



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2019; 7(6): 726-729

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Received: 07-09-2019

Accepted: 09-10-2019

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## Synthesis and characterization of feed grade nano dicalcium phosphate for poultry feeding

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### Abstract

Dicalcium phosphate nanoparticles (nano-DCP) were synthesized by physical method using ball mill. The synthesized DCP nanoparticles were characterized by various techniques viz. Particle size analyser, Zeta potential, Transmission Electron Microscopy (TEM), X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Energy Dispersive Analysis by X-ray (EDAX). The results revealed that synthesized nano-DCP had particle size between 10-20 nm and zeta potential value of  $-25.6 \pm 2.2$  mV. Crystal size of nano DCP analysed by XRD was 29.31 nm. Calcium and phosphorus content of synthesized nano-DCP were 23.22 and 17.67 per cent. Ball milling, a popular physical method and is easy to operate, economic, time saving and higher yield. Based on its bioavailability, the synthesized feed grade nano DCP can be utilized for *in ovo* feeding of broiler embryos, livestock and poultry feeding.

**Keywords:** Nano dicalcium phosphate, synthesis, characterization, ball mill, poultry

### Introduction

Calcium and phosphorus are involved in many vital biological activities of the chicken. Requirement of such nutrient is more in case of poultry to perform these activities (Hassan *et al.*, 2016) [1]. Use of costly inorganic source of phosphorus in poultry production results in increase of feed cost which in turn cost of production and also leads to excess amount of excretion in the droppings which leads to wastage of nutrients and negative effect on environment.

Nanotechnology, new emerging era of poultry nutrition becoming a popular trend because of its versatile advantages like increased bioavailability, absorption and reduction in excreta and its minimum quantity required in feed compared to conventional sources (Swain *et al.*, 2015) [2]. Dicalcium phosphate (DCP) is a dibasic calcium phosphate commonly used as a feed supplement and also as an important source of calcium and phosphorus for livestock and poultry.

Calcium phosphate nanoparticles can be synthesized by various method viz. physical (Ramesh, 2014) [3], wet chemical (Huang *et al.*, 2004) [4], solid state (Ota and Iwashita, 1998) [5], sol-gel (Samy *et al.*, 2015) [6], mechanochemical (Nakamura *et al.*, 2001) [7] and microwave processing (Sarig and Kahana, 2002) [8] methods. Out of these, physical method is more suitable because of its advantages viz., simple to operate, low cost of production and the possibility to scale it to produce large quantities (McCormick, 1995) [9].

Hence, an attempt has been made in this study to synthesize the nano dicalcium phosphate particles by using ball milling method to assess its suitability for livestock and poultry feeding.

### Materials and Methods

Feed grade dicalcium phosphate powder (DCP) was purchased from the Centre for Advanced Studies in Poultry Science, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, India.

### Synthesis of nano dicalcium phosphate

Nano particles of DCP were prepared by physical method using ball mill and it was synthesised by grinding feed grade bone based dicalcium Phosphate in a ball mill (8000D Mixer/Mill – Dual High Energy Ball Mill) at a rotatory speed of 1060 cycles/min using two 12.7 mm stainless steel ball in each jar of 75 ml capacity for 60 minutes.

### Characterization of nano dicalcium phosphate particles

Synthesized nano dicalcium Phosphate particles were characterized by different techniques for confirmation of its nanostructure and functional properties.

#### Particle size analyser and Zeta potential

Average particle size (nm) and Zeta potential (mV) of nano-DCP particles were determined based on the principle of photon correlation spectroscopy using Particle Size Analyser (HORIBA Scientific Nano partica SZ-100). Samples were diluted with MilliQ water and after 2 minutes of sonication, measurements were taken at a scattering angle of  $90^\circ$  and at a temperature of  $25^\circ\text{C}$ . Samples were taken in disposable plastic cuvette for particle size analysis and electrode cell for zeta potential.

