



RESEARCH ARTICLE

CHARACTERIZATION OF NANO DICALCIUM PHOSPHATE (NDCP) SYNTHESIZED BY SOL-GEL METHOD

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ABSTRACT

This study was conducted as an attempt to synthesize dicalcium phosphate in nano particle size (NDCP). A sol-gel method with deionized water or ethanol as a solvent was used.

The synthesized NDCP was characterized by means of Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM) and Energy Dispersive Analysis by X-ray (EDAX).

The results indicated that using ethanol solvent resulted in 81.8% NDCP and 18.2% calcium carbonate, but deionized water solvent gave 100% NDCP. Crystal sizes of NDCP synthesized using deionized water or ethanol as solvent were 26 nm and 34 nm, respectively. The NDCP synthesized using deionized water solvent is better than that synthesized by ethanol solvent in purity, size and cost of synthesis.

The proposed method is environmentally friendly, economic, time-saving and could perform at room temperature.

Further applied feeding studies on poultry will be conducted to determine the bioavailability of P on nano particle size.

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INTRODUCTION

Phosphorus and calcium are essential macro minerals; they are the most abundant mineral nutrient in the body.

Phosphorus is essential for bone formation, acid-base balance, protein synthesis and growth of body tissues. It is a part of phospholipids (fat molecules), such as lecithin, which cells use to make cell membranes. It also involves in the active coenzyme form of certain B vitamins and is an important consistent of the high-energy compound Adenosine tri-Phosphate (ATP), nucleic acids (DNA and RNA) that are responsible for cell reproduction (Soetan *et al.*, 2010).

Calcium functions as a constituent of bones and regulation of nerve and muscle function. In blood coagulation, calcium activates the conversion of prothrombin to thrombin. It plays a vital role in enzyme activation. Calcium activates a large number of enzymes such as Adenosine Tri-Phosphatase (ATPase), succinic dehydrogenase, lipase etc....(Soetan *et al.*, 2010).

Poultry diets consist of plant products often contains large amounts of unavailable phosphorus known as phytates which

accounts for 60-80% of the total phosphorus present in plant feeds. The Phytate-bound phosphorus is largely unavailable to monogastric animals as they do not naturally have the enzyme (phytase) needed to break it down (Bozkurt *et al.*, 2006). To meet the dietary phosphorus requirements of poultry, diets are often supplemented with inorganic P for optimal growth and production, which increases the cost of the diets. The unused portions of the supplemental P as well as the indigestible phytate are excreted by poultry, resulting in high concentrations of P in the excreta cause increases the cost of the diets and contributes to environmental pollution (Ravindran *et al.*, 2000).

Dorozhkin (2013) reported and tabulated the different materials of calcium phosphates including the Ca/P molar ratio, compounds and their typical abbreviations, chemical formula and properties.

Dicalcium phosphate (DCP) is a dibasic calcium phosphate as a dietary supplement; it is commonly used as source of Ca and P for both human and animal. It is obtained as a result of an acid-base reaction, DCP the most stable one at pH 2-6.5 (Dorozhkin, 2009; Ginebra *et al.*, 2012 and Rogina *et al.*, 2013).

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The recent strategy has been explored is nano minerals, which are increasing the absorption, bioavailability and efficiency of utilization by increasing the surface area leading to a reduction in the quantity of supplements, thus a reduction in feed cost and reduce environmental pollution.

The transition from micro to nano composites has led to a great improvement in the properties attributed to the higher surface area and interfacial energies associated with the nanoparticles.

Calcium phosphate materials in nano-size have higher specific surface area and surface roughness compared to conventional calcium phosphate materials. Therefore, nano-sized calcium phosphate materials have stronger interaction with organic materials (Chan et al., 2006 and Gross et al., 2014).

Rajendran (2013) reported that supplementation of mineral in the form of nano (Se, Cr and Zn) increases bioavailability and efficiency of utilization by increasing the surface area. Thus, calcium phosphate materials in nano-size are expected to have better bioactivity compared with conventional materials. The advantages of synthetic calcium phosphate materials in nano-size include higher biocompatibility, good biodegradability in situ (Poinern et al., 2009).

