Surfactant-Assisted Hydrothermal Synthesis of Hydroxyapatite Nanopowders

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Rod-like hydroxyapatite nanoparticles (n-HAp) with a highly ordered nanostructure were prepared by hydrothermal synthesis from calcium chloride, and phosphoric acid, as calcium and phosphorus sources, respectively. Various surfactant families such as cationic (CTAB), anionic (SDS) and non-ionic (Triton X-100) were used as regulators of the nucleation and crystal growth. The synthesized nanopowders were characterized using X-ray diffraction (XRD), Fourier transform infrared spectrograph (FTIR) and transmission electron microscopy (TEM). The rod-like morphology was obtained regardless of the surfactant used during the hydrothermal treatment, but the aspect ratio of the crystals was found to be surfactant dependent. The mechanism of crystal growth as well-oriented nanostructure is discussed.

Keywords: Hydrothermal, Hydroxyapatite, Nanopowder, Surfactant, Morphology, Electron Microscopy.

1. INTRODUCTION

Hydroxyapatite (denoted as HAp), with the chemical formula Ca₁₀(PO₄)₆(OH)₂, was introduced by Albee in 1920 for the first time and applied in a variety of forms. Currently, it is commonly used as raw material for the preparation of artificial bone grafts.¹,² It is also a promising material as reinforcing filler for composites,³,⁴ sensor agents,⁵,⁶ and chromatofluor for simple and rapid fractionation of proteins and nucleic acids.⁷,⁸ The features of nano-hydroxyapatite powders (n-HAp), including the small size of particles/crystallites, chemical and mineral compositions analogue to those of natural bone, make n-HAp an important material for applications in bone regeneration⁹ and tissue engineering.¹⁰ The properties of n-HAp, including bioactivity,¹¹ bioocompatibility,¹²-¹⁴ solubility,¹⁵,¹⁶ osteoconductivity,¹⁷,¹⁸ osteoinduction¹⁹,²⁰ and absorption usually depend on the control of the particles’ composition, particle size distribution, morphology and surface area, etc., and therefore, on the powder preparation method. For these reasons, it is of great importance developing synthesis methods focused on the precise control of particle size, morphology and chemical composition of n-HAp.

A number of techniques have been developed to prepare hydroxyapatite nanopowders with various morphologies. They include solid state reaction,²¹,²² sol–gel,²³-²⁵ hydrothermal,²⁶-²⁸ and microemulsion or emulsion methods.²⁹,³⁰ Recently, surfactant-mediated hydrothermal processes have shown great promises for the elaboration of nanoparticles with controlled morphology.³¹ While conventional methods tend to originate irregular morphologies and particle aggregates larger than 100 nm, the hydrothermal method proved to have some advantages in preparing crystalline materials (including salts, metal oxides, etc.) at relatively low temperatures, but the control on morphology is still unsatisfactory.³²

Surfactant template systems offer the opportunity of exploring the complementarity geometric, electrostatic and stereochemical molecular/ionic interactions in an attempt to control the nucleation and growth of inorganic materials.³³ Shanthi and coworkers³⁴ reported the preparation HAp nanorods with ≈20 nm diameter and 100–120 nm length by co-precipitation at ambient temperature and pressure in the presence of a cationic surfactant.
The same research group has successfully prepared shell-like nano-HAp spheres of varied morphology, with uniform size (≈200 nm) using Cetrimide as surfactant. Saha coworkers synthesized HA nanopowder by reverse microemulsion technique, using calcium nitrate and phosphoric acid as precursor salts dissolved in water. It was found that CTAB played a significant role in regulating the morphology of the nanoparticles.

This work reports on the synthesis of n-HAp nanoparticles in the presence of different kinds of surfactants to explore the effect of the type of surfactant (cationic, anionic, and non-ionic) on the formation of calcium phosphates. Cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulphate (SDS) and Triton X-100 [C14H22O(C2H4O) were used as surfactants. In our experiment, we investigated the behaviour of different surfactants in preparation and discussed the difference between them. Their influence on the nucleation, crystal growth and morphology of n-HAp is discussed.

2. MATERIALS AND METHODS

2.1. Materials

Calcium chloride (CaCl2 > 97%) and cetyltrimethylammonium bromide (CTAB: CH3(CH2)15N(CH3)Br) were purchased from Fluka. Sodium dodecyl sulfate > 99% (SDS: CH3(CH2)11SO3Na) was purchased from Merck. Phosphoric acid H3PO4 (85%) and ammonia NH4OH (25%) were used as precursors to prepare n-HAp Nanopowders. However, Triton X-100 was added drop wise to PO43− solution. However, Triton X-100 was added directly to the anionic solution.

