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Nano zinc oxide synthesized by physical method as alternative to inorganic and organic form of zinc oxide in poultry feed

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Abstract
A study was conducted to synthesize nano zinc oxide (nZnO) using ball milling technique to incorporate in poultry feed as alternative to other forms of ZnO. The synthesized nZnO was characterized by particle size analysis, zeta potential, X-Ray Diffraction (XRD), Fourier Transform Infra-Red spectroscopy (FTIR) and Transmission Electron Microscope (TEM). The particle size analyser and transmission electron microscope were used to analyse the size, shape and particle size distribution of the nanoparticles. The particle size and zeta potential of synthesised nZnO was 88.3 nm and -26.9, respectively. XRD result showed the peaks corresponded to the zinc oxide and the average particle size analysed by Scherrer's formula was 26.42 nm. The identity of functional group and chemical bonding of nZnO unveiled by FTIR technique. TEM revealed the particle size and shape of nZnO was 24.02 nm and hexagonal, respectively. Hence, it can be concluded that the synthesized product of nZnO can be used in the poultry feed to lower the dose and optimize the production performance of chicken.

Keywords: Ball milling technique, characterization, nano zinc oxide, TEM, XRD, FTIR

1. Introduction

Zinc is the essential mineral involved in the antioxidant defence mechanism, boost immune function and growth performance of poultry. Zinc is involved in the intermediary metabolism, hormone secretion pathways and immune defense systems (Dieck *et al*., 2003) [6]. It can be supplemented in feed as inorganic (Zinc Sulphate, Zinc Oxide, etc.), organic (Zinc methionine, Zinc Proteinate, etc.) and nano forms. The sulphate form is highly water soluble, allowing reactive metal ions to promote free radical formation that can lead to the breakdown of nutrients and ultimately results in degradation of fats and oils, decreasing the nutrient value of the diet. The most supplemental form of Zinc (80 to 90%) is ZnO as oxide form and it is less reactive, but it is less bioavailable for poultry than reagent-grade or feed-grade Zn sulphate (Sandoval *et al.*, 1997) [16].

Excess dietary inorganic Zn required for poultry growth, but high-Zn residues in the excreta negatively impact the environment (Leeson and Caston, 2008) [10]. Organic Zn sources, i.e., Zn methionine and Zn proteinate, has been shown to have a higher bioavailability than inorganic Zn sources and can replace them at lower doses without negative impact on either poultry production or the environment, despite some reports showing inconsistent findings regarding the comparative efficacy of both Zn sources for production and metabolic indices (El-Hack *et al.*, 2017) [7]. The bioavailability of organic zinc is higher than that of inorganic zinc, but the application of organic zinc in animal feed is limited due to its higher cost (Zhao *et al*., 2014) [20].

Nano form of minerals increase the surface area interaction with biological agents and improve bioavailability of the minerals, thereby lowers the effective dose of minerals. This study was conducted to investigate the characteristics of the nano zinc oxide (nZnO) produced by physical method.

2. Material and Methods

2.1 Synthesis of nano zinc oxide

The ball milling technique is the physical method of synthesizing nanoparticles through top-down approach in which planetary ball mill is used to reduce the particle size (Rajendran *et al.*, 2013) [12]. Inorganic ZnO was utilized for the preparation of nZnO. The amount of inorganic ZnO used, duration of grinding, number and diameter of balls used in planetary ball mill were standardized as outlined by Amirkhanlou *et al*. (2012). Nano form of zinc oxide was prepared by grinding 6 grams of inorganic zinc oxide in zirconium jar of 50 ml capacity with fifty zirconium balls of 5 mm diameter for 8 hours at the rate of 250 cycles per minute. Nanoparticle yield (%) was determined by dividing the nanomaterial obtained after grinding by quantity of mineral source used for grinding in the ball mill, multiplied by 100.

