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pH-dependent loading of Pt nanoparticles protected by dendrimer in calcium phosphate matrices



Yakub Fam^a, Toyoko Imae^{a,b,*}, Jonathan Miras^c, Maria Martinez^c, Jordi Esquena^c

^a Department of Chemical Engineering, National Taiwan University of Science and Technology, 43 Section 4, Keelung Road, Taipei 10607, Taiwan ^b Graduate Institute of Applied Science and Technology, National Taiwan University of Science and Technology, 43 Section 4, Keelung Road, Taipei 10607, Taiwan ^c Institute for Advanced Chemistry of Catalonia (IQAC-CSIC) and CIBER en Bioingenieria, Biomateriales y Nanomedicina (CIBER-BBN), C/ Jordi Girona 18-26, 08034 Barcelona, Spain

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ABSTRACT

Composites (CaP-DENPtNPs) of calcium phosphate particles (CaPs) with Pt nanoparticles protected by dendrimers (DENPtNPs) were synthesized at different pH following a hydrothermal synthesis method. The CaP matrix in CaP-DENPtNPs composites possessed hydroxyapatite crystalline structures at pH 12 and 8, but monetite crystals were obtained at pH 4. The particle sizes of the composites increased with decreasing pH, and at the same time the amount of PtNPs in the composites also increased. Furthermore, it was confirmed that CaP-DENPtNPs were mesoporous nanocomposites, and DENPtNPs nanoparticles were incorporated mainly on the surface of CaP particles at high pH, but most of DENPtNPs were encapsulated inside CaPs at low pH. The uptake of well-dispersed Pt nanoparticles up to 60 wt% in CaPs suggests the possibility of these materials as effective catalysts.

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1. Introduction

Research in the field of nanomaterial engineering is currently facing greatest challenges, owing to the wide variety of novel potential applications of nanomaterials in biomedical, environmental, optical, energy, and electronic fields [1,2]. It is well known that nanoparticles have a much larger surface area per weight than large particles, which causes them to be more active than macroscopic structural systems [3]. One of those materials with valuable potential applications is hydroxyapatite (HAp), which has been nowadays utilized particularly for biomedical purposes as materials for bone tissue engineering and matrices for drug delivery system [4,5] due to its biocompatibility, in situ slow degradability, and osteoconnectivity [6,7]. HAp nanoparticles also possess an exceptional characteristic biocompatibility with soft tissues (skin, muscle, and gum) [8], allowing HAp to be an ideal candidate as a constituent of orthopedic and dental implants. Especially, HAp is promoted their sinterability and densification owing to the greater surface area, which also improves their mechanical properties [7]. Moreover, HAp nanoparticles exhibit better bioactivity than coarse

E-mail address: imae@mail.ntust.edu.tw (T. Imae).

crystals [9], and hence nanosized HAp with large surface area attracts attention in this era of nanotechnology.

HAp nanoparticles can be prepared by a variety of techniques such as mechanochemical synthesis [10], thermal treatment [11], sol-gel synthesis [12], solid state reaction [13], co-precipitation [14], hydrothermal reaction [15], and various wet-chemical techniques [16,17]. Environmentally responsible techniques [18], namely, molten-salt synthesis, hydrothermal processing, biomimic synthesis and template synthesis have been implemented as viable techniques for the synthesis of a large variety of materials [19,20]. Especially, the hydrothermal method has been developed to be an effective and convenient process to prepare various inorganic materials with diverse, controllable morphologies and architectures [21–24]. Besides, this method also possesses other essential advantages such as easily controllable reaction conditions, relatively large scale, high yield, and aqueous medium [18].

Researches regarding the combination of nanoparticles with other molecules/materials, called nanocomposites, are notable. A large amount of investigations have been performed in respect to HAp-based nanocomposites [18,25,26], but only a few studies have been reported about nanocomposites of HAp with poly(amido amine) (PAMAM) dendrimer [27–31]. Khopade et al. [27] have proposed that COOH-terminated half-generation PAMAM dendrimers and/or their supramolecular aggregates might serve as nucleation sites, where Ca^{2+} ions of HAp precursors physically bound and then the binding of PO_4^{3-} ions was followed. Yang and his coworkers

^{*} Corresponding author at: Graduate Institute of Applied Science and Technology, National Taiwan University of Science and Technology, 43 Section 4, Keelung Road, Taipei 10607. Taiwan.

[28–30] also have suggested that Ca^{2+} ions of HAp precursors might be adsorbed on the surface or interior of half-generation PAMAM dendrimers through electrostatic interactions. On the contrary, Pramanik and Imae [31] adopted amine-terminated PAMAM dendrimer, where PO_4^{3-} ions of HAp precursors electrostatically interacted first with NH₂ groups from dendrimer and then with Ca^{2+} ions. Finally they have successfully synthesized porous HAp with dendrimer porogen. The different generations and functional groups of the dendrimers used in those reports indicated that dendrimers affect the shape and size of HAp, which is also influenced by pH. Overall, dendrimers are able to modify the original needlelike particles of HAp into elliptical or rod-like particles.