2.3. Characterization of n-HAp Samples

The crystalline phases of all synthesized samples were identified by X-ray diffraction (XRD) using a D8 Discover X-ray diffractometer. Scans were run from 10° to 80° (2θ) with a step of 0.02° using CuKα (wavelength of 1.5418 Å) incident radiation. Cell parameters of the HAp phase were calculated through TOPAS 4.2 (Bruker AXS). The average crystal size, D, was calculated using the Debye-Scherrer equation:

$$D_{hkl} = \frac{K \lambda}{\beta_{1/2} \cos(\theta)}$$

where $D_{hkl}$ is the length of the crystallite in a direction perpendicular to the reflection planes (hkl), K is the Sherrer constant approximately equal to unity and related to crystallite shape and chosen as 0.9 for the elongated apatite crystallites, $\lambda$ is the X-ray wavelength equal to 1.5418 Å for CuKα radiation, θ the Bragg’s diffraction angle (in degree), $\beta_{1/2}$ the full width at half maximum (FWHM) expressed in radians for the peak of the (300) plane, representative of the crystal size along the a-axis, and of the peak of (002) plane, which represents the crystal size along the c-axis. Fourier transform IR spectra were collected on a FTIR 2000 within the range of 4000–400 cm−1 to confirm the presence of OH and PO4 functional groups. The morphology of the particles was assessed by TEM (Tecnai G2 Series from FEI Company) observations.

3. RESULTS

The wide angle (2θ > 10°) X-ray diffraction patterns of the obtained samples are shown in Figure 1. The patterns have been normalized to enable a better comparison. It can

![Fig. 1. XRD patterns of calcined (550 °C, 6 h) n-HAp nanopowders prepared at 90 °C for 24 h in the presence of different surfactants: (a) CTAB, (b) SDS, (c) Triton X-100. The three patterns have been normalized.](image-url)
be seen that all the diffraction peaks could be indexed to hexagonal HAp crystals. There is a good consistency between the data from our samples prepared by hydrothermal surfactant assistance and those reported in the standard data base, with lattice dimensions of \( a = b = 9.418 \) Å, \( c = 6.884 \) Å, with space group \( P6_3/m \) (176, (Joint Committee for powder Diffraction Standards JCPDS No. 09-0432). No crystalline phases other than pure HAp were detected in the XRD patterns, confirming the formation of single phase n-HAp crystals in all samples. Thus, standard HAp with hexagonal structure was formed during synthesis, which remained stable after calcination.

The FTIR spectra presented in Figures 2(a)–(c) provide further confirmation about the apatite nature of the hydrothermally synthesized samples in the presence of CTAB, SDS, and Triton X-100, respectively. All n-HAp powders exhibit characteristic bands due to \( \text{PO}_4^{3-} \) ions \( (\nu_1 - 966 \text{ cm}^{-1}, \nu_2 - 1020 \text{ and } 1120 \text{ cm}^{-1}, \nu_3 - 574 \text{ and } 609 \text{ cm}^{-1}) \), which tend to coincide with the results of the previous study, with the strong PO stretching bands at about 1100 and 600 cm\(^{-1}\) being particularly well noticed. The presence of hydroxyl group, characteristic of the standard HAp, could be confirmed in the case of n-HAp powder synthesised in the presence of the non-ionic surfactant from the stretching vibration at 3570 cm\(^{-1}\) and libration mode at 630 cm\(^{-1}\). The peak at 3570 cm\(^{-1}\) is attributed to stretching vibration of \( \text{OH}^- \) ions in the HAp lattice. However, it is difficult to explain why the presence of hydroxyl group is not apparent in the n-HAp powders synthesised in the presence of the other surfactants. The highly sensitive FTIR results indicated that no surfactant molecules eventually adsorbed in the as synthesized powders remained in the samples after the heat treatment.

The crystallite size data derived from the XRD peaks 002 (conscripted as \( X_{002} \)) and 300 (conscripted as \( X_{300} \)) are collected in Table I.\(^1\) The crystallite size measured from \( X_{002} \) matches to \( c \) axis, while \( X_{300} \) value corresponds to \( a \) axis. The results of these calculations show clearly that all the values are comprised between \( \sim 13 \) and \( \sim 46 \) nm size for all the powders.

