

Biomimetic Fabrication of Nanoengineered Hydroxyapatite/Polyelectrolyte Composite Shell

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Biomimetic synthesis of biocompatible ceramic material, calcium hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, was performed exclusively inside poly(allylamine)/poly(styrene sulfonate) polyelectrolyte capsules of micron scale. Calcium hydroxyapatite mainly forms on the inner side of the capsule shell resulting in empty composite hydroxyapatite spheres of micron size. Depending on the amount of precipitated hydroxyapatite, the thickness of the shell of composite spheres can be varied between 25 and 150 nm. Hydroxyapatite spheres were characterized by TEM, SEM, and WAXS techniques. The influence of the inner capsule composition on the characteristics of the resulting hydroxyapatite material was also investigated.

Introduction

Biomimetalization and biomimetic synthesis are among the upcoming lines of investigations in modern material chemistry.^{1,2} On the basis of knowledge of the synthesis of inorganic materials by natural organisms, a wide range of new inorganic hybrid composites with unique properties can be obtained.^{3,4} For instance, CaCO_3 particles of different morphology and crystal modification (calcite, vaterite) can be synthesized in water solution at ambient conditions adding different amphiphilic block copolymers.^{5,6}

Polymeric capsules, recently proposed as a novel type of nano-engineered multifunctional material,^{7,8} can be utilized as microreactors for biomimetic synthesis. Layer-by-layer assembly of polyelectrolyte molecules on the surface of decomposable micron and submicron sized templates (inorganic or organic particles, biological cell^{9–11}) results in fabrication of hollow polyelectrolyte

capsules. The possibility of tailoring different functionalities, impregnating inorganic and organic substances both inside polyelectrolyte capsules and in their polyelectrolyte shell, and controlled release of encapsulated compounds provided continuous scientific and industrial interest in employing capsules as microcages for protection, increased solubility, and delivery of different compounds.

Polyelectrolyte capsules can be employed as excellent microreactors for carrying out chemical synthesis in their spatially restricted volume where one of the reagents can be entrapped. Magnetic ferrites and magnetite, ferric oxide, and CaCO_3 were synthesized exclusively inside polyelectrolyte capsules.^{12,13} It was found that the confined volume of the capsule microreactor affects crystallinity, surface morphology, and particle size of the resulting substances.

On the other hand, apatites, which are metal basic phosphates with chemical formula $\text{Me}_{10}(\text{PO}_4)_6(\text{OH})_2$ (Me is divalent metal), have received attention regarding biomimetalization because they are the main constituents of vertebrate bones and teeth.^{14,15} Apatites are of interest in biocompatible ceramics, and as catalysts, adsorbents for biomaterials, and ion exchangers. The characteristics of apatite material are influenced by the size and morphology of particles, crystallinity, and water content, etc.^{16,17} Hence, spatially restricted synthesis of

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(1) Mann, S.; Hannington, J. P.; Williams, R. J. P. *Nature* **1986**, 324 (6097), 565.

(2) Rautaray, D.; Sainkar, S. R.; Sastry, M. *Langmuir* **2003**, 19 (3), 888.

(3) Kulak, A.; Davis, S. A.; Dujardin, E.; Mann, S. *Chem. Mater.* **2003**, 15 (2), 528.

(4) Grassmann, O.; Muller, G.; Lobmann, P. *Chem. Mater.* **2002**, 14 (11), 4530.

(5) Cölfen, H.; Antonietti, M. *Langmuir* **1998**, 14 (3), 582.

(6) Sedlak, M.; Antonietti, M.; Cölfen, H. *Macromol. Chem. Phys.* **1998**, 199 (2), 247.

(7) Sukhorukov, G. B.; Donath, E.; Davis, S.; Lichtenfeld, H.; Caruso, F.; Popov, V. I.; Möhwald, H. *Polym. Adv. Technol.* **1998**, 9 (10–11), 759.

(8) Donath, E.; Sukhorukov, G. B.; Caruso, F.; Davis, S.; Möhwald, H. *Angew. Chem., Int. Ed.* **1998**, 37 (37), 2202.