The main objective in the present study is the adjunction of catalytic functionality, namely Pt nanoparticles (PtNPs), in composites consisting of calcium phosphate (CaP) and dendrimer (DEN). To the best of our knowledge, the preparation of functional composites consisting of dendrimer-stabilized Pt nanoparticles in a CaP matrix has never been studied. The optimization for the preparation of the composites, the characterization of the products and the identification of the PtNPs can be considered of high importance. Thus, in the present research, PtNPs were encapsulated into dendrimers as the size/shape-controller as well as the stabilizer and then loaded to CaPs, which play the role of a three-dimensionally structured scaffold. Since the resulting composites are expected to load large amounts of Pt nanoparticles, they may be valuable as catalysts on many reaction processes.

2. Experimental section

2.1. Materials

All chemicals such as calcium nitrate $(Ca(NO_3)_2 \cdot 4H_2O, 99+\%)$, diammonium hydrogen phosphate $((NH_4)_2HPO_4, 99+\%)$, fourth generation amine-terminated PAMAM dendrimer (DEN, 10 wt% solution in methanol), sodium hexachloroplatinate hexahydrate $(Na_2PtCl_6 \cdot 6H_2O, 98\%)$, an aqueous ammonia solution $(NH_4OH, 35\%)$, an aqueous hydrochloric acid solution (HCl, 35%), sodium borohydride $(NaBH_4, 98\%)$, and sodium hydroxide (NaOH, 98%)were analytical grade and available from commercial sources. Ultrapure water $(18.2 M\Omega \text{ cm} \text{ resistivity})$ was utilized through whole of the experiments.

2.2. Synthesis of CaP-DENPtNPs composites

PtNPs loaded in DEN (DENPtNPs) were prepared according to the previously reported procedure [32,33]. In an aqueous solution of DEN, Na₂PtCl₆·6H₂O was added at a mixing molar ratio of Pt precursor:NH₂ group in DEN = 0.2:1. The solution was adjusted at around pH 4 by adding an HCl solution before and after adding Pt precursor, and the yellowish solution was stirred for 3 days. After that, a reducing agent, NaBH₄, in a 0.3 M NaOH solution was added at a mixing molar ratio of reducing agent:Pt precursor of typically 10:1. The dark-brown solution was stirred for 1 day.

The synthesis of composites (CaP-DENPtNPs) of CaP and DEN-PtNPs through a hydrothermal method followed the procedure previously reported [31]. In a typical procedure, an aqueous DEN-PtNPs solution was mixed with an aqueous solution of 0.1 M (NH₄)₂HPO₄ (at mole ratio of PO₄³⁻:NH₂ = typically 1:1) and kept for typically 3 h with stirring. Subsequently, an aqueous solution of 0.1 M Ca(NO₃)₂·4H₂O was dropwise added to the DENPtNPsphosphate solution at a Ca²⁺/PO₄³⁻ mole ratio of 1.67 with vigorous stirring at room temperature. The mixed solutions were adjusted at pH 12, 8, and 4 by adding an NH₄OH or HCl solution. Darkbrown gelatinous precipitates were generated. For the preparation of CaP powders, the same procedure was carried out without an addition of the DENPtNPs solution. A white gelatinous precipitate was obtained. All resultant precipitates were then annealed in an autoclave at 150 °C for 15 h. Afterward, the powders were separated by centrifugation, rinsed with water, and dried overnight in an vacuum oven at 70 °C.

