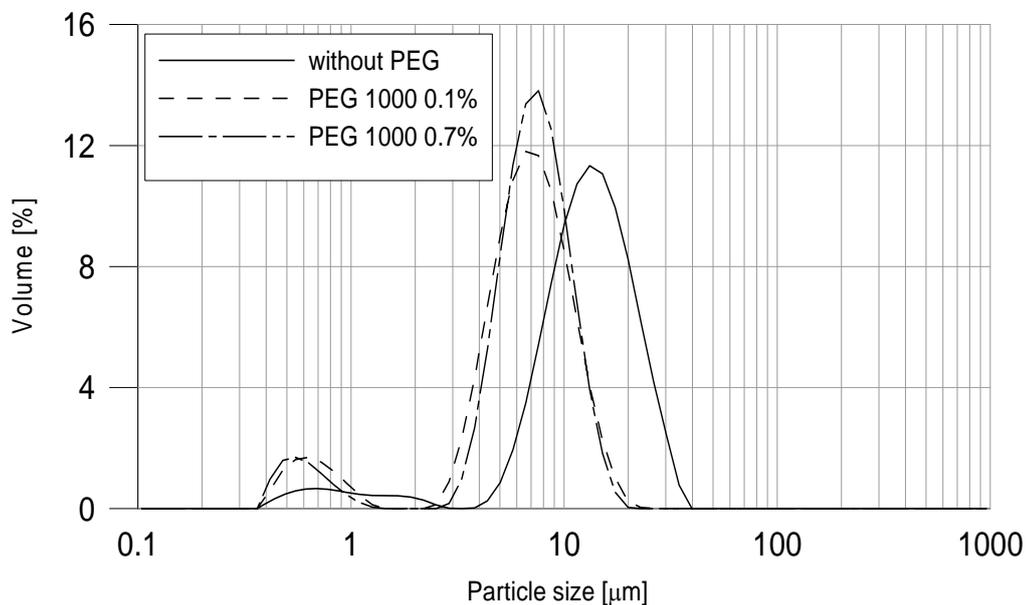


Fig. 2. The effect of PEG concentration on the size distribution curves of CaCO_3

The effect of molecular weight of polymer (PEG) at the same concentration (0.1%) was tested at the next series of experiments. As can be seen in Fig. 3 a and b, small changes of size distribution can be observed when the molecular weight of polymer has been changed from 1000 to 5000000.