2.2 Characterization of nZnO

The synthesized nano zinc was diluted with milliQ water at the concentration of 1mg / ml, sonicated for 1 hour 15 minutes and centrifuged for 15 minutes at 5000 rpm. The particle size of nano zinc was measured at 90˚ scattering angle by utilising disposable plastic cuvettes and zeta potential was measured in an electrode cell using particle size analyser (Horiba scientific nanoparticle SZ-100) based on photon correlation spectroscopy technique.

Structural aspect of prepared nZnO nanoparticles was determined by X-Ray Diffraction technique using Rigaku-Mini Flex-II Desktop X- ray Diffractometer as explained by Theivasanthi and Alagar (2010) [18]. The surface chemistry and functional group was investigated using Fourier Transform Infra-Red (FTIR) spectroscopy according to the method of Chattopadhyay *et al.* (2014) [4]. The transmission electron microscopy was used to determine the size and shape of the synthesized zinc nanoparticles, following the approach of Bisht *et al.* (2005) [3]. Using a micro pipette, one drop of 1% phosphotungstic acid was combined with two drops of aqueous nanoparticles dispersion on a parafilm. A copper grid was laid over the liquids' surface and left for 2 minutes. The extra fluid was absorbed using tissue paper once the copper grid was lifted. The copper grid was air dried at 37 °C in an incubator. The dried copper grid was viewed under transmission electron microscope (HT7800 RuliTEM).

3. Result and Discussion

The ball milling process used in the preparation of the ZnO nanoparticles below 100 nm, as determined by particle size analysis, XRD and TEM. The product yield of nZnO produced by physical method is 96.87%.

3.1 Particle size analysis and Zeta potential

Particle size distribution of synthesized nano ZnO is depicted in Figure 1. The particle size of the nano ZnO is measured by technique dynamic light scattering (DLS).

DLS technique, also known as photon correlation spectroscopy, is a fast and relatively affordable tool to determine the mean size and size distribution of a nanoparticle sample as stated by Lu *et al*. (2017) [11]. The nano-particle size of ZnO under this study was found to be 88.3 nm. Similar to the present findings, Balamurugan *et al.* (2015) [2] produced the nano zinc oxide with average particle size of 13.07 nm by using ball milling technique.

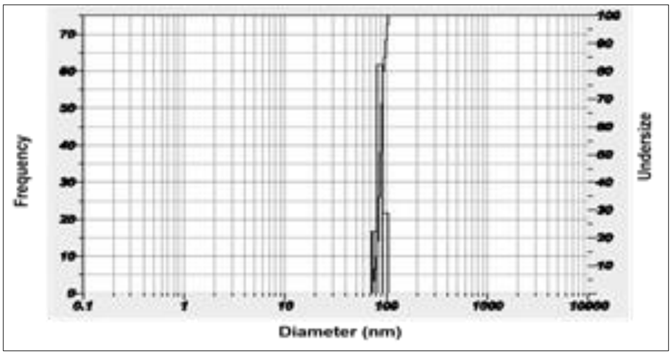

Fig 1: Particle size distribution of synthesized nano ZnO

The Zeta potential value of nano ZnO is depicted in Figure 2. Zeta potential is an essential parameter to study the stability of the nanoparticles. In our study, the zeta potential of synthesized nano zinc oxide was -26.9 mV. Xu (2012) [19] and Clogston and Patri (2011) [5] suggested that the zeta potential of +30 to-30 mV considered to have high degree of stability in anionic and cationic nano-particles. The similar finding was observed by Reda *et al.* (2021) [13] who found that the zeta potential of nano zinc oxide was - 28.7 mV.