2.3. Equipments

The Fourier transform infrared (FTIR) absorption spectroscopic analysis was carried out on a Nicolet 6700 for samples in KBr pellets. The thermal analysis was carried out on a thermogravimetric analysis (TGA) thermal analyzer (TA Instruments Q500, UK) under an air flow at 100 cm³/min with constant temperature rise at 10 °C/ min. The particles were visually observed on a Hitachi H-7000 transmission electron microscope (TEM) with an acceleration voltage of 100 kV. The specimens for TEM were prepared by placing a drop of the aqueous suspension of sample powder on a carboncoated copper grid and drying it at room temperature. Dynamic light scattering (DLS) and laser Doppler electrophoresis (LDE) measurements were performed at 25 °C in a 6 mm carbon electrode cell on a nanoparticle analyzer (Horiba SZ-100, Japan), equipped with a 10-mW DPSS laser light source and a photo-multiplier tube detector. Elemental analysis was performed by means of an energy dispersive X-ray (EDX) analyzer (JEOL, JSM-6500F, Japan). The crystallographic analysis was done by a wide-angle X-ray diffraction (XRD) analyzer (Bruker D2 Phaser, USA) using 10 mA current and 30 kV voltage with a monochromatic CuK α radiation (λ = 1.5405 Å) at a 2θ step size of 0.05°/sec and a scan range from $2\theta = 20$ -80°. Small angle X-ray scattering (SAXS) patterns were recorded using an X-ray system instrument (Hecus S3 MICRO, Austria), which was operated at 50 kV and 1 mA with monochromatic CuKa radiation. The porous texture was analyzed by the nitrogen adsorption determination (Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH)) at 77 K using a surface area analyzer (Quantachrome Autosorb-iQ porosimeter, UK) after degassing the samples at 55 °C for 12 h. The specific surface area (S_{BFT}) was calculated from the adsorption curve of the BET, the pore volume $(V_{\rm p})$ was evaluated from the final point in the adsorption isotherm (at approximately 0.995 relative pressure), and the pore diameter (D_{BIH}) was taken as the maximum in the pore size distribution calculated by the BJH method applied to the desorption curve.

3. Results and discussion

3.1. Effect of pH on Fabrication of CaP-DENPtNPs composites

Composites, CaP-DENPtNPs, were synthesized by incorporating dendrimer-stabilized Pt nanoparticles, DENPtNPs, into a calcium phosphate, CaP, ceramic matrix. The CaP plays the role of dispersion medium, which prevents the aggregation of DENPtNPs. The process started from the fabrication of DENPtNPs nanoparticles by encapsulating PtNPs into the PAMAM dendrimer with the aim of controlling and stabilizing the particle size of PtNPs [32,33]. Subsequently, CaP precursors (PO_4^{3-} and Ca^{2+}) were mixed into the solution containing DENPtNPs, and then CaP-DENPtNPs composites were synthesized [31].

On the synthesis of CaP-DENPtNPs composites through the hydrothermal method, the optimum condition was determined, as reported in Supporting Information (with Figs. S1–S5). The most loading of DENPtNPs took place after 3 h of stirring (reaction) time on the mixing process of DENPtNPs nanoparticles with PO_4^{3-} precursors, and the PtNPs nanoparticles spread in the whole CaP matrices. The uptake of DENPtNPs nanoparticles in the CaP matrices was also investigated by increasing the PO_4^{3-} concentration against the constant DEN concentration, and the 1:1 mol ratio of

 PO_4^{3-} precursor:NH₂ group of DEN was the effective optimum mole ratio. When the content of reducing agent to Pt precursor was varied, the homogeneity of DENPtNPs solutions were maintained at higher NaBH₄ content, but the PtNPs nanoparticles prepared at lower content of reduced agent were dispersed well in CaP-DENPtNPs composites, indicating the effective 1:1 mol ratio of NaBH₄:Na₂PtCl₆·6H₂O. The CaP-DENPtNPs composites, which were prepared following the optimum conditions described above, were utilized hereafter.

On the synthesis of CaP-DENPtNPs composites, the pH adjustment of the composite solution after adding each CaP precursor is of high importance, since it will lead to different outcomes in terms of the loading of DENPtNPs, the distribution of PtNPs, the largeness of surface area, and the variation of crystallinity. In order to reach the desired destination, the pH is the key factor, since the DEN belongs to a polyprotic molecule, where the pK of peripheral primary amines ($pK_1 = 9.2$) are more alkaline than the pK of internal tertiary amines ($pK_2 = 6.65$) [34]. Thus, DEN is almost neutral in an aqueous solution at pH 12, neutral in its interior and protonated in its periphery at pH 8, and protonated in almost all amino groups at pH 4. Then, CaP particles and CaP-DENPtNP composites were prepared at these pH conditions.

As seen in Fig. 1(a), transmission electron microscopic (TEM) images of CaPs at pH 12 and 8 exhibited particles with different aspect ratio, which was larger at pH 8 than at pH 12. For the case

of CaP at pH 4, the particles with various shapes and sizes coexisted and the large particles were in sub-micrometer to micrometer sizes. The morphologies of CaP-DENPtNP composites are also compared in Fig. 1(b). CaP matrices at pH 12 and 8 still maintained similar size and shape to those without DENPtNPs. However, the CaP matrices at pH 4 varied to the infinite structure owing to the abundant uptake of DENPtNPs, as shown in Fig. 1(b): The uptake of DENPtNPs nanoparticles occurred at every CaP matrices and increased in number with decreasing pH. The abundant uptake in acid environment brought in the infinite morphological structure of CaP matrices.