(9) Sukhorukov, G. B.; Donath, E.; Lichtenfeld, H.; Knippel, M.; Budde, A.; Möhwald, H. *Colloids Surf., A* **1998**, 137 (1–3), 253.

(10) Sukhorukov, G. B. Designed Nano-engineered Polymer Films on Colloidal Particles and Capsules. In *Novel Methods to Study Interfacial Layers*; Mobius, D., Miller, R., Eds.; Elsevier Science B. V.: Amsterdam, The Netherlands, 2001; pp 384–414.

(11) Wang, D. Y.; Caruso, F. *Chem. Mater.* **2002**, 14 (5), 1909.

(12) Shchukin, D. G.; Radtchenko, I.; Sukhorukov, G. B. *Mater. Lett.* **2003**, 57 (3), 1743.

(13) Shchukin, D. G.; Radtchenko, I.; Sukhorukov, G. B. *J. Phys. Chem. B* **2003**, 107 (1), 86.

(14) Elliott, J. C. *Structure and Chemistry of the Apatite and Other Calcium Orthophosphates*; Elsevier: Amsterdam, The Netherlands, 1994.

(15) Cheng, Z. H.; Yasukawa, A.; Kandori, K.; Ishikawa, T. *J. Chem. Soc., Faraday Trans.* **1998**, 94 (10), 1501.

(16) Mortier, A.; Lemaitre, J.; Rodrique, L.; Rouxhet, P. G. *J. Solid State Chem.* **1989**, 78 (2), 215.

(17) Kandori, K.; Horigami, N.; Yasukawa, A.; Ishikawa, T. *J. Am. Ceram. Soc.* **1997**, 80 (5), 1157.

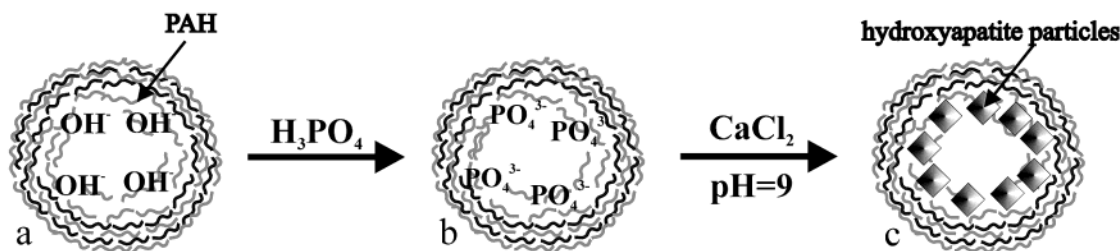


Figure 1. Schematic illustration of the calcium hydroxyapatite synthesis inside polyelectrolyte capsules.

apatites can result in formation of the material with new interesting properties.

In this paper a biomimetic synthesis of the most typical apatite, calcium hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, exclusively inside micron scale poly(allylamine hydrochloride)/poly(styrene sulfonate) polyelectrolyte capsules is accomplished employing the poly(allylamine hydrochloride)/ PO_4^{3-} complex captured in the capsule volume as a source of phosphate anions. The deposition of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ was performed from alkali water solution containing CaCl_2 .

Experimental Section

Materials. Poly(allylamine hydrochloride) (PAH, MW $\sim 50\,000$), sodium poly(styrene sulfonate) (PSS, MW $\sim 70\,000$), citric acid, CaCl_2 , NaCl , NaOH , and H_3PO_4 were obtained from Aldrich. All chemicals except PSS were used as received. PSS was dialyzed against Milli-Q water using a Spectra/Por membrane (MWCO 14 000) and then lyophilized. The water used in all experiments was prepared in a three-stage Millipore Milli-Q Plus 185 purification system and had a resistivity higher than $18\,\text{M}\Omega\cdot\text{cm}$.