Nano-sized calcium phosphates offer a higher surface area with improved reactivity, a greater drug loading capacity and interaction with the biological environment (Gross et al., 2014).

Calcium phosphate particles could be prepared by a variety of techniques such as mechano chemical synthesis, co-precipitation, sol-gel synthesis, hydrothermal reaction, mechanical milling, combustion preparation, and wet chemistry techniques, etc., by which calcium phosphate particles with various shapes and sizes can be obtained (Gu et al., 2007; Jadalannagari et al., 2011; Lee et al., 2010 and Niu, 2007).

This study aimed to synthesize the nano dicalcium phosphate inorganic particles (NDCP) using the sol-gel method with deionized water or ethanol as solvent. The synthesized NDCP was characterized by Fourier transform infrared spectroscopy (FTIR), X-Ray diffraction (XRD), Transmission electron microscope (TEM) and Energy Dispersive Analysis by X-ray (EDAX) using scanning electron microscope.

MATERIALS AND METHODS

MATERIALS

Calcium carbonate (CaCO₃) and phosphoric acid (H₃PO₄) were used as starting calcium and phosphorous precursors. Deionized water and ethanol were used as solvent materials.

METHODS

Two samples of dicalcium phosphate were prepared from the same source of calcium and phosphorus (CaCO₃ and H₃PO₄) using two different solvents (deionized water - ethanol) using the sol-gel method.

NDCP with a Ca/P ratio of 1 was produced as follows: A solution of 0.5M phosphoric acid (H₃PO₄) was drop wise to 0.5M CaCO₃ solution, while, stirring using deionized water or ethanol as solvent.

After all the H₃PO₄ solution had been added, the solution was stirred for a further six hours at room temperature, resulting in white precipitates settling at the bottom of the beaker.

The precipitate was separated from the solution using What man® ashless, Grade 42 filter paper and washed repeatedly using deionized water to remove the impurities. The obtained DCP particles were dried at 60 °C for 24 h in a dry oven.

Measurements Techniques

The synthesized products were measured by different methods:

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR measurements were carried out using a Jasco instrument (Model 6100, Japan). Analyses were performed after mixing 2 mg of sample powders with 198 mg of KBr powder, followed by compacting those into a thin pellet in a stainless steel die of 1 cm inner diameter. FTIR spectra of the samples were obtained in the spectral range of 4000- 400cm⁻¹.

X-Ray Diffraction (XRD).

X-ray diffraction was used to detect the phase analysis of the produced particles using Shimadzu 7000, Japan equipped at Cu-K radiation (λ = 0.1540 nm), the tube operated at 30kV, Bragg's angle (2θ) in the range (5-60°).

Crystal Size (D)

The mean crystal size (D) of the particles was calculated from the XRD line broadening measurement from Scherrer's equation (Azaroff, 1968):

$$D = \frac{0.89\lambda}{n \cos\theta}$$

Where: D is diameter of the dicalcium phosphate particle, λ is wavelength of CuK radiation (λ = 1.5406 Å), Δ is full width at half maximum (FWHM) of the intensity peak and θ is the diffraction angle at the maxima of high intensity peak in the patterns.

Transmission Electron Microscope (TEM)

Transmission Electron Microscope (TEM) (JEOL - JEM-1011, Japan) was used to observe the morphology and the particle size. Samples were prepared by putting the carbon grid in the deposition chamber and particles deposited on the grid, and then the images were recorded.

Energy Dispersive Analysis by X-ray (EDAX)

The EDAX spectrum was performed using Scanning electron microscope (JEOL-JSM 6100) using the OXFORD X-ray

microanalysis software was used to examine the elemental composition of the sample.