The particle size and zeta potential were evaluated from dynamic light scattering (DLS) and laser Doppler electrophoresis (LDE) measurements, respectively. It was confirmed from Table 1 that sizes of both CaP particles and CaP-DENPtNPs composites increased as the pH decreased. Especially, in acidic condition, two or three kinds of particles with different sizes coexisted. These tendencies in particle size supported TEM results in Fig. 1 regarding the effect of pH on size, but particle sizes from DLS seemed to be larger than those obtained from TEM. This indicates that the aggregation of both CaP particles and CaP-DENPtNPs composites proceeds with decreasing pH.

It has been reported that CaP materials (particularly HAp) display negative zeta potentials [35,36]. The result in the present study (Table 1) was in accordance with those reports, supported



Fig. 1. TEM images of (a) CaP particles and (b) CaP-DENPtNPs composites at different pH.

Sample	pH 12	pH 8	pH 4	H 4	
			Particle 1	Particle 2	Particle 3
Size (nm)					
CaP	58.9 ± 1.8	513 ± 31	-	370 ± 43	>7 µm
CaP-DENPtNPs	65.9 ± 2.2	573 ± 31	121 ± 10	505 ± 93	>7 µm
Zeta Potential (mV)					
CaP	-2.85 ± 0.05	-1.75 ± 0.25		-0.85 ± 0.05	
CaP-DENPtNPs	-0.15 ± 0.05	1.65 ± 0.05		-0.85 ± 0.15	
variation	2.70 ± 0.05	3.40 ± 0.15		0.00 ± 0.10	

 Table 1

 Size and zeta potential of CaP particles and CaP-DENPtNPs composites at different pH.

by a notion that PO_4^{3-} ions prefer to be located on the periphery of HAp [37]. Table 1 signified that the zeta potential of CaPs approached to zero as the pH was lowered, because PO_4^{3-} was protonated with decreasing pH. After the DENPtNPs was loaded, CaPs at pH 12 and 8 diminished the negative character, and the variation was smaller at pH 12 than at pH 8. This may be due to the surface charge of DEN, that is, the positive charge density of DEN is smaller at pH 12 than at pH 8, because the pK value of the peripheral primary amines of DEN is 9.2 [34], suggesting that large amounts of DENPtNPs were adsorbed on the surface of CaP at pH 8. On the other hand, no variation of zeta potential was observed at acidic pH, although TEM showed a huge incorporation of DENPtNPs inside the CaP matrix, and consequently, the surface charge was not affected.

The identification of functional groups in CaP and CaP-DEN-PtNPs was performed by Fourier transform infrared (FTIR) absorption analysis. As seen in Fig. 2(A) and Table S1, the FTIR spectrum of CaP at pH 4 was different from those at CaPs at pH 12 and 8 regarding positions and intensities of the IR bands. Actually, due to the protonation at acidic pH, PO₄ stretching vibration bands were blue-shifted, PO₄ bending vibration bands were red-shifted and relative intensities of these bands were varied.

Fig. 2(B) and Table S1 showed that IR absorption spectra of CaP-DENPtNPs composites maintained IR bands of CaPs in Fig. 2(A). The principal difference in the IR spectra of the composites against CaP particles came from the presence of DEN, as proved by amide I (1630 cm^{-1}) and II (1540 cm^{-1}) vibration modes [38]. The intensity of these bands slightly increased with decreasing pH from 12 to 8, but drastically strengthened at acidic pH. Furthermore, the CaP-DENPtNPs composites also exhibited new characteristic bands at 1370 and 1320 cm⁻¹. These bands, which were assigned to the CH₂ bending vibration modes in the backbone of DEN, had relatively high intensity at pH 4. These results indicate that larger amounts of dendrimers are incorporated into CaP at acidic pH than at higher pH, which is consistent with the observations by TEM.

Elemental analysis was performed by energy dispersive X-ray (EDX) analysis. EDX results in Fig. S6 displayed P and Ca peaks at 2.0 and 3.7 keV, respectively, and the intensity ratio between them changed depending on pH. Quantitative results (Table 2) showed that CaP particles retained the ideal Ca/P mole ratio of HAp at pH 12 and 8, but this ratio severely dropped down to 1.20 at pH 4, pointing out that CaP acquires a HAp structure at high pH but its crystallinity becomes rather different at acidic pH. Incidentally the HAp (Ca₅(PO₄)₃(OH)) particles are characterized by a calculated Ca/P mole ratio of 1.67 [31].

The EDX results of CaP-DENPtNPs composites in Fig. S6 and Table 2 indicated a tendency comparable to those of CaP particles in absence of DENPtNPs. That is, the composites at pH 12 and 8 retained the ideal Ca/P mole ratio of HAp, but this ratio significantly decreased at pH 4. It should be remarked that the value



Fig. 2. FTIR absorption spectra of (A) CaP particles and (B) CaP-DENPtNPs composites at different pH.

