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A facile and sustainable method based on deep eutectic solvents toward synthesis of amorphous calcium phosphate nanoparticles: The effect of using various solvents and precursors on physical characteristics

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ABSTRACT

Amorphous calcium phosphate (ACP) nanoparticles were synthesized using a sustainable method based on precipitation of calcium and phosphate precursors in deep eutectic solvents (DESs). To this aim, three types of DESs, i.e., choline chloride–urea, choline chloride–ethylene glycol, and choline chloride–glycerol were prepared by simple heating-mixing method. The DESs were used as synthesis media for synthesis of ACP nanoparticles from Calcium nitrate tetrahydrate/calcium chloride and di-potassium hydrogen orthophosphate/di-ammonium hydrogen orthophosphate precursors. Characterization of the synthesized ACP nanoparticles by X-ray diffraction, field emission scanning electron microscopy, transmission electron microscopy–selected area electron diffraction, energy dispersive X-ray spectroscopy, and Fourier transform infrared spectroscopy confirmed the formation of amorphous nanoparticles with spherical morphology and a high elemental/structural purity. Based on the results, with the change of DES from choline chloride–urea to choline chloride–ethylene glycol the diameter and Ca/P molar ratio of ACP nanoparticles changed from 24 to 39 nm, and 1.15 to 1.01 respectively. No significant changes in particle size of ACP nanoparticles were detected with change of precursors. After synthesis, DESs were recovered and re-used for synthesis of ACP nanoparticles. Analysis results suggested successful synthesis of ACP nanoparticles with high phase/elemental purity in the recovered DESs.

1. Introduction

Amorphous calcium phosphates (ACPs) are a unique class of CaP minerals of biological organisms with chemical and structural similarities to mammalian bones and teeth. ACPs can promote the in vivo formation of apatite layer analogous to bone mineral that leads to their various biomedical applications for bone regeneration [1–4]. In addition, the promising biocompatibility and bioresorbability of ACPs make them potential candidates for manufacturing artificial bone grafts [2,3]. As an intermediate phase in the preparation of several CaPs, ACPs are synthesized by chemical precipitation method [5], freeze-drying [6], sol–gel processes [7], quenching of calcium phosphates melted at high temperatures [8] or physical deposition techniques [9].

With growing environmental awareness, material scientists have focused their attention on developing alternative routes for the processing of materials using both renewable resources and "green" solvents [10]. Developing new green solvents is one of the key subjects in Green Chemistry. Recently, ionic liquids (ILs] and deep eutectic solvents (DESs) have been paid great attention to replace molecular solvents such as water and highly volatile organic solvents and have been applied to many chemical processing [11,12]. DESs share many characteristics with ILs and have added advantages of low price, low toxicity, biodegradability, environmental friendliness, ease to prepare for large scale and the elimination of the preliminary purification step [13,14]. In general, a DES is a fluid generally composed of two or three cheap and safe components that are capable of self-association, often through hydrogen bond interactions, to form a eutectic mixture with a melting point lower than that of each individual component. Thanks to their attractive properties, DESs are now of growing interest in many fields of research such as synthesis [15]. In our previous report [16], we successfully synthesized calcium phosphate nanoparticles in choline chloride-urea DES. The effect of synthesis temperature on phase characteristics and physical properties of the nanoparticles were examined. Here, the synthesis of amorphous calcium phosphate nanoparticles in DESs including choline chloride-urea, choline chloride-ethylene glycol, and choline chloride-glycerol has been explored. The effect of various DESs and calcium/phosphate precursors on crystalline, particulate and structural properties of nanoparticles has been investigated by X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), transmission electron microscopy–selected area electron diffraction (TEM–SAED), energy dispersive X-ray spectroscopy (EDS), and Fourier transform infra-red spectroscopy (FTIR).

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2. Experimental

2.1. Materials

Calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, ≥98.0%), calcium chloride anhydrous ($CaCl_2$, ≥98.0%), di-potassium hydrogen orthophosphate (K_2HPO_4, 98.0–100.5%), di-ammonium hydrogen orthophosphate ($(NH_4)_2HPO_4$, ≥99.0%), Choline chloride ($C_5H_{14}ClNO$, 98.0–100.5%), urea (CH_4N_2O, ≥99.0%), ethylene glycol ($C_2H_6O_2$, ≥99.5%), and glycerol ($C_3H_8O_3$, ≥99%) were supplied by Merck Chemicals (Germany).All the chemicals were used as received without any purification.

2.2. Preparation of deep eutectic solvents (DESs)

The DESs were prepared by stirring the two components in molar ratios of 1 choline chloride:2 urea (referred as CU), 1 choline chloride:2 ethylene glycol (referred as CE), 1 choline cloride:2 glycerol (referred as CG) under 100 °C until a homogeneous colorless liquid was formed. To obtain a homogeneous liquid at room temperature and form a perfect eutectic mixture, the aforementioned molar ratios were selected.

2.3. Synthesis of ACP nanoparticles

Amorphous calcium phosphate (ACP) nanoparticles were prepared by a solution method using the prepared DESs of CU, CE, and CG as solvent. Firstly, a 0.12 M solution containing one of the calcium precursors of calcium chloride anhydrous, $CaCl_2$, or Calcium nitrate tetrahydrate, $Ca(NO_3)_2{\cdot}4H_2O$, in DES was prepared. Then, a 0.81 M aqueous solution (with minimum water content) including one of the phosphate precursors of di-potassium hydrogen orthophosphate, K_2HPO_4, or di-ammonium hydrogen orthophosphate, $(NH_4)_2HPO_4$, was added to the DES (containing calcium ion). The solution was stirred vigorously at room temperature for 20 min by a magnetic stirrer, allowing the formation of gel-like precipitates of ACP. The resultant precipitates were filtered, washed several times with deionized water and finally freeze dried at −57 °C for 65 h. The dried powder was kept in a freezer to hinder any phase transformation. To recover the DESs, the filtrate was heated in oven at 105 °C for 48 h. After this time, the emerged crystalline deposits (probably related to by-product salts such as KCl) in solution were separated and the solvent was further dried in desiccator for 72 h. After solvent recovery, the ACP nanoparticles were synthesized in the recovered CU solvent according to aforementioned procedure. The synthesized ACP nanoparticles were labeled as shown in Table 1.

