Chemisorption of silane compounds on hydroxyapatites of various morphologies

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Functionalized-inorganic powders were obtained by the chemisorption of a series of substituted trimethoxysilanes on the surface of spherical and hexagonal hydroxyapatite crystals. The highest total loading for all trimethoxysilanes was achieved for the hydroxyapatite (CaHAP) of spherical morphology. For hexagonal CaHAP, the loading decreased with an increase in the length to diameter ratio from 0.5 to 11. Thus, the different faces of the crystal are not equally reactive: {0001} planes are more reactive than {10$\bar{1}$0} planes.
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As the main mineral component of mammal bones, calcium hydroxyapatite (CaHAP) $Ca_{10}(PO_4)_6(OH)_2$ is attracting attention of various research groups around the world. In particular, chemisorption of organic substances on CaHAP surfaces may convert it from a hydrophilic to a hydrophobic material and/or may improve its mechanical properties [1,2]. Such materials can be used in a wide variety of applications, for example as filler in polymer composites [3], catalysts [4], adsorbents for heavy metals in environmental applications [5], immobile phases in high-performance chromatographic systems [6], carriers for bioactive and pharmaceutical ingredients or components for slow-release formulations [7–12]. Due to their ion-exchange ability, adsorption capacity and acidic properties, such hybrid organic-mineral materials can also be used as effective ion exchangers, sorbents, composites for tissue engineering and carriers for immobilized enzymes. In addition, modified hydroxyapatite particles have been suggested as carriers for drugs, such as interferon alpha, testosterone enanthate and cyclosporin A [13].

However, to date, most materials were obtained via the formation of hydrogen or ionic bonds between the CaHAP surface and organic substrates, or via physical adsorption [14,15]. Only a few publications are devoted to the synthesis of these materials based on the formation of covalent bonds by grafting of organic substances on the CaHAP surface [3,16–19]. However, the widespread application of tailored CaHAP-based materials (e.g. as drug carriers or biocompatible slow-release materials) requires an in-depth investigation of the grafting mechanisms and the interconnection between the chemistry of the organic modifiers and the CaHAP morphological microstructure.

A well-known technique for the covalent grafting of organic molecules on surfaces is the reaction of surface hydroxyl groups with substituted trimethoxysilanes [20,21]. In the present work we studied the covalent bonding of three substituted trimethoxysilanes (3-amino propyltrimethoxysilane, *N*-[3-(trimethoxysilyl)propyl]-ethylenediamine and 2-(2′-pyridyl)-ethyltrimethoxysilane) to the surface of CaHAP particles of different morphology. The first trimethoxysilane is widely used for the functionalization of surfaces of other materials (silica gel, MCM-41, etc.) by NH_2 groups, which allows further immobilization of a large variety of organic molecules. The second compound serves as a ligand for metal complexes that makes it possible to immobilize metal cations on CaHAP surface. Such organic/inorganic hybrid materials may then be used as effective catalysts or sorbents. The last compound containing pyridine

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groups can be used for obtaining metal complexes that are reactive to primary or secondary amines (e.g. metal carbonyls).

A high density of the functional group on the carrier's surface is important for obtaining increased activity and/or improved adsorption capacity for metals. However, different faces of the CaHAP crystals (side and end) may have different surface densities of P–OH groups, which can react with trimethoxysilanes. Thus, one objective of this study is to obtain functionalized CaHAP materials and to determine the effect of CaHAP morphology on its surface reactivity with trimethoxysilanes.

We used four types of samples of CaHAP with different morphology, i.e. spherical (type I) and hexagonal (types II–IV) crystals. The samples were synthesized by the hydrothermal method (described in the supplemental data). The X-ray diffraction (XRD) patterns of CaHAP powders contain only CaHAP peaks. No significant amount of amorphous phase was detected [22], and all four samples are crystalline materials. However, some differences between the patterns of the spherical porous CaHAP (type I) and the hexagonal nonporous CaHAP (types II–IV) are observed: the peaks in the pattern of type I are broader, indicating smaller crystallites.