 (1.04 ± 0.10) was somewhat lower than that of CaP at pH 4, meaning that the crystal structure transition of CaP at acidic pH was promoted by the presence of DENPtNPs.

It was noticeable from the TGA profiles in Fig. 3 that CaP and CaP-DENPtNPs, obtained at pH 4, decreased greatly their weight at temperatures between 400 and 500 °C. This behavior was different from those of CaP and CaP-DENPtNPs obtained at pH 12 and 8, which gradually lost the weight till 700 °C and suggested us a difference in crystallinity of CaP depending on pH. The finding of a crystalline transition induced between pH 4 and 8 was consistent with the results from FTIR and EDX.

Table 2

Elemental analyses of CaP particles and CaP-DENPtNPs composites at different pH from EDX results.

	Content (atom%)				
	pH 12	рН 8	pH 4		
CaP					
Ca	37.5 ± 0.7	38.1 ± 0.7	45.5 ± 1.0		
Р	62.5 ± 0.7	61.9 ± 0.7	54.5 ± 1.0		
Ca/P	1.67 ± 0.05	1.62 ± 0.05	1.20 ± 0.05		
CaP-DENPtNPs	5				
Ca	60.3 ± 2.6	59.4 ± 0.2	48.4 ± 4.6		
Р	36.0 ± 0.4	36.2 ± 2.2	46.6 ± 0.3		
Ca/P	1.68 ± 0.06	1.65 ± 0.11	1.04 ± 0.10		



Fig. 3. TGA results of CaP particles and CaP-DENPtNPs composites at different pH.

Based on thermogravimetric analysis (TGA) results in Fig. 3, the content of DEN in CaP-DENPtNPs composites at each pH was quantitatively calculated from the weight difference (w_{DEN}) at 700 °C between CaP-DENPtNPs composites and CaP particles, as listed in Table 3. Then, the contents (w_{Pt} , w_{CaP}) of PtNP and CaP in composites can be evaluated from the following relations and procedures:

$$w_{\rm DEN} = 0.11 w_{\rm Pt} \tag{1}$$

$$w_{CaP} + w_{DEN} + w_{Pt} + w_{H_2O/OH} = 1$$
(2)

where $w_{H_2O/OH}$ is the content of H_2O/OH . Eq. (1) was obtained from a TGA result (Fig. 3) of the DENPtNPs nanoparticles, which indicated 2 steps of weight loss, namely the evaporation of adsorbed water from 30 to 120 °C and the decomposition of DEN from 120 to 600 °C. This result revealed that the DEN content was 9.60 wt% and the remaining substance (PtNPs) was 87.25 wt%. Hence, the mass ratio of DEN to Pt can be obtained as 0.11:1. Then, the mass value of CaP in CaP-DENPtNPs could be calculated using the observed mass of $w_{CaP} + w_{Pt}$ (see Fig. 3), and $w_{H_2O/OH}$ was from Eq. (2). Thus, the mass percentages of every component in the CaP-DENPtNPs composites were obtained at different pH as listed in Table 3. The results showed that the content of PtNPs (and

 Table 3

 Component analyses in CaP-DENPtNPs composites at different pH from TGA results.

Component	Content (wt%)			
	pH 12	pH 8	pH 4	
НАр	$81.0 \pm 0.0_5$	74.3 ± 0.4	28.2 ± 0.8	
DEN	1.67 ± 0.09	1.91 ± 0.43	6.63 ± 1.03	
Pt	$15.2 \pm 0.0_4$	17.4 ± 0.2	60.2 ± 0.5	
H ₂ O & OH	2.18 ± 0.01	6.44 ± 0.51	4.97 ± 0.88	

DENPtNPs) in CaP matrices increased slightly with decreasing pH from 12 to 8 and became remarkably high at acidic pH, which are evidently supported by the TEM results.

3.2. Structures of CaPs in composites

Since the results described above demonstrated that powders of CaP and CaP-DENPtNPs were pH-dependent on their crystallographic structure, wide-angle X-ray diffraction (XRD) analysis was performed. The XRD peaks of CaPs at pH 12 and 8 were exactly consistent with the theoretical Bragg peaks of HAp crystal (see Fig. 4(A)). However, the observed XRD of the acidic sample at pH 4 displayed a completely different combination of Bragg peaks, which were assigned to a crystal of monetite (CaHPO₄, in majority) and brushite $(CaHPO_4 (H_2O)_2)$, in minority). In order to verify the transition that occurred at 400-500 °C for a sample at pH 4 (see Fig. 3), the samples heated up to 1000 °C in TGA were then analyzed by XRD. The results in Fig. 4(B) showed that as for the sample at pH 12, even after heating there was no significant change or modification on its HAp crystalline structure, while for pH 8, tricalcium phosphate $(Ca_3(PO_4)_2)$ crystal appeared as the side product and the sample at pH 4 consisted of calcium pyrophosphate (β -Ca₂P₂O₇) with a side product $(Ca_3(PO_4)_2)$. This thermal transition of crystal follows the reaction proposed in a literature [39];

 $2CaHPO_4 \rightarrow \beta$ - $Ca_2P_2O_7 + H_2O$

suggesting that monetite can dehydrate to calcium pyrophosphate at temperature up to 520 °C.