#### Transmission Electron microscopy (TEM)

The shape and size of dicalcium phosphate nanoparticles were analysed by using Transmission Electron Microscopy (TEM). Samples were prepared by mixing 1% phosphotunistic acid with aqueous dispersion of nano particles on a para film. A copper grid was placed over the surface of the liquids. After drying of the copper grid in an incubator and it was examined under a transmission electron microscope and the image for taken to analyse its size and shape.

#### X-ray diffraction (XRD)

Structural aspects of prepared nano form of dicalcium phosphate were determined by X-Ray Diffraction technique using Rigaku Mini Flex-II Desktop X-Ray Diffractometer as per the protocol explained by Cengiz *et al.* (2008) [10]. The mean crystal size of particles was calculated from Scherrer's equation (Azaroff, 1968) [11].

#### Fourier Transform Infrared Spectroscopy (FTIR)

The surface chemistry of the synthesised nano form of dicalcium phosphate was investigated by Fourier Transform Infra-Red (FTIR) spectroscopy (Agilent Model Cary 630). The FTIR spectra of the samples were obtained in the spectral range of  $4000\text{-}400\text{cm}^{-1}$ .

#### Energy Dispersive Analysis by X-ray (EDAX)

The percentage of calcium and phosphate present in prepared nano form of dicalcium Phosphate was estimated using Energy Dispersive Analysis by X-ray, using scanning electron microscope (EDAX: SUPRA 55-CARL ZEISS, Germany).

### Results and Discussion

Nano dicalcium phosphate 47.5 g/hr was produced from 50 g of dicalcium phosphate coarse particles using ball mill method. The production efficiency of this method was 95 per cent. The results of the present study was confirmed by the findings of Ramesh, (2014) [3] and Singh and Karmakar, (2011) [12] who reported that optimum quantity of nano particles can be produced by physical method using ball mill.

#### Particle size analyser and Zeta potential

The results of particle size analyser and zeta potential revealed that synthesized nanoparticle had average particle size of  $62.6\pm 3.4$  nm (Fig.1) and zeta potential of  $-25.6\pm 2.2$  mV (Fig.2). This range clearly indicated the uniform distribution of particles in the sample. Ramesh (2014) [3] reported that the nano form of dicalcium phopshxae can be produced by ball milling with an average diameter of 46.60 nm and confirmed by characterization under particle size

analyser. Zeta potential value of nano-DCP ( $-25.6\pm 2.2$  mV) obtained in this study was in agreement with Nanocomposix, (2012) [13] which explained that zeta potential values greater than  $+25$  mV or less than  $-25$  mV typically have high degree of stability.