RESULTS

Fourier Transform Infrared Spectroscopy (FT-IR)

The results of Fourier Transform Infrared Spectroscopy (FT-IR) showed no differences among all treatments (particles obtained by water solvent, A; ethanol solvent, B and the commercial dicalcium phosphate particles, C) and the Standard dicalcium phosphate in the range 4000- 400cm⁻¹ (Fig. 1). These results confirmed the chemical compositions of the obtained dicalcium phosphate.

Table 1 IR assignments of most intense absorption bands for particles obtained using water solvent (A), ethanol solvent (B) and the commercial (C) dicalcium phosphate inorganic particles.

IR wavenumbers (cm ⁻¹)			IR vibration modes
A	B	C	
3440	3420	3428	O-H stretching of residual free water
2854	2858	2855	PO-H stretching
2446	2425	2428	PO-H stretching
1644	1624	1628	O-H bending and rotation of residual free water
1402	1424	1439	P-O-H in-plane bending
1132	1130	1130	P-O stretching
1067	1066	1068	P-O stretching
999	998	998	P-O stretching
899	876	881	P-O(H) stretching
575	569	570	O-P-O(H) bending mode

The different absorption bands are shown in Table 1. The absorption bands at 3420- 3440 cm⁻¹ are attributed to the O-H stretching of residual free water. The absorption bands at 2854-2858 and 2425-2446 cm⁻¹ are attributed to the PO-H stretching. The absorption bands at 1624-1644 cm⁻¹ are attributed to the H-O-H bending and rotation of residual free water. The absorption bands at 1402-1439 cm⁻¹ are attributed to the P-O-H in-plane bending. The absorption bands at 1130-1132, 1066-1068 and 998-999 cm⁻¹ are attributed to the P-O stretching. The absorption bands at 876-899 cm⁻¹ are attributed to the P-O(H) stretching. The absorption bands at 569- 575cm⁻¹ are attributed to the O-P-O(H) bending mode.

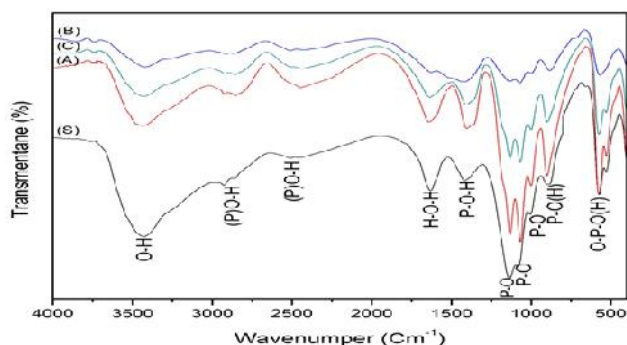


Figure 1 FT-IR absorption spectra of the particles obtained by water solvent (A), ethanol solvent (B), the commercial (C) and the standard (S) dicalcium phosphate inorganic particles.

X-ray Diffraction (XRD)

XRD analysis is very useful in knowing the structure of the materials.

The XRD patterns of the nano dicalcium phosphate inorganic particles obtained by water solvent (A), by ethanol solvent (B) and the commercial dicalcium phosphate inorganic particles (C) in the scanning range 2θ 5-60° are shown in Fig. 2.