From XRD results of CaP-DENPtNPs composites (Fig. 4(C)), it is apparent that the crystallinity of CaPs at pH 12 and 8 was preserved even after the uptake of DENPtNPs. On the other hand, the peaks corresponding to monetite were still present in the composite at pH 4, but the peaks of brushite were diminished. The variation of crystallinity, as observed by XRD, can be compared to the Ca/P ratios obtained from EDX. The Ca/P ratio (1.20) of CaP at pH 4 was higher than the calculated value of monetite (CaHPO₄, Ca/P = 1.0) due to contamination of brushite, while the Ca/P ratio (1.04) of CaP-DENPtNPs at pH 4 was consistent, within experimental error, with the value of monetite.

The noteworthy difference of CaP-DENPtNPs from CaP on XRD results was the existence of crystal PtNP, namely, the appearance of broad Bragg peaks at around 39°, 46° and 67° of (111), (200) and (220) planes, respectively. These peaks were more intense at pH 4 than at pH 12 and 8, due to the incorporation of larger amount of PtNPs at pH 4, in consistency with TEM and TGA results.

Fig. 5 presents small-angle X-ray scattering (SAXS) patterns of CaP-DENPtNPs composites at different pH. For the composites at pH 12, an almost imperceptible peak appeared. However, at pH 8, an intensified broad (100) peak emerged at a 2θ value centered around 2.41°, corresponding to the d-spacing, d_{SAXS} , value of 3.66 nm. It was further strengthened at pH 4, taking place at a 2θ value of approximately 2.33°, which corresponded to the d_{SAXS} value of 3.79 nm. Such broad peaks might originate from the average correlation distance (center-to-center distance) between neighbor DENPtNPs nanoparticles. It might be noted that the broad peaks in SAXS were not sharp enough to be associated with highly ordered crystals. However, the higher intensity may also indicate that ordered domains of nanoparticles are formed as pH decreases. This is relative to TEM results showing the large uptake of DEN-PtNPs nanoparticles in the acidic pH (Fig. 1(b)). In addition, it was discernible from Fig. 1(b) (see round marks) that the arrangement of such nanoparticles in small domain was hexagonal. Therefore, the d_{SAXS} values can be used to calculate the center-to-center distances ($a_0 = (2/\sqrt{3})d_{SAXS}$) of 4.23 nm for pH 8 and 4.38 nm for pH 4 based on the geometrical definition (Fig. 6(A)). These results



- \triangle Ca₃(PO₄)₂ (Tricalcium phosphate ~ JCPDS #09-16)
- Pt (JCPDS #04-0802)

Fig. 4. XRD results of (A) CaP particles, (B) burned CaP particles and (C) CaP-DENPtNPs composites at different pH.



Fig. 5. SAXS results of CaP-DENPtNPs composites at different pH.

were also consistent with TEM analysis, where a_o was 4.20 ± 0.31 nm for pH 8 and 4.31 ± 0.62 nm for pH 4 (Table 4).

The textural qualities of DENPtNPs nanoparticles in CaP matrices at three pH values were investigated through nitrogen sorption isotherms, and the results are shown in Fig. 7. The nitrogen adsorption and desorption isotherms of CaP-DENPtNPs composite



Fig. 7. Adsorption/desorption $N_{\rm 2}$ isotherms for CaP-DENPtNPs composites at different pH.

powders corresponded to a type IV physisorption isotherm cycle for mesoporous materials according to the BDDT (Brunauer– Deming–Deming–Teller) system [40] with well-defined steps from $P/P_0 = 0.85-0.95$ for a sample at pH 12, from $P/P_0 = 0.85-0.97$ for



Fig. 6. Illustrations of (A) a geometry of hexagonal array and (B) the possible model of CaP-DENPtNPs composites at different pH.

Numerical values obtained from BET, BJH, SAXS, and TEM analyses for CaP-DENPtNPs composites at different pH.