These results show that although pure HAp has been obtained irrespective of the kind of added surfactant, the crystallite size and the intensity of diffraction peaks are dependent on type of surfactant used. The crystallite size scale reasonable well with the molecular size of the surfactants/micelles. But the XRD peaks are sharper in the case n-HAp-Triton X-100 powder (Fig. 1(c)), followed by that prepared in the presence of cationic surfactant (CTAB, Fig. 1(a)). Accordingly, the XRD peaks are broadest for the HAp-SDS sample (Fig. 1(b)).

The morphological features of calcined hydroxyapatite nanopowders are revealed in the Transmission Electron Microscopy (TEM) micrographs shown in Figure 3. It can be seen that the average dimensions of calcined n-HAp-CTAB particles (Fig. 3(a)) are about 15 nm diameter and 75 nm length, corresponding to an aspect ratio (i.e., length/diameter) of about 4.8. The n-HAp-SDS particles are much thinner and longer (Fig. 3(b)) with average diameter and length of about 11 nm and 137 nm, respectively, and an aspect ratio of about 12.4. The morphology of the particles formed in the presence of Triton X-100 (Fig. 3(c)) vary between the sphere-like shape with dimensions in the range of 19–30 nm and the needle shape with the lengths and widths of 79 nm and 21 nm, respectively. The aspect ratio is about 4.4 for the needle shaped particles. This less distinctive morphology might be attributed to the fact that the non-ionic surfactant is less prone to form an ordered structure in the solution to template the synthesized inorganic particles. These results are roughly consistent with those obtained from the XRD data.

The corresponding crystallite size distributions are also shown in Figure 4. In the TEM measurements (using “Image J” software) more than 20 particles were randomly chosen in different areas in each powder sample. Two main features emerge from this figure: (i) the XRD size distributions determined along the directions 002 and 300 are

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Refined lattice data for HAp phase (Å)</th>
<th>Crystallite size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( a ) axis (Å)</td>
<td>( c ) axis (Å)</td>
</tr>
<tr>
<td>ICDD no. 09-432</td>
<td>9.4180</td>
<td>6.8840</td>
</tr>
<tr>
<td>n-HAp-CTAB</td>
<td>9.4856</td>
<td>6.8573</td>
</tr>
<tr>
<td>n-HAp-SDS</td>
<td>9.4374</td>
<td>6.8543</td>
</tr>
<tr>
<td>n-HAp-TX-100</td>
<td>9.4740</td>
<td>6.8947</td>
</tr>
</tbody>
</table>

Fig. 2. FTIR spectra of n-HAp nanopowders prepared in the presence of different surfactants: (a) CTAB, (b) SDS, (c) Triton X-100.
very different, accounting for the anisotropy nature of the particles as observed by TEM; (ii) there is an excellent qualitative agreement between the distributions determined by XRD and TEM. The quantitative differences between the X-ray and the TEM data are probably due to the fact that the particles might not be monocrystalline but instead result from the coalescence of some growing nuclei. While TEM images reflect the size of the polycrystalline entities, XRD is able to discriminate the features of the composing individual crystallites.

4. DISCUSSION

The surface active agents, also called surfactants, are generally referred to as being amphiphilic. They usually consist of a hydrophilic polar head with high affinity for water and a hydrophobic tail with a completely opposite behaviour towards water, as schematized in Figure 5(a). Their first property is the ability bridge two immiscible phases. For example, in an aqueous solution, surfactants have the tendency to migrate to the interfaces, particularly
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Fig. 4. Bar diagram of the size distributions obtained from XRD and TEM micrograph. For each sample more than 20 particles were counted. Lm is the average length. Dm is the average diameter.

Surfactants are broadly classified into the following four groups based on the ionic charges they carry on the hydrophilic head group:

(i) Anionic, with the hydrophilic group carrying a negative charge, such as carboxyl (RCOO⁻) or sulphate (ROSO₃⁻);
(ii) Cationic, with the hydrophilic group bearing a positive charge, as for example, the quaternary ammonium salts (RN⁺X⁻) or amine salts;
(iii) Amphoteric and zwitterionic, in which the surface—active position bears both positive and negative charges;
(iv) Non-ionic, where the hydrophilic group has no net electrical charge.

This is the case of synthetic products like betaines or sulfobetaines and natural substances such as aminoacids and phospholipids. For the present study we selected the cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulphate (SDS), and Triton X-100 as cationic, anionic and non-ionic surfactants, respectively, in an attempt to shed further light on the mechanisms involved in the formation of HAp nanoparticles. Their structures are schematised in Figure 6.