MnCO_3 template particles of $3.6\text{-}\mu\text{m}$ diameter for polyelectrolyte capsule assembly were synthesized by adding $0.5\,\text{mL}$ of ethanol to $99.5\,\text{mL}$ of $0.008\,\text{M}$ MnSO_4 and $0.08\,\text{M}$ NH_4HCO_3 solution.¹⁸

Formation of Hollow Polyelectrolyte Capsules. Hollow PAH/PSS capsules loaded with PAH were employed as microreactors for hydroxyapatite synthesis. A mixture of MnCO_3 suspension ($5\cdot 10^8\,\text{mL}^{-1}$) with $5\cdot 10^{-3}\,\text{M}$ solution of citric acid was taken and then the PAH solution ($1\,\text{mg/mL}$) was stirringly dropped. To obtain capsules containing $0.1\,\text{monoM}$ (molar concentration of aminogroups) PAH inside, $0.7\,\text{mL}$ of PAH solution was mixed with $1\,\text{mL}$ of MnCO_3 suspension; for $0.04\,\text{monoM}$ PAH inside, $0.28\,\text{mL}$ of PAH solution was added. The deposition of the water insoluble PAH/citrate complex on the surface of template particles was followed by layer-by-layer assembly¹⁹ of polyelectrolyte PAH/PSS multilayers using $1\,\text{mg/mL}$ PAH solution and $2\,\text{mg/mL}$ PSS solution. After formation of PAH/PSS shells, the MnCO_3 core was dissolved in $0.1\,\text{M}$ HCl and hollow capsules composed of the inner thick PAH/citrate layer and the outer PAH/PSS layer were formed. More detailed description of the synthesis of hollow PAH/PSS capsules can be found elsewhere.^{7–10} Before loading the initial PAH/PSS capsules with PO_4^{3-} anions they were kept in NaOH solution, pH 9, for 24 h.

Characterization. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) were performed to investigate the original structure and inner composition of the synthesized polyelectrolyte shell/hydroxyapatite particles using a Gemini Leo 1550 instrument. To view the interior of the capsules, the samples were embedded in poly(methylmetacrylate) and then ultrathin sections ($30\text{--}100\text{-nm}$ thick) were obtained by a Leica ultracut UCT ultramicrotome. Carbon-coated or noncoated copper grids were used to support

the thin sections, and a Zeiss EM 912 Omega transmission electron microscope (TEM) was employed for analysis. The crystal structure of the resulting apatite was determined from wide-angle X-ray scattering with an Enraf-Nonius PDS-120. To measure the quantity of hydroxyapatite, we weighed an equal number of empty and loaded capsules with a quartz crystal microbalance (QCM). Equal quantities of empty and loaded capsules were estimated taking into consideration different concentration of capsule suspension before and after hydroxyapatite synthesis measured by confocal fluorescent microscope operating in bright field mode.

Results and Discussion

The shell of polyelectrolyte capsules was shown to be permeable for ions and small organic molecules, whereas large polymer molecules as well as nanoparticles can be captured inside.⁹ This enables introduction of polyelectrolytes possessing ion-exchange properties in capsule volume. Thus, the interior of the polyelectrolyte capsule can be filled with desired anions (cations) participating in further chemical reaction in the capsule volume. PAH/PSS capsules with positively charged PAH macromolecules inside can act as microcontainers for PO_4^{3-} anions. Poly(allylamine hydrochloride) forms stable complexes with multi-charged anions such as PO_4^{3-} preventing their diffusion from the capsule volume to the surrounding solution.⁹

Figure 1 schematically illustrates a procedure of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ hydroxyapatite synthesis exclusively in the capsule volume. At first, polyelectrolyte capsules doped with OH^- ions were treated for 24 h with $0.1\,\text{M}$ H_3PO_4 . Preliminary doping of polyelectrolyte capsules with OH^- ions is necessary to ensure complete filling of the capsule volume with PO_4^{3-} via performing a neutralization reaction. Then the polyelectrolyte capsules were washed and added to the solution containing $1\,\text{M}$ CaCl_2 at pH 9 (adjusted by NaOH). A similar synthesis of hydroxyapatite was developed previously for water solutions.^{14,15} Capsules were immersed in alkali CaCl_2 solution for 48 h. Such a long reaction time is required to consume all PO_4^{3-} anions from the inner PAH/ PO_4^{3-} complex. If the reaction time exceeds 48 h the amount of synthesized hydroxyapatite does not change (as confirmed by QCM weighing of the samples) which evidences the end of the reaction inside the capsule volume. During the reaction no hydroxyapatite precipitate was observed in solution. The average weight of one polyelectrolyte capsule after finishing the reaction was $\sim 30\,\text{pg}$; for comparison the average weight of the initial PAH-loaded PAH/PSS is around $10\,\text{pg}$.