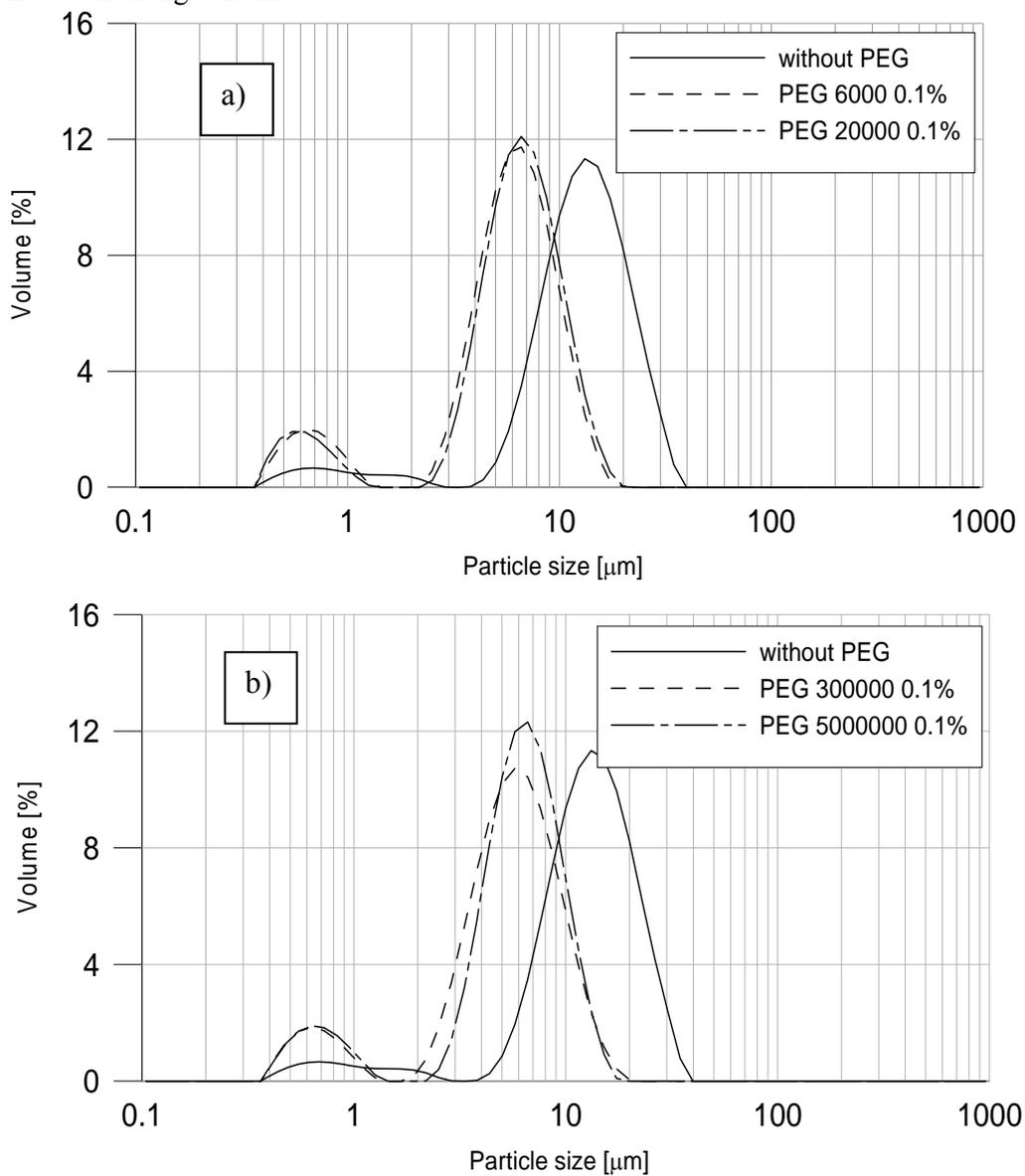


Fig. 3 a and b. The effect of the molecular weight of PEG on the size distribution of CaCO₃

The same procedure of salt solutions preparation was used for calcium ions precipitation in the presence of microbial filtrates (B4 and B6). Figure 4 (a,b) shows

the influence of both biosurfactant solutions (B4 and B6) on the size distribution of precipitated calcite particles. It is clear that the size distribution curve of calcium carbonate in the presence of B4 broth has changed as a function of time. This phenomenon can be explained by a specific interaction between biosurfactant and inorganic materials. It is known that biosurfactants play a role of the template to synthesize lithium iron phosphate (Li et al., 2009) or mesoporous hydroxyapatite (Zhao et al., 2008).