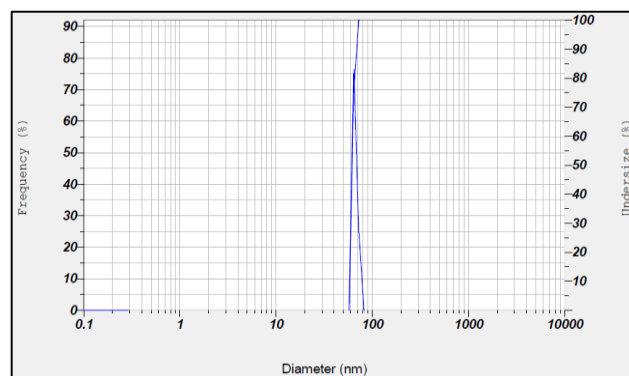


Fig 1: Size distribution pattern of nano-DCP

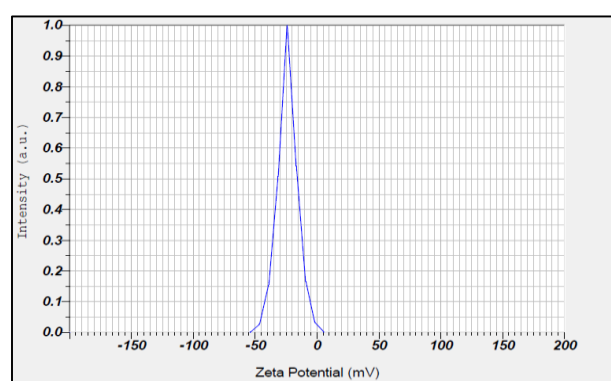


Fig 2: Zeta potential value of nano-DCP

#### Transmission Electron microscopy (TEM)

The size of the nano DCP particles analysed through TEM (Fig.3) revealed that nano-DCP powder contain finer particles of 10-20 nm in size and spherical in shape. Similar results were observed by Welzel *et al.* (2004) [14]; Sokolova *et al.* (2006) [15], Qing *et al.* (2012) [16] and Ramesh, (2014) [3] who reported spherical shape nano calcium phosphate particles with particle size between 20-30 nm under TEM. Whereas, Xiaochen *et al.* (2012) [17] observed rod shaped nano hydroxyapatite by transmission electron microscopy with average particle size of  $23\pm 5$  nm.

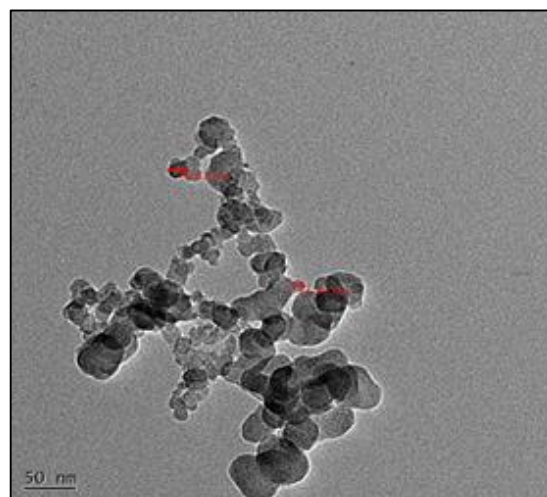


Fig 3: Image of nano DCP under transmission electron microscopy

### X-ray diffraction (XRD)

The XRD pattern (Fig.4) of nano-DCP particles revealed that the synthesized nano particles are crystalline in nature and matches very well with that of the standard dicalcium phosphate inorganic particles. The data recorded in the  $2\theta$  was analysed using Jade 6.0 software and the average crystallite size obtained was 29.31 nm. Similar findings were reported by Singh and Karmakar (2011) [12] and Cengiz *et al.* (2008) [10] who observed crystal size of 21 nm and 15.88 to 16.12 nm respectively.