Table 4

CaP-DEN(PtNP)s	S_{BET} (m ² /g)	V _p (cm ³ /g)	D _{BJH} (nm)	d _{SAXS} (nm)	a _{o, SAXS} (nm)	a _{o, TEM} (nm)	D _{Pt, TEM} (nm)
pH 12	105	0.69	3.06	-	-	-	2.75 ± 0.94
рН 8	83.4	0.84	3.06	3.66	4.23	4.20 ± 0.31	2.69 ± 0.62
pH 4	62.2	0.45	3.60	3.79	4.38	4.31 ± 0.62	2.62 ± 0.62

pH 8, and from $P/P_o = 0.94-0.97$ for pH 4. Due to those characteristics and the large hysteresis loop extending from relatively low pressures, it can be noted that the composites possess a mesostructural nature. Besides, Fig. 7 also indicated the existence of a weak adsorbate (N₂)-adsorbent (composite) interaction from the hardly visible rounded-knee line shape during the initial relative pressure. Consequently, it can be suggested that micropores (<2 nm) are scarcely present in the composites materials.

Moreover, according to the International Union of Pure and Applied Chemistry (IUPAC) classification [40], those isotherms also displayed particular types (H1-H4) of a hysteresis loop. The hysteresis behavior from composites at pH 12 and 8 could be assigned to type H1, consistent with TEM observations that the CaP particles were approximately uniform and possessed a low aspect ratio like spheres and bear cylindrical pores. However, the composite at pH 8 produced a type H3 hysteresis loop, since the CaP matrices at pH 8 also took a platelet-like structure with a high aspect ratio, giving rise to slit-shaped pores besides cylindrical ones. The composite at pH 4 exhibited the plate-like structure and, therefore, belonged to type H4, featuring narrow slit-like pores. Overall, the specific surface areas (S_{BET}) were lowered from 105 m²/g at pH 12 to 62 m^2/g at pH 4, as listed in Table 4. This comes from the difference of the degree of CaP crystallinity, as known from the variation of Bragg peaks in XRD depending on pH (see Fig. 5). The low degree of crystallinity at alkaline pH indicates the coexistence of microcrystal domains, giving rise to the large surface area [41].

The pore sizes, D_{BJH} , of CaP-DENPtNPs composites listed in Table 4 were common at three pH, indicating the existence of mesopores (2–50 nm). The obtained D_{BJH} values are rather close to the diameter of DENPtNPs nanoparticles and larger than the diameters of PtNPs observed by TEM. Moreover, D_{BJH} values are smaller than the center-to-center distances (a_o) from SAXS and TEM analyses. These parameters are related to the DENPtNPs nanoparticles and their arrangement in the composites.

Supposing that CaP itself also has irregular macroporous (>50 nm) structures besides mesopores, the pore volume (V_p) should vary, depending on type and number of the pores. The V_p value depends on the void volume inside composites, where the adsorbate (N_2 gas) fills. The irregular tendency in V_p values depending on pH (see Table 4) may be induced by the competition of variables with opposite effects. Macropores are majority at alkaline pH but they decreases with decreasing pH, relating to the decrease of surface area. Meanwhile, mesopores increase with the encapsulation of DENPtNPs inside the CaP, that is, with decreasing pH. The variation of whole pore volume results from the competition between the opposite effects of the variation of macro- and mesopores.

Various analyses were carried out regarding the fabrication of CaP-DENPtNPs, especially their morphology and the location of DENPtNPs in CaP matrices at different pH as described above. Fig. 6(B) is a schematic representation based on the analytical results. CaP matrices in composites increased their size but decreased their surface area and surface charge with decreasing pH, although the pore volume was the maximum at pH 8. The incorporation of DENPtNPs increased with decreasing pH, but DEN-PtNPs mainly adsorbed on the periphery of CaP matrices at alkaline environment, where the negative surface charges of CaP matrices were compensated by the positive charges on the exterior of basic DENPtNPs. The incorporation of DENPtNPs took place inside CaP matrices in an acidic pH, resulting in the unchanged surface charge of CaP matrices. The abundant coating by DEN over the surface of CaP nanoparticles at alkaline pH and the decreasing coating thickness with decreasing pH have been reported already [31]. These previous results are of a great support for the current finding that the DENPtNPs are distributed on periphery of CaP matrices at high pH.

3.3. Formation mechanism of CaP-DENPtNPs composites

Regarding the formation mechanism of CaP-DENPtNPs composites, for the case of pH 12 ($pK_1 < pH$), the less protonation of primary NH₂ groups in DEN causes the less binding with PO₄^{3–} precursor, resulting in the least uptake of DENPtNPs nanoparticles inside CaP matrices. However, DEN can adsorb on the external surface of CaP matrices, probably by hydrogen bonding. When the pH was adjusted to 8 ($pK_2 < pH < pK_1$), protonated primary NH₂ groups bind with PO₄^{3–} precursor ions through the electrostatic interaction, inducing the uptake of DENPtNPs nanoparticles into CaP matrices. The CaP-DENPtNPs at pH 4 ($pH < pK_2$) can incorporate a large number of DENPtNPs nanoparticles inside CaP, since the protonated tertiary and primary amines are abundantly able to bind with PO₄^{3–} ions.

