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Three-dimensional flower-like brushite crystals prepared from high internal phase emulsion for drug delivery application

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ABSTRACT

In the present study, three-dimensional brushite crystals were prepared through palm olein-in-water high internal phase emulsion processing route for the first time. X-ray diffraction patterns revealed that the powders possessed brushite crystalline phase with trace amount of hydroxyapatite. Unique morphologies of the brushite crystals were obtained as a result of tailoring the precursor concentration, surfactant concentration and oil volume fraction. These factors governed the rate of nucleation and crystal growth, resulting in flower-like morphologies. The petal-like flakes grew radially from the centre which gave rise to porosity of less than 2 μm. A plausible mechanism of crystal growth is discussed and postulated schematically. Sodium ampicillin, a broad spectrum antibiotic, was loaded into the pores of the crystals, which was subsequently released in vitro. The controlled release ability for up to 14 days indicated the potential of using these brushite crystals as drug delivery agents for localized treatments.

1. Introduction

Calcium phosphates analogy to the mineral component of bone makes these biocrystals desirable as drug delivery agents because of their biocompabitility and osteoconductive properties. They are salts of tribasic phosphoric acid, H_3PO_4 and its ionic compounds. Hydroxyapatite (HAP) $Ca_5(PO_4)_3(OH)$, octacalcium phosphate (OCP) $Ca_8H_2(PO_4)_6{\cdot}5H_2O$, monetite $CaHPO_4{\cdot}2H_2O$, and brushite $CaHPO_4{\cdot}2H_2O$ are different crystalline orthophosphates that have been extensively studied for their relevance in biological mineralization [1].

Drugs embedded into the pores of calcium phosphates lead to sustained release of drug [2]. This could overcome the lack of neoformative blood vessel in the bone fraction site which makes it difficult for drugs to reach the bone defect orally or by injection [3]. Furthermore, these materials have a dual function as osteoconductive bone grafts after release of drug, which eliminates the need for a second operation to remove the implant. The removal of implant is necessary if non-resorbable drug loaded polymers are used as antibiotic delivery systems in clinical use for the treatment of infected bone [2].

Results from literature have noted the significant influence of pore size on drug delivery behaviour in order to provide prolonged sustained release [2,4–6]. The processing method used greatly influences the extent of porosity, pore morphology and pore size distribution of the final material [7]. Various methods have been developed to introduce pores in calcium phosphates including incorporation of volatile organic particles [8], gel casting [9], replication of a polymer sponge or reticulation [10], salt leeching [11] and electrospinning [12]. Most of these methods produced calcium phosphates with pore size of more than 10 μm.

High internal phase emulsion (HIPE) is gaining considerable interests amongst researchers as a reaction medium for the preparation of meso/macroporous materials [13–15]. This particular emulsion consists of disperse phase which exceeds the close packing volume limit of 0.73, the point where the droplets just touch each other [16]. Emulsion droplets of sizes between 0.5 and 5.0 μm offer a reaction medium fit for the creation of micron-sized pores into an inorganic or organic matrix [17].

In this work, three-dimensional porous calcium phosphate crystals with pore size of less than 2 μm with unique morphologies were successfully synthesized through palm olein-in-water (O/W) HIPE. The porous calcium phosphates were resulted from the continuous phase of the HIPE. Calcium and phosphate reactants were introduced into the structure of HIPE, which acted as a reaction medium. This was followed by removal of the medium in order to obtain the porous structure. Formation mechanism of porous calcium phosphates is discussed. The resulting crystals were then loaded with sodium ampicillin, a broad spectrum antibiotic that is active against both gram-positive and gram-negative bacteria

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Table 1
Parameters to fabricate calcium phosphates using the HIPE processing route.

Sample	Precursor concentration		Surfactant concentration (wt%)	Oil volume fraction, Ø[a]	Cumulative pore volume (cm^3/g)	Morphology of calcium phosphates
	$CaCl_2$ (M)	Na_2HPO_4 (M)				
Bulk	0.50	0.30	-	-	0.000875	Merged large plates
BR1	0.50	0.30	5.0	0.80	0.994	Carnation-like
BR2	0.30	0.18	5.0	0.80	0.871	Bud-like
BR3	0.10	0.06	5.0	0.80	0.0565	Lotus-like
BR4	0.50	0.30	2.0	0.80	0.135	Carnation-like
BR5	0.50	0.30	8.0	0.80	0.146	Spider lily-like
BR6	0.50	0.30	5.0	0.75	0.997	Carnation-like
BR7	0.50	0.30	5.0	0.85	0.732	Carnation-like
BR8	0.50	0.30	5.0	0.90	0.591	Carnation-like

[a] $Ø = (m_o/\rho_o)/[(m_o/\rho_o)+(m_w/\rho_w)]$, where m_o/ρ_o is the volume of oil and m_w/ρ_w is the volume of water [32].

which is widely used for the treatment of infections [18]. The sodium ampicillin loaded calcium phosphates are potential for application as local drug carriers with controlled release properties.