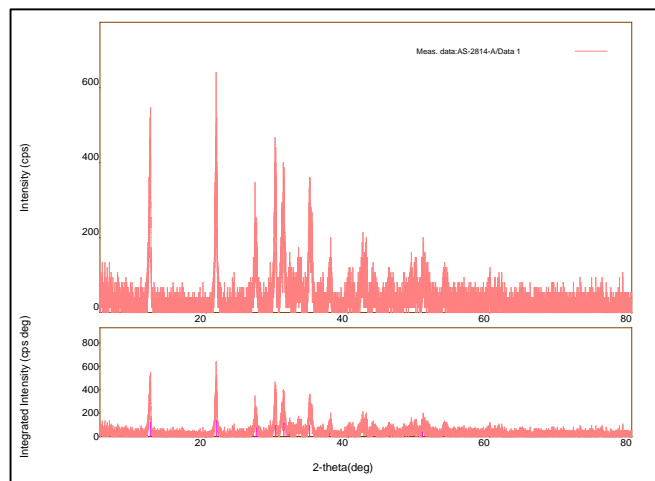


Fig 4: XRD pattern of nano –DCP

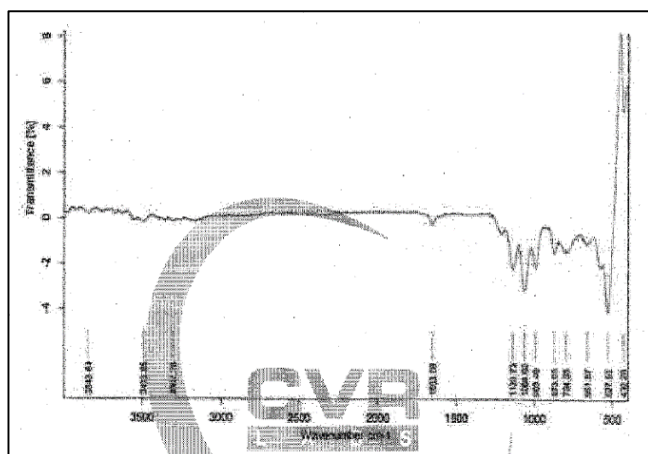


Fig 5: FTIR spectra of nano-DCP

### Fourier Transform Infrared Spectroscopy (FTIR)

The typical FTIR spectra of synthesized nano particles of dicalcium phosphate showed well defined peaks between  $4000-400\text{ cm}^{-1}$  (Fig. 5). The appearance of this peak was due to the presence of hydroxyl groups. The observed FTIR results confirmed that dicalcium phosphate nano particles can be synthesized without any significant impurities. Major peaks noticed in FTIR spectra were 3500, 3440, 1644, 1132, 1067, 999 and 575  $\text{cm}^{-1}$  and these peaks corresponds to stretching and vibrational bending of O-H stretching of residual free water, O-H bending and rotation of residual free water, P-O stretching and O-P-O(H) bending mode. Samy *et al.* (2015) [6] observed similar findings for nano-DCP particles under FT-IR spectroscopy.

### Energy Dispersive Analysis by X-ray (EDAX)

The result of EDAX (Fig.6) showed that nano dicalcium phosphate powder is composed of 23.22% Calcium and 17.67 % phosphorus and Ca/P ratio of 1.31. This finding was in

concurrent to the reports of Ramesh (2014) [3] who observed 23.12 and 16.93 per cent calcium and phosphorus content and Ca/P ratio of 1.31 in nanoparticles prepared by ball milling method. In contrary to this Sagadevan and Dakshnamoorthy (2013) [18] observed Ca/P value of 1.68.

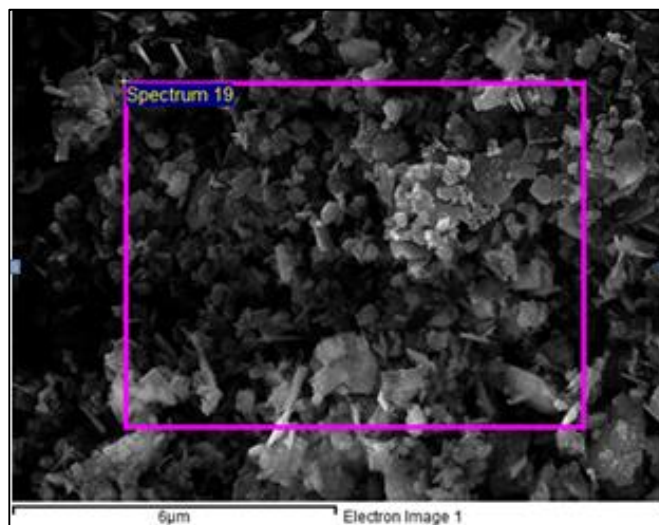


Fig 6: E-DAX spectra of nano-DCP

### Conclusion

The present study indicates that the ball milling method can be successfully and effectively used for the synthesis of 47.5 g/hr feed grade dicalcium phosphate (DCP) nanoparticles from 50 g of coarse DCP and it was found to be quick, easy to perform and economically feasible under field conditions. Based on its bio availability through biological trials it can be recommended in livestock and poultry feeding. It can also be supplemented to fast growing poultry species like broilers, turkey and meat type Japanese quail feed to prevent various leg and bone problems

### Acknowledgement

The authors greatly acknowledged the Tamil Nadu Veterinary and Animal Sciences University, Chennai-51 for providing all infra structural facilities and research grant to carry out this work.

### Conflict of interest statement

The author expresses no conflict of interest with any other individual or organisation regarding the information discussed in the manuscript.

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