SEM images of the samples show different sizes and morphologies of the crystals (Fig. 1). Type I particles have almost spherical shape and are relatively small. Sample types II–IV consist of much bigger hexagonal particles with different aspect ratios. Density data (see
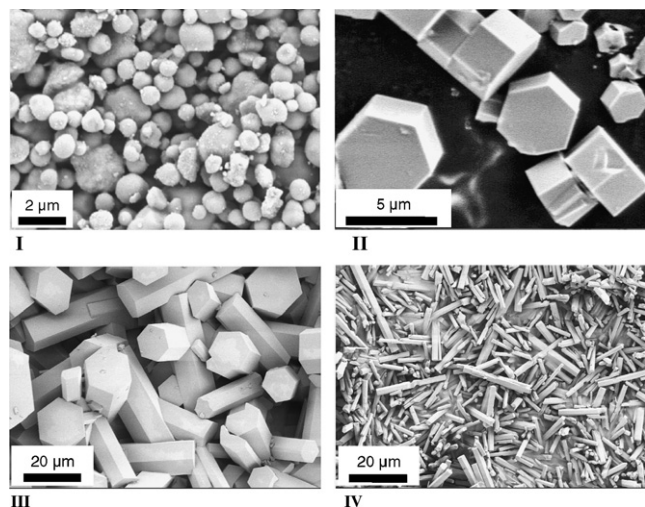
Table 1) show significant differences between type I and types II–IV. Taking into account the theoretical density of CaHAP ($3.15 \, g \, cm^{-3}$), it can be concluded that all hexagonal particles are single crystals and do not exhibit any porosity. In contrast, spherical particles (type I) contain a significant void volume. The XRD data together with density data suggest a complex structure of the spherical particles. Consequently the internal structure of type I was investigated by high-resolution transmission electron microscopy (TEM) (Fig. 2). The image shows that the spherical particles consist of randomly packed tiny platelets with a size of about 10–15 nm.

The effect of crystal morphology on the properties of CaHAP was illustrated by the measurement of electrophoretic mobility, which is proportional to the surface charge. The data in Table 1 show that all hexagonal particles (types II–IV) possess negatively charged surfaces. Types III and IV have similar values of the aspect ratio and show quite similar negative values of the electrophoretic mobility. In contrast, the surface of the spherical particles (type I) is positively charged. It is known that hydroxyl and phosphate groups on hydroxyapatite are responsible for negative surface charges, while calcium-rich surfaces gain a positive charge [23]. Charge differences between morphologically different CaHAP samples can be attributed to a different distribution of the charged groups on the surface of the crystals.

The presence of two different HA surfaces (planes {0001} and {1010}) with different reactivity was first confirmed during the separation of proteins and nucleic acids of different nature using column chromatography [24]. It was shown that acidic compounds were mainly adsorbed on one type of surface, while basic proteins are adsorbed on another. Further studies of protein
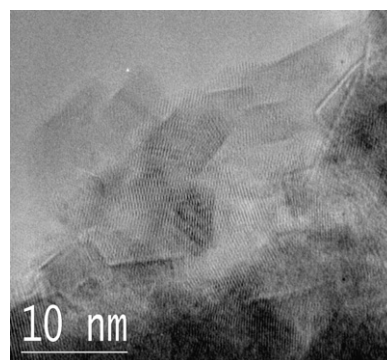

Figure 1. SEM images of CaHAP sample types I–IV.

Figure 2. TEM image of sample type I.