The aforementioned analyses confirmed that the CaP matrices are apparently affected by the reaction pH. In order to understand the formation of CaP crystals as the 3D-structured matrix at different pH, it is essential to take account of the chemical reaction based on the fundamental stepwise dissociation of phosphoric acid (H₃PO₄) [42]. The reaction at the alkaline and neutral conditions is likely as follows:

$$\begin{split} & 6(NH_4)_2HPO_4 + 10Ca(NO_3)_2 + 8NH_4OH \\ & \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20NH_4NO_3 + 6H_2O \end{split}$$

 $Ca_{10}(PO_4)_6(OH)_2 \xrightarrow{\Delta} Ca_{10}(PO_4)_6(OH)_2$

 HPO_4^{2-} ions in a $(NH_4)_2HPO_4$ solution are turned into PO_4^{3-} ions, since an NH_4OH solution is added to adjust the pH. The resultant PO_4^{3-} ions combine with Ca^{2+} ions in an added $Ca(NO_3)_2$ solution to create stoichiometric HAp $(Ca_{10}(PO_4)_6(OH)_2)$ precursors with the by-product of ammonium salts. Then, the HAp keeps its molecular structure even after the hydrothermal treatment.

On the other hand, the possible reaction at acidic environment is

$$\begin{array}{l} 2(\mathsf{NH}_4)_2\mathsf{HPO}_4 + \mathsf{Ca}(\mathsf{NO}_3)_2 + 2\mathsf{HCl} \rightarrow \mathsf{Ca}(\mathsf{H}_2\mathsf{PO}_4)_2 + 2\mathsf{NH}_4\mathsf{NO}_3 \\ + 2\mathsf{NH}_4\mathsf{Cl} \end{array}$$

$$Ca(H_2PO_4)_2 \xrightarrow{\Delta} CaHPO_4 + H_3PO_4$$

 $Ca(H_2PO_4)_2 + 2H_2O \xrightarrow{\Delta} CaHPO_4(H_2O)_2 + H_3PO_4$

 $H_2PO_4^-$ ions are formed from HPO_4^{2-} ions in a $(NH_4)_2HPO_4$ solution, since an HCl solution is added to adjust the pH. When the Ca $(NO_3)_2$ solution is added, Ca²⁺ ions bind with $H_2PO_4^-$ ions to be monocalcium phosphate anhydrous $(Ca(H_2PO_4)_2)$ with byproducts of ammonium salts. When the hydrothermal treatment is performed, 2 types of products are produced, namely, monetite $(CaHPO_4)$ and brushite $(CaHPO_4(H_2O)_2)$. The CaHPO_4 predominates in the product (Fig. 4), since it is easier for Ca $(H_2PO_4)_2$ to be crystallized into CaHPO_4 than to undergo a hydration into CaHPO_4(H_2O)_2 during the hydrothermal procedure at high temperature and pressure.

4. Conclusions

CaP-DENPtNPs composite particles were obtained through the successive mixing of DENPtNPs nanoparticles with PO_4^{3-} and Ca^{2+} precursors of CaP, followed by aging at 150 °C for 15 h. The pH played a key role both on the distribution of DENPtNPs nanoparticles and the morphological/crystal structures of CaP matrices. The lowering in pH improved the incorporation of DENPtNPs into CaP matrices and brought on the variation of the size of the matrices as well as their crystalline structure from HAp to monetite. Such

pH lowering also promoted the formation of ordered domains of DENPtNPs nanoparticles in composites. Moreover, the specific surface area decreased together with decreasing pH because of the increasing crystallinity of CaP, while the pore volume in the composite was highest at pH 8, since it was influenced competitively by the variation of contribution of meso- and micropores. The particle charge (zeta potential) of the composites was also affected by the pH, resulting in the maximum value at pH 8, because it depends on the surface charge of CaP matrices and the adsorption of DENPtNPs on the surface of CaP matrices. Ultimately, it can be concluded that CaP-DENPtNPs are mesoporous nanocomposites, where DENPtNPs nanoparticles are distributed on the surface and/or the interior of CaP matrices. The limited amount of supply as well as high cost is still a challengeable subject on the catalytic usage of Pt that is required on its efficient applications. The high loading (60%) of Pt on calcium phosphate will satisfy this demand. Moreover the manipulation of Pt materials with nanoscaled size can contribute to increase the catalytic efficiency of Pt and to achieve the necessary cost reduction. Pt is particularly focused on the catalytic and electrocatalytic reactions such as methanol oxidation [43], and oxygen reduction [44] in applications to automotive converters [45], sensors [33], and fuel cells [46].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.micromeso. 2014.07.003.

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