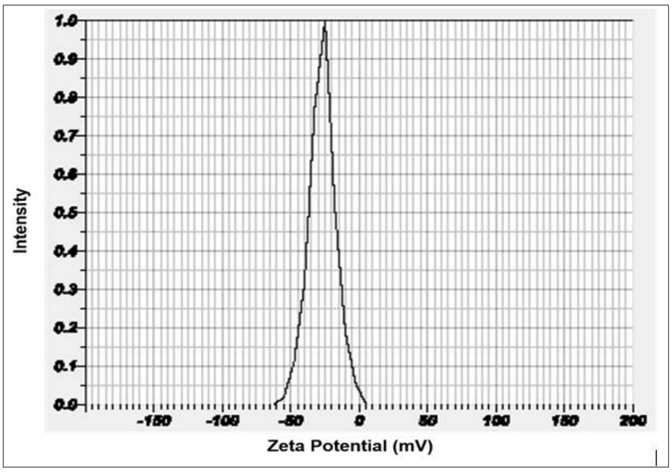

Fig 2: Zeta potential of nano ZnO

XRD analysis

It was performed to check the particle size, purity and crystallinity of the nanoparticles. The typical XRD pattern of synthesized nZnO is depicted in the figure 3. The sharp diffraction peaks indicates that zinc oxide nanoparticles have high crystallinity. No other characteristic peaks were detected which confirmed that the synthesized nZnO was free of impurities. Similar finding was reported by Sadraei (2016) [14], the sharp diffraction peaks indicated the high crystallinity of nanoparticles and all diffraction peaks in the pattern indexed the hexagonal shape.

The average particle size has been estimated by using Debye-Scherrer formula

$$D = \frac{0.9\,\lambda}{\beta \cos\,\theta}$$

Where 'λ' is wave length of X-Ray (0.1541 nm), 'β' is FWHM (Full Width at Half Maximum), 'θ' is the diffraction angle and 'D' is particle diameter size. The particle size obtained from the study was 26.42 nm. The present results of the study are in agreement with Talam *et al*. (2012) [17] who derived the average particle size of nZnO was 16.21 nm using the Debye-Scherrer formula.

In our study, the diffraction peaks produced by nZnO were observed at 31.93°, 34.58°, 36.41°, 47.70°, 56.75°, 63.01°, 66.55° and 69.26° indexed as hexagonal wurtzite phase of ZnO and similar results was also reported by Jayarambabu *et al*. (2014) [9].

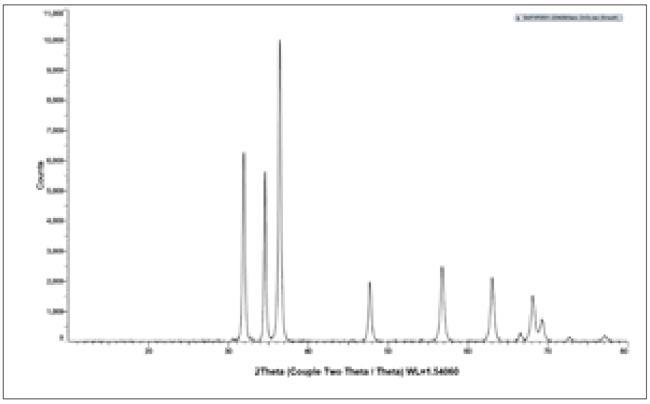

Fig 3: Powder XRD patterns of ZnO

FITR

The FTIR spectrum of synthesized nano particle source of ZnO is depicted in figure 4. The peaks indicate the characteristics functional group present in the synthesized zinc oxide nanoparticles. The FTIR spectrum of synthesized nanoparticles source of zinc revealed well-defined peaks at around 552.30, 868.68, 983.29, 1625.92, 2851.85, 2923.48 and 3437.18cm^{-1}. The absorption peak at 552.30 cm^{-1} corresponds to metal-oxygen (ZnO stretching vibrations) vibration mode. The peaks at 2923.8 cm^{-1} and 3437.18 cm^{-1} are ascribed to the stretching vibration of hydroxyl compounds. The observed FTIR results confirmed that synthesized zinc nanoparticle was without any significant impurities. Jayarambabu *et al*. (2014) [9], who synthesized the ZnO nanoparticle from mungbean seeds, are in support of our present study.