A lower PAH concentration (and, as follows, PO_4^{3-} concentration) inside polyelectrolyte capsules decreases the amount of the synthesized $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. For example, at $0.1\,\text{monoM}$ PAH concentration the average

(18) Hamada, S.; Kudo, Y.; Okada, J.; Kano, H. *J. Colloid Interface Sci.* **1987**, *118* (2), 356–365.

(19) Decher, G. *Science* **1997**, *277* (5330), 1232.

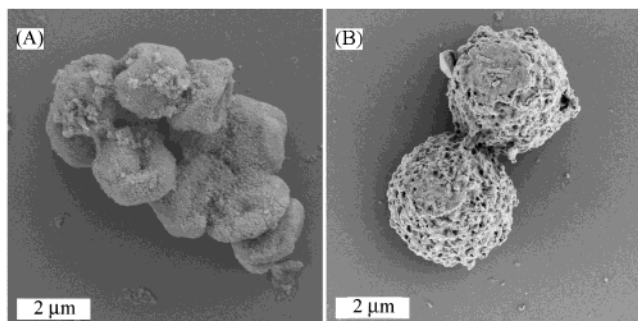


Figure 2. Scanning electron microscopy images of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ precipitated inside polyelectrolyte capsules containing 0.04 monoM PAH (a) and 0.1 monoM PAH (b).

weight of hydroxyapatite-loaded capsules is 30 pg; at 0.04 monoM PAH concentration the average weight of hydroxyapatite-loaded capsules is 20 pg. A similar effect of the PAH concentration inside polyelectrolyte capsules on the structural properties of hydroxyapatite-loaded capsules was observed during scanning and transmission electron microscopy investigation. As shown in previous studies,⁹ initial PAH-containing PAH/PSS capsules collapse upon drying resulting in overlapping flat structures with folds and creases, but the presence of inorganic nanoparticles inside maintains a bulky shape forming a rigid scaffold inside the capsule.¹³ Hydroxyapatite particles also stabilize the bulky shape after drying (Figure 2), however, the diameter and morphology of the resulting solid particles drastically depend on the amount of synthesized hydroxyapatite inside. Capsules containing 10 pg of hydroxyapatite (0.04 monoM PAH) are considerably shrunken (Figure 2a) and have a diameter of around $1.8 \mu\text{m}$ (diameter of initial PAH/PSS capsules was $3.6 \mu\text{m}$). Moreover, these dried capsules are partially collapsed and agglomerated. In contrast, capsules containing 20 pg of hydroxyapatite inside (0.1 monoM PAH) have a size close to that of initial capsules in solution (Figure 2b). Such effect of decreasing the capsule diameter was previously observed in ref 20. Considerable differences between sizes of dried hydroxyapatite-containing polyelectrolyte capsules can be explained by a different content of both apatite material and PAH inside which increase the stability of the capsules against osmotic pressure.

Figure 3 shows TEM images of polyelectrolyte capsules of different PAH concentration with $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ hydroxyapatite inside. On these images hydroxyapatite particles are seen as dark spots; the inserts in Figure 3 depict the capsule wall composed of hydroxyapatite nanoparticles at higher magnification. TEM analysis establishes the preferable formation of the hydroxyapatite nanoparticles on the inner side of the PAH/PSS shell resulting in empty hydroxyapatite spheres. This phenomenon can be explained by several reasons: higher PAH concentration near the inner side of the capsule wall (as confirmed by confocal microscopy); lower energy required for heterogeneous nucleation of hydroxyapatite on the capsule wall compared to that required for the homogeneous nucleation process in solution; and blockage of the shell pores by hydroxyapatite precipitate preventing, thereby, Ca^{2+} diffusion to

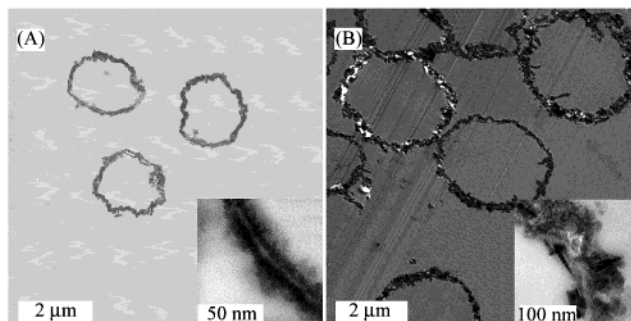


Figure 3. Transmission electron microscopy images of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ spheres precipitated inside polyelectrolyte capsules containing 0.04 monoM PAH (a) and 0.1 monoM PAH (b).