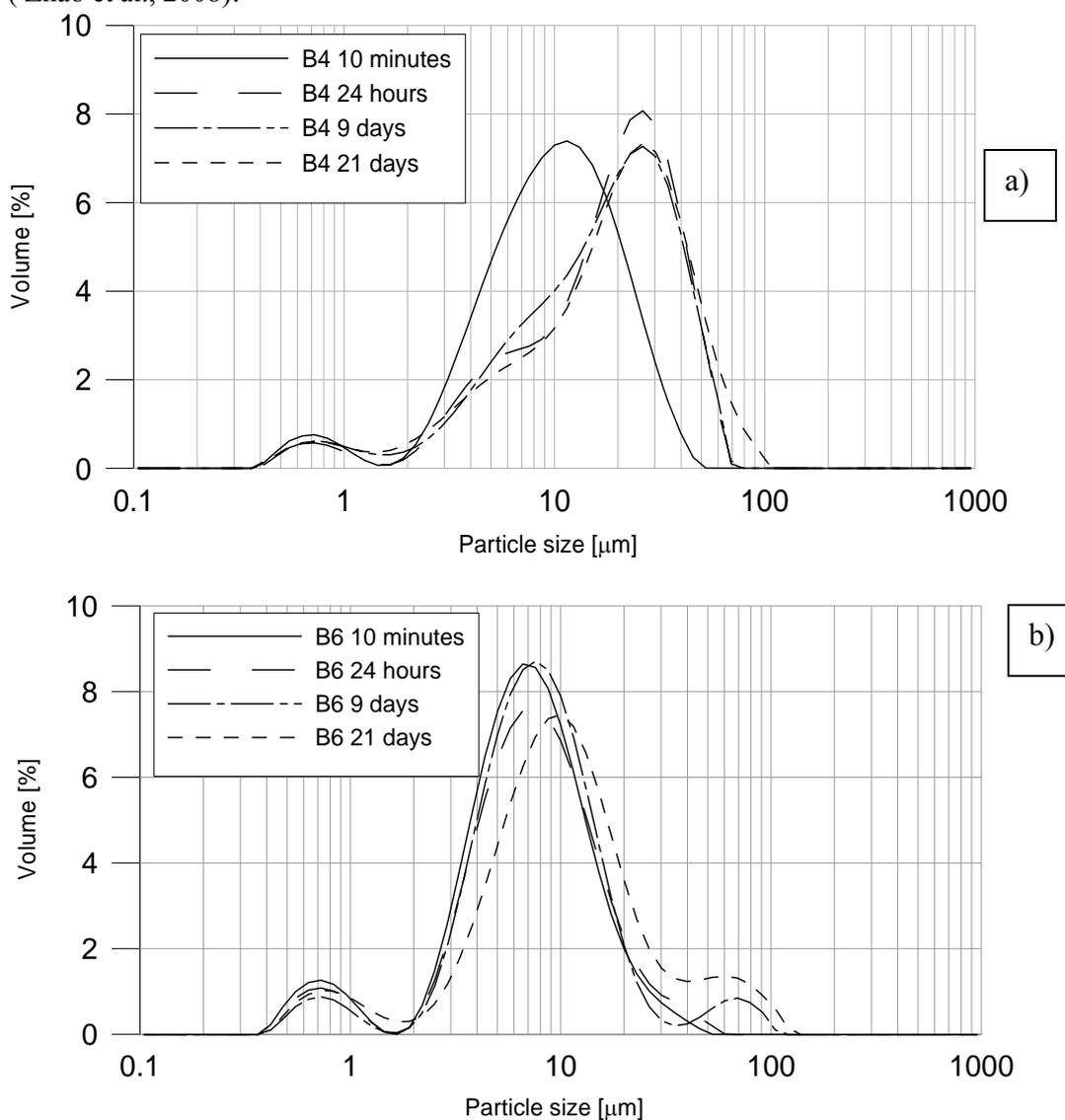


Fig. 4 a and b. The size distribution curves of CaCO_3 precipitated in the presence of the microbial filtrate

CONCLUSIONS

The calcium carbonate precipitation experiments were conducted to examine the effect of physical and chemical conditions of calcite synthesis on the size distribution of precipitated particles. From these experiments the following conclusions can be drawn:

1. The length of mixing period and the stirring speed have an essential influence on the size distribution of precipitated calcium carbonate.
2. The homogenization caused the aggregates' destruction.
3. The polymer (PEG) addition brings about smaller particles of CaCO₃. The size of particles depends on the polymer concentration. The molecular weight of polymer does not affect the particles size significantly.
4. The particles of CaCO₃ synthesized in the presence of microbial filtrate (B4) showed an increase of size in time. It can suggest a specific interaction between biosurfactant and precipitated particles.

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Zaprezentowano biomimetyczną procedurę zarodkowania wzrostu CaCO_3 przeprowadzając chemiczną syntezę „na mokro” koloidalnych cząstek CaCO_3 . Dokonano charakterystyki wytrąconych cząstek przez wyznaczenie ich składu ziarnowy. Badano też fizyczne i chemiczne parametry wytrąconych ziarn CaCO_3 . Badania te mogą dostarczyć nowego spojrzenia na precypitację węgla wapnia.

słowa kluczowe: węglan wapnia, nanoziarna, synteza, skład ziarnowy