Table 1. Properties of hydroxyapatites and trimethoxysilanes loading

| CaHAP	BET surface area ($m^2 \, g^{-1}$)	Density ($g \, cm^{-3}$)	Porosity (%)	Particle size (µm)	Aspect ratio	Electrophoretic mobility ($µm \, s^{-1}$)/(V cm^{-1})	Loading of organic compounds (mmol g^{-1})		
							1	2	3
I	168	2.80	8.9	0.8	1.00	+0.84	0.73	1.23	1.34
II	12	3.10	<1	5.3	0.51	−1.95	0.21	0.44	0.35
III	15	3.14	<1	10.2	6.7	−2.76	0.12	0.37	0.31
IV	13	3.12	<1	19.4	11.1	−2.62	0.11	0.31	0.26

adsorption on the surface of different calcium phosphates showed that these materials contain two types of adsorbing sites: positively charged calcium sites and negatively charged phosphate sites [25]. Considering our data of the surface charges of the samples, we suggested that sample type I possesses a Ca-rich surface, while the surfaces of the types II–IV are phosphate rich.

As shown before, the surface of CaHAP contains P–OH groups of an acidic nature, while the Ca atoms are covered by excess phosphate ions [26]. These surface hydroxyl groups are also the reactive centers for the chemisorption of trimethoxysilanes. Thus, we can interpret the difference in charge between hexagonal and spherical CaHAP in terms of the variation in the fraction of acidic surface hydroxyl groups. The difference between the hexagonal types II–IV might be interpreted in terms of the ratio between the surface contributions by the $\{10\bar{1}0\}$ plane (sides) and $\{0001\}$ plane (ends) faces of the crystals. If these crystal planes (side and end faces) possess different charges (and consequently different affinity to the organic substrates), one can expect the CaHAP morphology to affect the chemisorption of organic groups and the chemical reactivity of the various samples.

In the case of spherical polycrystalline CaHAP, particles are comprised of tiny platelets (see Fig. 2). We believe that these tiny platelets may be attracted to each other due to differences in surface charges on the $\{0001\}$ and $\{10\bar{1}0\}$ faces of the hexagonal nanocrystals which may be responsible for the agglomeration. The Ca-rich surface of type I is expected to have the lowest amount of hydroxyl groups and, respectively, the lowest reactivity to trimethoxysilanes.

Surface modification was conducted by the following method. Dry CaHAP (1 g) was suspended in 100 ml of dry toluene. Then 10 m mol of trimethoxysilane was added and the reaction mixture was refluxed for 6 h (Scheme 1). Next, the modified CaHAP was filtered, washed with toluene and diethyl ether and dried in vacuum overnight.

Chemisorption of trimethoxysilanes was confirmed by Raman studies of the samples. A Raman spectrum of parent CaHAP (type I) contains the characteristic bands associated with the PO$_4$ vibrations (Fig. 3). The
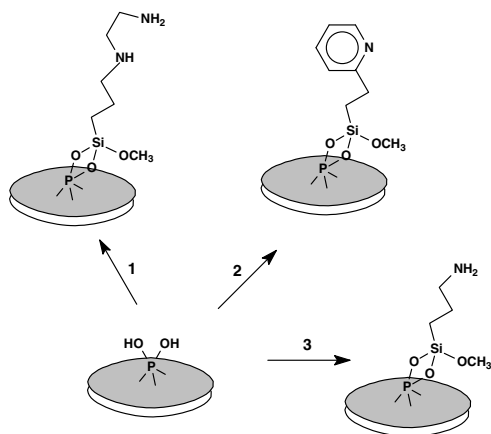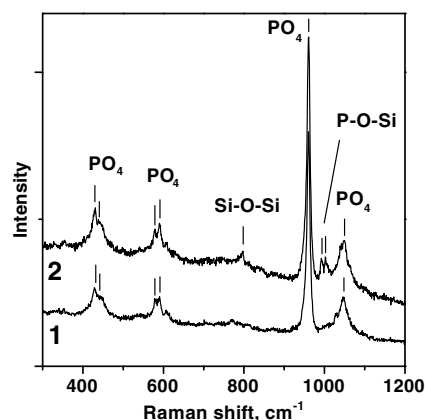
Figure 3. Raman spectra of sample I (1, parent; 2, modified by 2-(2′-pyridyl)-ethyltrimethoxysilane).