2. Experimental

2.1. Calcium phosphate fabrication

In order to fabricate calcium phosphate crystals through the HIPE processing route, two aqueous solutions, containing (a) 5.0 wt% Brij 35 (Fluka) and 0.50 M calcium chloride, $CaCl_2$ (Sigma–Aldrich) and (b) 5.0 wt% Brij 35 and 0.30 M disodium hydrogen phosphate, Na_2HPO_4 (Sigma–Aldrich) were prepared. Calcium and phosphate concentrations were prepared with calcium-to-phosphate ratio of 1.67, similar to that of natural bone [19]. Refined-bleached-deodorized (RBD) palm olein (Moi Foods Malaysia Sdn. Bhd.) as oil phase was added dropwise into each of the aqueous phase equally while stirring. The mixtures were then mixed and homogenized at 1500 rpm for 15 min using a Multimix CKL mixer at room temperature to form HIPE, which was allowed to age at 40 °C in an MMM Vacucell vacuum oven for 7 days for the formation of calcium phosphate crystals. In order to retrieve the precipitates, ethanol (98%, Fluka) was added to demulsify the HIPE system. The demulsified HIPE was centrifuged using a Hettich centrifuge at 4500 rpm for 30 min to separate the precipitates from the medium. The washing process was repeated three times with ethanol followed by deionized water. The precipitates were then calcined at 600 °C in a Carbolite furnace for 2 h to obtain white powders. Precursor concentration, surfactant concentration and oil volume fraction (Ø) were varied as shown in Table 1. Bulk calcium phosphate was prepared using conventional wet chemical processing route whereby 0.50 M $CaCl_2$ aqueous solution was titrated with 0.30 M Na_2HPO_4 aqueous solution under constant stirring, as a comparison to the HIPE-prepared calcium phosphates.

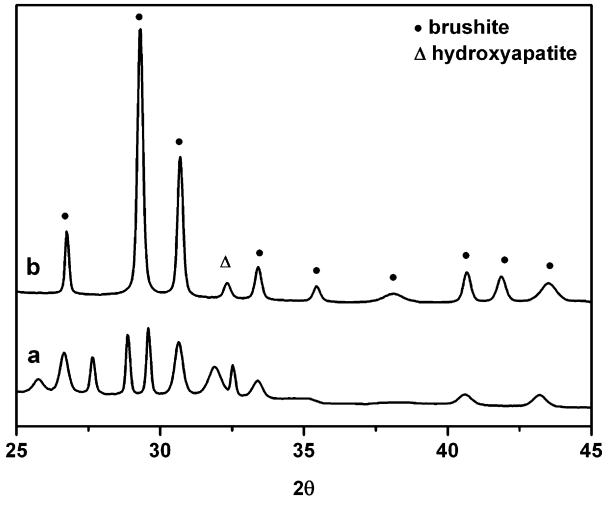

Fig. 1. XRD patterns of (a) bulk brushite and (b) BR1.

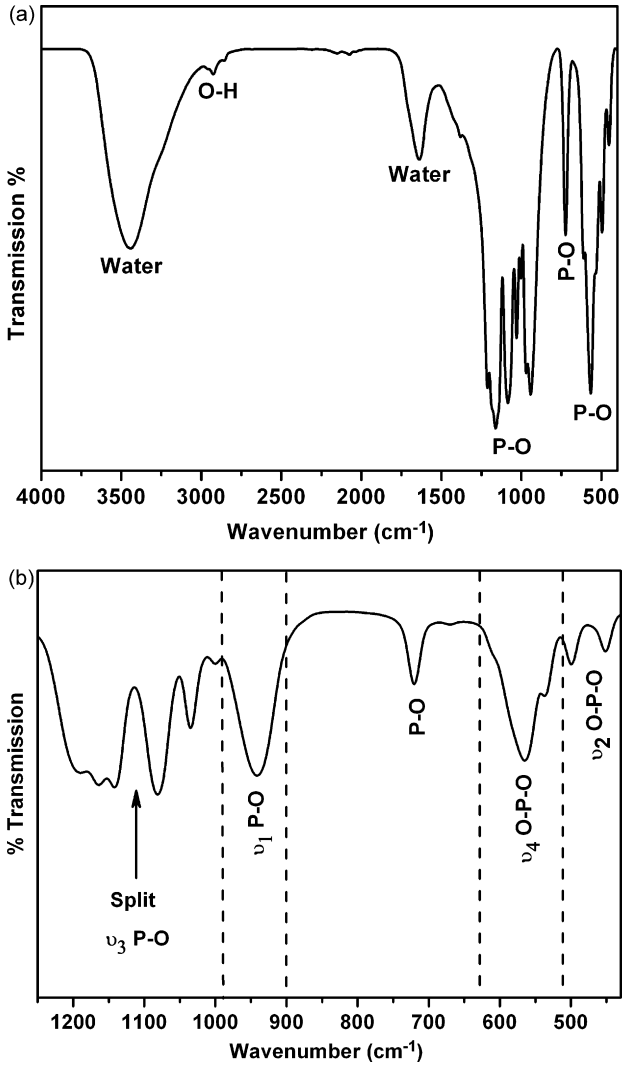

Fig. 2. FTIR spectra of (a) brushite and (b) highlights of chemical bonding of BR1.