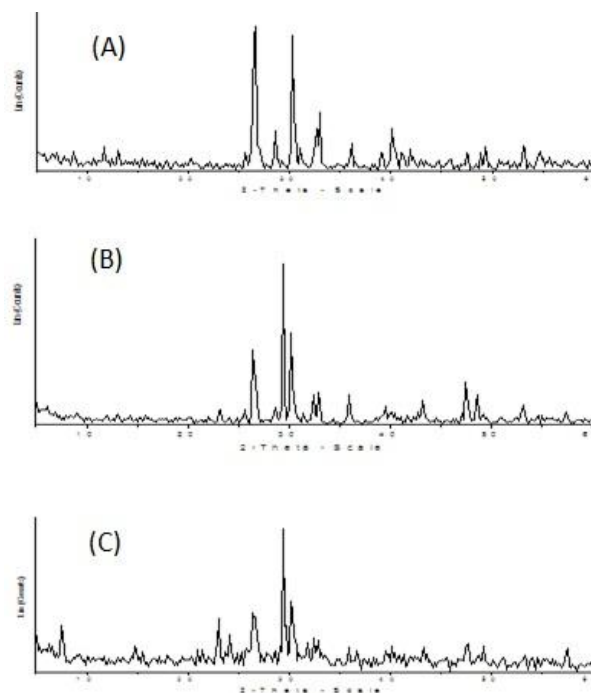


Figure 2 X-ray diffraction of the nano obtained by water solvent (A), by ethanol solvent (B) and the commercial (C) dicalcium phosphate inorganic particles.

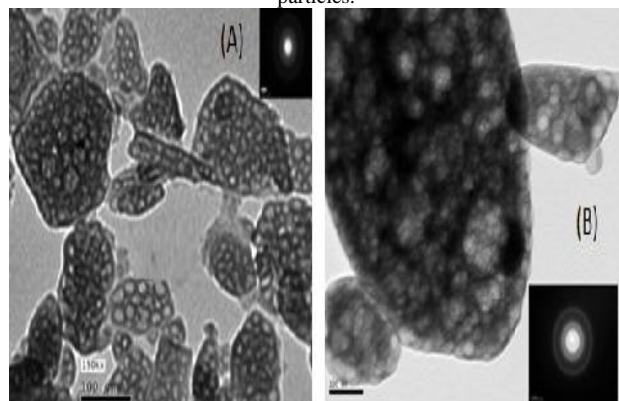


Figure 3 TEM images of the nano obtained by water solvent (A) and by ethanol solvent (B) dicalcium phosphate inorganic particles.

In the A pattern the diffraction peaks are almost identical with standard graphics of dicalcium phosphate anhydrous (reference code: 70-0359), by 92.8%, which further indicates DCP formed indeed and dicalcium phosphate dihydrate, CaHPO₄ (H₂O)₂ (reference code: 72-0713) by 7.2%. In the B pattern the diffraction peaks are almost identical with standard graphics of dicalcium phosphate anhydrous (reference code: 70-0359), by 81.8%, which further indicates DCP formed indeed and calcite, CaCO₃ (reference code: 88-1807) by 18.2%.

In the C pattern, the diffraction peaks are identical with standard graphics of dicalcium phosphate anhydrous, CaHPO₄ (reference code: 70-0359) by 43.5%, calcite, CaCO₃ (reference code: 88-1807) by 14.5%, Calcium oxide, CaO (reference code: 17-0912) by 10.3%, Bassanite CaSO₄.0.5H₂O (reference

code: 33-0310) by 18% and calcium hydrogen phosphate hydrate, $\text{Ca}(\text{H}_2\text{PO}_4)_2(\text{H}_2\text{O})$ (reference code: 70-0090) by 13.7%.