Surfactants have been used as additives to template the crystallization processes, but their mechanisms of action are still poorly understood. The presence of surfactants tends to exert an overall inhibitory action on the overall crystallization process, with specific effects depending on the type and concentration of surfactant. Within the pH values of interest in the present work, CTAB molecules dissociate completely in the aqueous solution, forming cationic CTAB⁺ species. Above CMC, these species tend to self-assemble in direct cylindrical micelles exposing the positive shells to water molecules, as schematized in Figure 7(a). Therefore, when the PO₄⁻ solution is mixed with the CTAB solution, the PO₄⁻ ions are readily attracted towards ionized heads forming CTAB⁺–PO₄⁻ complexes (which might be mono-, bi-, multi-dentate) onto the surface of the cylindrical micelles and decreasing the total charge and the freedom of the PO₄⁻ ions (Fig. 7(b)). Upon the subsequent addition of the Ca²⁺ containing solution, Ca₆(PO₄)₆ clusters are likely formed, driven by
factant molecules onto crystal faces are thermodynamically driven and may greatly govern the crystallization and crystal growth.\[^5\,\[^6\] The molecular structure of Triton X-100 (Fig. 6(c)) consists of a hydrophilic polyethylene oxide group (head) and a hydrophobic or lipophilic hydrocarbon (tail). Being a non-ionic surfactant, the possibility of electrostatic interactions may be eliminated. Above the CMC, it forms an isotropic colloidal mixture of spherical micelles and hexagonally ordered cylinders, with the polyethylene oxide group located at the outer shell and the hydrophobic tail at the micelle core.\[^47\] Lee coworkers\[^48\] suggested that the polyethylene oxide group could coordinate with Ca\[^{2+}\] ions through hydrogen bonds, forming calcium complexes that mediate the reaction with the PO\(^{3−}\) solution. These intermolecular interactions would enable nucleation and particle growth to proceed, upon mixing the reagent solutions, according to the template micelles. These theoretical arguments are in good agreement with our experimental TEM observations that reveal a mixed morphology for the n-HAp particles from spherical to rod-like.\[^57\]

Concerning the size of the produced nanoparticles, we perceive that this factor is strongly dependant on the surfactant used in the experiment. Each surfactant has its sharp and characteristic critical micelle concentration above which the dissolved species combine to form micelles.\[^52\,\[^53\] The structure of these micelles was assumed to be an aggregate of from 50–100 molecules with a radius approximately equal to the length of the hydrocarbon chain of the surfactant. Thus, for ionic surfactants, the average size of CTAB micelles is expected to be bigger than that of SDS micelles. Further, the CMC effectively decreases with increasing alkyl chain length. These different features explain why nanoparticles with smaller diameters were obtained in the presence of SDS. In the case of the non-ionic surfactant (Triton X-100), its lower solubility in comparison to the ionic ones makes the CMC to decrease (Table II). The shape and the structure of the resulting micelles are not so quite defined and their average size increases. The space between micelles also increases, thus inducing a steric hindrance that further contributes to increase the size of the produced particles (Fig. 8).\[^51\]

The expected stronger interactions between the ionic surfactants and the HaP-forming ions, in comparison to those involving the non-ionic surfactants, mean that the Ca\(^{2+}\) and PO\(^{3−}\) ions are likely to form from mono- to multi-dentate complexes. This stronger sequestering effect

![Fig. 7. Scheme of the formation process of crystalline HAp nanorods in the presence of CTAB.](Image 87x566 to 317x759)

the remaining electrostatic interactions, thus leading to the nucleating and growth of rod-like n-HAp crystals (Fig. 7(c)). The formation of ionic-surfactant complexes means that the effectiveness of the collisions between the hydroxyapatite forming ions decreases, explaining their inhibitory action on the overall crystallization process.\[^34\,\[^35\] The formation of ionic-surfactant complexes and the consequent decreased efficiency of the relevant collisions towards the formation of hydroxyapatite means that ions are added to the surfaces of n-HAp particles more slowly but in a more ordered manner, resulting in crystalline powders (Fig. 1). The addition of ions to the crystal surfaces is helped by the high temperature/pressure used in the hydrothermal treatment. The n-HAp-CTAB complexes might coalesce and contribute to control the size and morphology of the obtained nanoparticles and account for their polycrystalline nature.\[^34\,\[^35\] This coalescence would explain the quantitative differences observed in Figure 4 between the size distributions data determined from XRD and TEM.