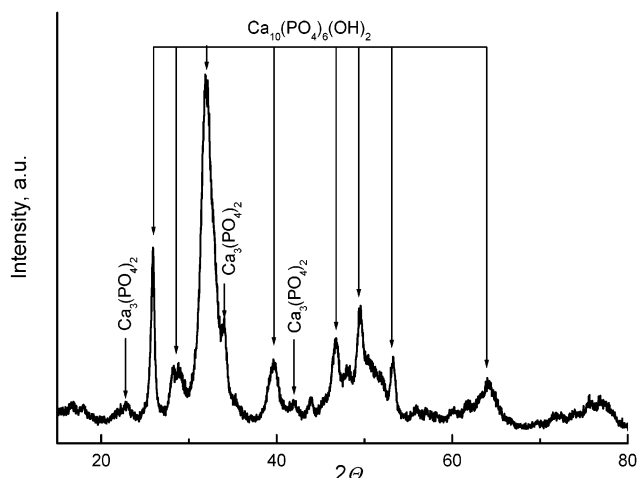


Figure 4. XRD pattern of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ formed in capsule volume.

the capsule center. Such changes in polyelectrolyte membrane permeability were also observed during nanoparticle precipitation inside flat polyelectrolyte films.²¹ Thickness and particle size of the $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ layer greatly depend on the amount of PAH, and, as a consequence, on the quantity of synthesized hydroxyapatite material. For 0.1 monoM PAH a continuous 100–120-nm layer made of 12–16-nm particles is observed while a 25-nm thin layer built from 5–8-nm particles is formed in the capsules containing 0.04 monoM PAH.

Identification of hydroxyapatite material inside polyelectrolyte capsules was made using X-ray scattering. As shown in Figure 4, the apatite material obtained is crystalline calcium hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Several peaks corresponding to the $\text{Ca}_3(\text{PO}_4)_2$ phase were found, indicating the presence of calcium phosphate as minor component. The Ca/P ratio, estimated from EDX data, is 1.66, which is in close agreement with the Ca/P value for pure hydroxyapatite (1.67).

Conclusions and Outlook

In this presented work we demonstrated the possibility of performing biomimetic synthesis of a biocompatible ceramic material, calcium hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, exclusively inside polyelectrolyte capsules

(20) Leporatti, S.; Gao, C.; Voigt, A.; Donath, E.; Mohwald, H. *Eur. Phys. J. E* **2001**, 5 (1), 13.

(21) Joly, S.; Kane, R.; Radzilowski, L.; Wang, T.; Wu, A.; Cohen, R. E.; Thomas, E. L.; Rubner, M. F. *Langmuir* **2000**, 16 (3), 1354.

of micron scale. Calcium hydroxyapatite mainly forms on the inner side of the capsule shell resulting in empty composite hydroxyapatite spheres of micron size (so-called "nanoeggs"). Depending on the amount of precipitated hydroxyapatite, the thickness of the shell of composite spheres can be varied between 25 and 150 nm. Fabricated hydroxyapatite particles have shape and surface morphology different from the particles synthesized by common methods in solution. Other peculiar properties of hydroxyapatite composite hollow shell such as surface acidity, catalytic and biological activity, etc. can be expected. The developed approach of hydroxyapatite formation inside the polyelectrolyte capsules illustrates perspectives to employ these capsules as

microreactors for biomimetic synthesis of biocompatible materials and also can be used for modeling biomineralization processes in natural cells. Hydroxyapatite composite capsules can find application as medical agents for bone repairing, catalytic microreactors, etc.

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