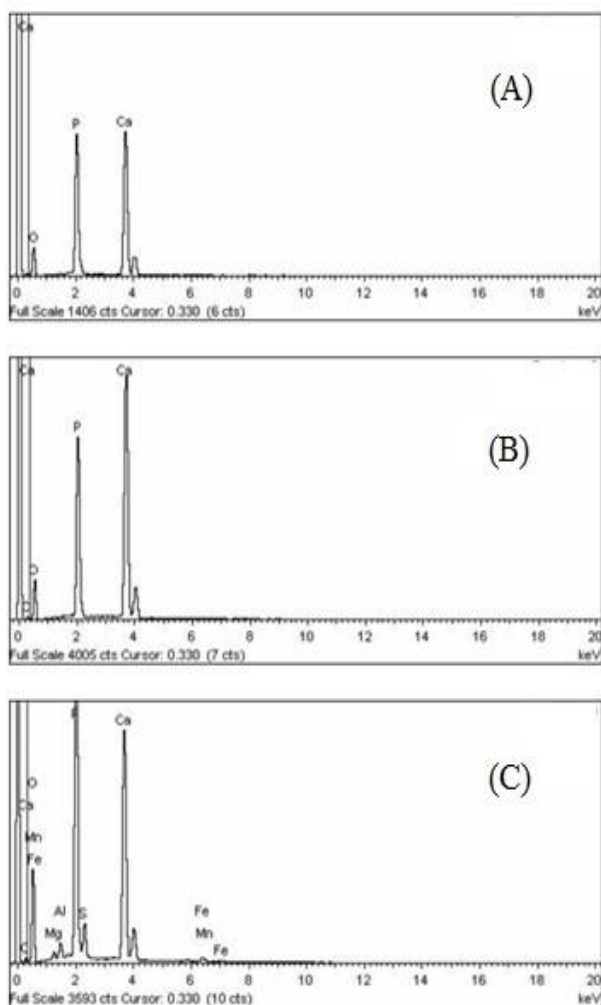


Figure 4 EDAX of the nano obtained by water solvent (A), by ethanol solvent (B) and commercial (C) dicalcium phosphate inorganic particles.

Crystal Size (D)

The Scherrer's equation was adopted to calculate the mean crystal size (D) of the obtained nano dicalcium phosphate inorganic particles from the XRD line broadening. The results showed that the mean crystal sizes of the particles obtained by deionized water solvent (A), and ethanol solvent (B) were 26 nm and 34 nm, respectively.

Transmission Electron Microscope (TEM)

The results of TEM (Fig. 3) confirmed that the NDCP particles contained clusters comprised of still finer particles of 10 to 15 nm in size, and indicated that the material has an amorphous structure.

EDAX (Energy Dispersive Analysis of X-rays)

The results of Energy Dispersive Analysis of X-rays (EDAX) are shown in Fig.4. The powder obtained by water solvent (A) composed of Ca, O and P is present in only nano dicalcium

phosphate. This result is in agreement with the X-ray result. The powder obtained by ethanol solvent (B) composed of Ca, O, P and C is present in nano dicalcium phosphate and contains calcium carbonate. This result is in agreement with those obtained by X-ray. The composition of commercial dicalcium phosphate (C) showed Ca, O, P, Mg, Al, S, Mn and Fe because it contains DCP and impurities. This result is also in agreement with X-ray results.

The presented spectrums show that the Ca/P value of synthesized NDCP by water solvent (A), NDCP by ethanol solvent (B) and commercial (C) dicalcium phosphate have the Ca/P ratio close to dicalcium phosphate.

DISCUSSION

Since 1975 till now, There were attempts to develop new methods, which would facilitate a reduction in the mean particle size of CaHPO_4 (Aoki *et al.*, 1989; Chen *et al.*, 2005; Chenot,1975; Eshtiagh-Hosseini *et al.*, 2008; Jinawath *et al.*, 2001; Kong *et al.*, 2005; Louati *et al.*, 2005; Ma *et al.*, 2006; Sun *et al.*, 2010; Tas, 2009; Thomas and Dehbi, 1986 and Wächter *et al.*, 2013).

Chen *et al.* (2005) synthesized submicrometer particles of CaHPO_4 in oil-in-water (o/w) system and water-in-oil (w/o) system with a novel membrane microdispersion mixing technique. In this study, sodium dodecyl sulphate (SDS)-containing Ca-acetate aqueous solutions were used as the water phase, whereas butanol–phosphoric acid mixture was the oil phase.

Kong *et al.* (2005) prepared reverse microemulsions by adding calcium chloride and sodium hydrogen phosphate, aqueous solutions into a mixture of Span 80s, Tween 80s, and n-heptane to get two kinds of emulsions. n-buranol was used to adjust the emulsions to transparent state. Calcium phosphate was prepared by adding phosphate microemulsion to the calcium microemulsion. They obtained micron size needles of CaHPO_4 . Louati *et al.* (2005) synthesized large crystals of CaHPO_4 by slowly evaporating an aqueous solution of the stoichiometric mixture of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $\text{NH}_4\text{H}_2\text{PO}_4$ at around 80°C .