most intense band at 965 cm^{-1} corresponds to the symmetric stretching vibrations of phosphate ions [27]. Other phosphate modes are also present in the spectra at 430, 440, 576, 585 and 1050 cm^{-1}. After modification, a new doublet appeared at 990–1003. We associate this band with the vibrations of the Si–O–P bonds that were formed during the reaction of surface phosphate hydroxyls with trimethoxysilane groups. The vibration of this bond in modified CaHAP materials has never been studied by Raman spectroscopy. However, its position in infrared spectra was reported at 998 cm^{-1} [28]. Another new band appeared in all spectra of modified CaHAP at 796 cm^{-1}. The presence of this band may indicate a coupling between two or more molecules of trimethoxysilanes with the formation of Si–O–Si bonds. These bonds appear because of cross-linking reaction that involve one of many possible reactions between silane compounds. For example, one possible reaction could involve reaction of the third methoxy group of neighbouring trimethoxysilanes compound already attached to a CaHAP surface (Scheme 1).

As can be seen in Table 1, the specific surface of CaHAP varies considerably between type I and types II–IV. This variation strongly affects the chemosorption of trimethoxysilanes. The highest total loading for all three types of organic modifiers was achieved for the spherical CaHAP which has the highest specific surface. However, the data in Table 1 do not show direct proportionality between total loading and surface area. TEM, shows spherical CaHAP consists of nano-size platelets, these have a high affinity for chemisorption of trimethoxysilanes, as indicated by the surface charges. However, the surface density of the amine groups is relatively small. A likely explanation is the small amount of hydroxyl groups on the Ca-rich surface of type I.

In the hexagonal samples, the loading decreases from type II to type IV, i.e. with an increase of the aspect ratio and increase of the crystals' length. Thus, the different faces of the crystal are not equally reactive, and it can be concluded that end faces are more reactive and can be used to adsorb a higher loading of organic modifiers. These results are in good agreement with the data on electrophoretic mobility (see Table 1).

Scheme 1. Chemisorption of trimethoxysilanes on CaHAP surfaces.

It is known from the literature that the surface of CaHAP contains about 2.6 P–OH groups nm^{-2} [29]. Inspection of the data regarding amine adsorption in Table 1 suggests the existence of multilayer chemisorption of the trimethoxysilanes on CaHAP of types II–IV. Based on the results of Table 1, this multilayer can reach a thickness of at least 2–3 amine monolayers. Such phenomena for a similar process have been reported in the literature [3]. Formation of polysiloxane multilayers can be facilitated by surface chemical reactions, e.g. by the formation of Si–O–Si bonds. The small peak at $790 \, cm^{-1}$ in the Raman spectrum can be attributed to the formation of such bonds [30]. Investigation of the mechanism of multiplayer adsorption is a focus of future studies.

In summary, we reported the modification of CaHAP particles of different morphologies with trimethoxysilanes containing different substituents. For most CaHAP samples the maximum loading was observed for 2-(2′-pyridyl)-ethyl groups, while the loading of *N*-(2′-amino-ethyl)-3-aminopropyl groups was the lowest. It was shown that the morphology of CaHAP significantly affects the chemisorption of trimethoxysilanes on the surface: for hexagonal samples the total amount of chemisorbed compounds decreases with the increase of CaHAP aspect ratio. Thus, the different faces of the crystal are not equally reactive, and it can be concluded that {0001} faces are more reactive than side planes {1010}. The spherical sample possesses the lowest reactivity of the surface to trimethoxysilanes. Modified CaHAP particles might serve as intermediates for grafting various biologically active substances, such as coupling agents for biomedical composites.

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.scriptamat.2007.12.014.

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