A similar reasoning could be argued to interpret the anisometric growth of n-HAp particles in the presence of the other surfactants. The only difference expected when using SDS is the early formation of SDS−Ca\(^{2+}\) complexes that precedes the formation of Ca\(_2\)(PO\(_4\))\(_3\) clusters upon mixing the two HAp precursor solutions. It is likely that the specific interactions between the surfactant molecules and the different crystal surfaces, which might differ especially among the basal plans (perpendicular to c-axis) and the lateral plans (parallel to c-axis), also contribute in different extents to the anisometric growth of n-HAp particles.\[^46\] These effects related to the adsorption of surfactant molecules onto crystal faces are thermodynamically driven and may greatly govern the crystallization and crystal growth.\[^59\,\[^60\] The molecular structure of Triton X-100 (Fig. 6(c)) consists of a hydrophilic polyethylene oxide group (head) and a hydrophobic or lipophilic hydrocarbon (tail). Being a non-ionic surfactant, the possibility of electrostatic interactions may be eliminated. Above the CMC, it forms an isotropic colloidal mixture of spherical micelles and hexagonally ordered cylinders, with the polyethylene oxide group located at the outer shell and the hydrophobic tail at the micelle core.\[^47\] Lee coworkers\[^48\] suggested that the polyethylene oxide group could coordinate with Ca\(^{2+}\) ions through hydrogen bonds, forming calcium complexes that mediate the reaction with the PO\(^{3−}\) solution. These intermolecular interactions would enable nucleation and particle growth to proceed, upon mixing the reagent solutions, according to the template micelles. These theoretical arguments are in good agreement with our experimental TEM observations that reveal a mixed morphology for the n-HAp particles from spherical to rod-like.\[^57\]

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### Table II. Typical CMC values of the surfactants used and the respective crystallite sizes of the n-HAp powders derived thereof.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>CTAB (mM)</th>
<th>SDS (mM)</th>
<th>Triton X-100 (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC (mM)</td>
<td>0.92–1.0</td>
<td>8.5</td>
<td>0.55</td>
</tr>
<tr>
<td>Length of synthesized n-HAp (nm)</td>
<td>75</td>
<td>137</td>
<td>79</td>
</tr>
<tr>
<td>Diameter of synthesized n-HAp (nm)</td>
<td>15</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>Surfactant length (Å)</td>
<td>22.2</td>
<td>18.4</td>
<td>59.9</td>
</tr>
</tbody>
</table>

*Note: Calculation was done by Chem 3D Pro software.*
exerted by the ionic surfactants might hinder the immediate release of the surfactants species after an effective collision between two HAp-forming ions. Shall this happens, the adsorbed surfactants species will disturb the atomic arrangement upon building the HAp crystal lattice. Therefore, a less ordered structure is expected to form in the presence of ionic surfactants. Although the adsorbed surfactant species will be thermally degraded upon calcination, explaining why no surfactant molecules were detected in the samples heat treated at 550 °C for 6, Figure 2, it is likely that this disturbing role in the atomic arrangement will have reflexes in the degree of crystallinity obtained after being treated at this moderate temperature. This explains why the sharpness of XRD peaks of the powders n-HAp-CTAB (Fig. 1(a)) and n-HAp-SDS (Fig. 1(b)) is less in comparison to that n-HAp-Triton X-100 (Fig. 1(c)).

5. CONCLUSIONS

Rod-like nano-crystalline HAp particles have been successfully prepared by hydrothermal synthesis in the presence of different surface active agents. The obtained results show that the ionic surfactants are better suited to promote and control the anisometric growth of n-HAp particles. The strong interactions between the dissolved CTAB and SDS species and water molecules lead to the formation of well-defined cylindrical micelles that act as templates for the nucleation and growth of rod-like n-HAp particles. The low solubility of the smaller and non-ionic Triton X-100 molecules stimulates them to self-organize but not in a so quite defined manner leading to the formation of smaller sized micelles and resulting particles. Stronger specific interactions between the surfactant molecules and the different crystal surfaces tend to hinder atomic arrangement and lead to decreased crystallinity. The results from the different characterization techniques used (FTIR, XRD and TEM) show good consistency. The size and shape of the obtained self-assembled rod-like nano-crystalline HAp powders are similar to those of the hydroxyapatite mineral existing in human bones, making them good candidate materials for tissue engineering applications and drug delivery systems.

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References and Notes

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