Ma *et al.* (2006) synthesized CaHPO_4 particles (3–5 μm in diameter) by one-step microwave-assisted heating method at 95°C using $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, NaH_2PO_4 , and sodium dodecyl sulfate (SDS) in water/ethylene glycol (EG) mixed solvents.

Eshtiagh-Hosseini *et al.* (2008) slowly added a solution of H_3PO_4 diluted in methanol to a solution of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ dissolved in methanol, followed by aging the mixed solutions from 24 h to 6 days in sealed glass containers to obtain heavily agglomerated and CaCO_3 -containing CaHPO_4 particles. One of the reported disadvantages of this method is used methanol, which has an extremely toxic property.

Tas (2009) prepared submicron CaHPO_4 powders by reacting precipitated and submicron calcium carbonate powders together with phosphoric acid in a medium of pure ethanol. The solutions did not contain any water and the reaction was performed at room temperature. Sun *et al.* (2010) obtained

primary crystals (172 nm) of the nCaP with Ca/P = 1 prepared by spray drying process.

Wächter *et al.* (2013) studied dicalcium phosphate prepared by emulsion synthesis with different surfactants (Tween 80, Tween 50 and Brij 35) and concentrations (5 and 10%). They found that particles appeared to be agglomerates ($> 20 \mu\text{m}$) of fine-sized crystals with a size of the sub- μm range shown in the TEM. This was confirmed by particle size measurements demonstrating medium sizes d_{50} to be in the range of 6 – 10 μm , while the primary crystal size (calculated from peak widths of XRD data) was found to be approximately 30 – 90 nm.

In this study, synthesized NDCP by sol-gel with two different solvents (deionized water and Ethanol). The proposed method doesn't involve using or production of any toxic or environmentally hazardous solvents, surfactants, or organic chemicals and be economical, time-saving, and performed at room temperature.

According to the appearance of absorption peaks at Fourier Transform Infrared Spectroscopy (FT-IR), it could be concluded primarily that the inorganic Ca-P component obtained is dicalcium phosphate, which is identical with previous reports (Sun *et al.*, 2010 and Tas, 2009).

The X-ray patterns detect the structure of the materials synthesized by deionized water solvent is better than that synthesized by ethanol solvent in purity, they were 100% and 81.8% NDCP, respectively these results were confirmed by EDAX results.

All the FTIR and XRD profiles of nano dicalcium phosphate from deionized water and ethanol were in good agreement with the FTIR and XRD spectrums of standard dicalcium phosphate. Which is identical with previous reports (Sun *et al.*, 2010; Tas, 2009 and Wächter *et al.*, 2013).

The EDAX spectrums show that the Ca/P value of synthesized NDCP by water solvent (A), NDCP by ethanol solvent (B) and commercial (C) dicalcium phosphate have the Ca/P ratio close to dicalcium phosphate.

Crystal size of NDCP synthesized using deionized water or ethanol as solvent estimated by Scherrer's equation were 26 nm and 34 nm, respectively. This result is in agreement with the result of TEM that found the NDCP particles contained clusters comprised of still finer particles (Wächter *et al.*, 2013) of 10 to 15 nm in size. Results referred to success to synthesize of much smaller particles.

CONCLUSION

The sol-gel method provides a simple route and environmental friendly for synthesis of nano dicalcium phosphate. Nano dicalcium phosphate (NDCP) with a Ca/P ratio of 1 synthesized using a sol-gel method with deionized water solvent is better than that with ethanol solvent in purity, size and cost.

The structure of NDCP obtained by the sol-gel method allows the product to be used for nutrition. The proposed method doesn't involve using or production of any toxic or environmentally hazardous solvents, surfactants, or organic chemicals and be economical, time-saving, and performed at room temperature. Thus, the sol-gel process can be usefully utilized to synthesize NDCP under significantly mild conditions.

Further applied feeding studies on poultry will be conducted using the synthesized NDCP to determine the bioavailability of P on such nano particle size.

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