2.4. Characterization of ACP nanoparticles

The crystal structure, particle size, morphology, chemical composition, and characteristic functional groups of ACP samples were analyzed by X-ray diffraction (XRD, Siemens D-500 diffractometer), transmission electron microscopy (TEM, Philips CM 30), field-emission scanning electron microscopy (FESEM, Tescan Mira 3 LMU), energy dispersive X-ray spectroscopy (EDS, Bruker, Quantax 200), and Fourier transform infrared spectroscopy (FTIR, PerkinElmer Spectrum 400), respectively.

Table 1
Coding method of synthesized ACP nanoparticles along with synthesis conditions.

Code	DES	Precursors
ACP-CU-C	Choline chloride-urea	$CaCl_2 + K_2HPO_4$
ACP-CE-C	Choline chloride-ethylene glycol	$CaCl_2 + K_2HPO_4$
ACP-CG-C	Choline chloride-glycerol	$CaCl_2 + K_2HPO_4$
ACP-CU-K	Choline chloride-urea	$Ca(NO_3)_2{\cdot}4H_2O + K_2HPO_4$
ACP-CU-N	Choline chloride-urea	$CaCl_2 + (NH_4)_2HPO_4$

3. Results

XRD patterns of the nanoparticles synthesized by different precursors and in various DES are displayed in Fig. 1. According to the figure, all XRD patterns show a broad diffraction line at $2\theta = 30°$ that is indexed to the amorphous phase of calcium phosphate [10,17]. The nanoparticles synthesized in the CU solvent show a more intense diffraction pattern than those synthesized in CG and CE solvents. This can be related to the lower dielectric constant (ε) of CU solvent compared to CG and CE solvents (CE (ε: 32) > CG (ε: 22) > CU (ε: 12)) that induces a higher degree of amorphization because of less solvation of ions and increase in precipitation kinetic [4,18,19]. In case of ACP nanoparticles synthesized in CE solvent, two peaks located at $2\theta = 28.33°$ and 31.69° related to (102) and (211) planes of calcium deficient hydroxyapatite (CDHA) (JCPDS–9–432) can be observed over the broad diffraction [16]. This is due to the high dielectric constant of CE solvent, leading to evolution of crystalline apatite traces [4]. There is no peak correspond to other phases in XRD patterns revealing the high purity of ACP nanoparticles.

Fig. 2 shows FESEM and SAED-TEM micrographs of synthesized ACP nanoparticles. FESEM micrographs reveal the spherical nanoparticles with some degrees of agglomeration. The average diameter of the particles was calculated from FESEM micrographs using the *SemAfore* software (version 5.21). For this purpose, around thirty measurements were carried out on the micrographs for each sample. The SAED-TEM micrograph presented in Fig. 2(f) approves the results of XRD and FESEM. The hollow form of the SAED pattern represents the amorphous nature of calcium phosphate phase, whereas the TEM micrograph displays the spherical particles with an average size of 30 nm. As shown in Fig. 3, ACP nanoparticles synthesized in CU solvent are smaller in size than those synthesized in CG and CE solvents. This is in relation to the order of the solvent viscosities (CU (720 cP) > CG (259 cP) > CE (37 cP)) [14].

EDS analysis provided the composition of the ACP nanoparticles. The insets of Fig. 2 show the EDS patterns of the as-synthesized ACP nanoparticles. It can be clearly identified that the nanoparticles are composed of Ca, O, and P elements. Furthermore, the silicon element observed in the spectra comes from the glass substrate. There is no peak related to any contaminated element revealing the high purity of the synthesized ACP nanoparticles. The mean Ca/P ratio of the nanoparticles was calculated from EDS data obtained from three different points on each sample. As shown in Fig. 4, all ACP nanoparticles show a lower Ca/P ratio than initial ratio (~1.5). The decrease of Ca/P ratio is either due to the presence of HPO_4^{2-} ions which arise from the neutral nature of the eutectic solvents or surface-adsorbed soluble phases which can be washed away [4,10]. Furthermore, ACP nanoparticles synthesized in CU solvent show a higher Ca/P compared with those synthesized in CE and CG solvents. This can be attributed to the slightly alkaline nature of CU solvent because of the presence of urea in its structure [11]. A review of literature reveals that ACP nanoparticles can contain HPO_4^{2-} ions instead of

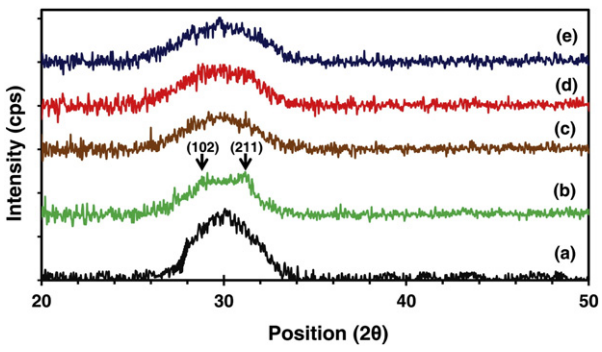

Fig. 1. XRD patterns of the synthesized ACP nanoparticles; ACP-CU-C (a), ACP-CE-C (b), ACP-CG-C (c), ACP-CU-K (d), and ACP-CU-N (e).