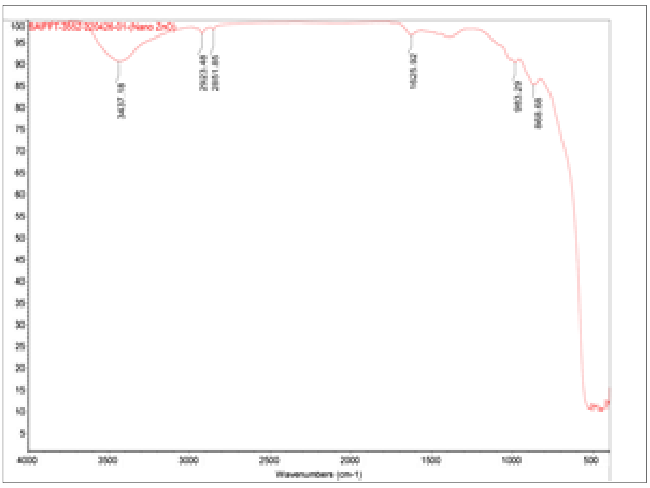

Fig 4: FTIR spectra of synthesized nano ZnO

TEM

TEM image of synthesized nZnO is depicted in the figure 5. The size of nano ZnO particle was analysed by TEM which was in the range of 20-30 nm. Similarly, Salah *et al*. (2011) [15] who investigated the particle size of nano zinc oxide synthesized by high energy ball milling of 10 gm zinc oxide with 5 balls for 50 hours contains ultrafine particles of sizes in the range 20-30 nm. Habib *et al*. (2009) [8] synthesized nano zinc oxide using high energy ball milling method and observed the TEM image was in the range of 5 to 10 nm, whereas the diameter of the nanoclusters is of several tens of nanometers.

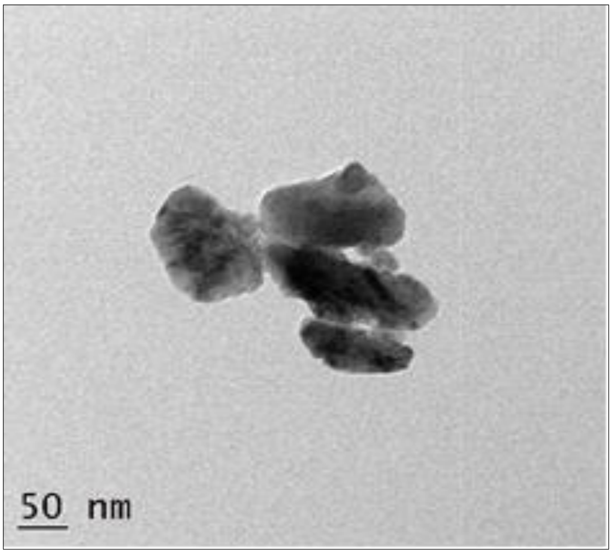

Fig 5: TEM micrographs of synthesized nano ZnO

Conclusion

ZnO nanoparticles have been prepared by using ball milling technique and were characterized by DLS, Zeta potential value, XRD, FTIR and TEM. XRD and TEM results confirmed the nanostructures for the prepared ZnO nanoparticles.

Nano zinc oxide synthesized by ball milling method had all the requisite characteristics of nanoparticles. So, it can be used as alternative to inorganic or organic form of zinc oxide in the poultry feed to increase the bioavailability and lowers the use of inorganic form, which affects the environment.

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References

1. Amirkhanlou S, Ketabchi M. and Parvin N. Nanocrystalline / nanoparticle ZnO synthesized by high energy ball milling process. Materials Letters. 2012;86:122-124.
2. Balamurugan S, Joy J, Anto Godwin M, Selvamani S and Gokul Raja TS. ZnO nanoparticles obtained by ball milling technique: Structural, micro-structure, optical and photo-catalytic properties. DAE Solid State Physics Symposium. 2015;1731:050121-1-050121-3.
3. Bisht S, Bhakta G, Mitra S and Maitra A. pDNA loaded calcium phosphate nanoparticles: highly efficient non-viral vector for gene delivery. International journal of pharmaceutics. 2005;288(1):157-168.
4. Chattopadhyay S, Dash SK, Tripathy S, Das B, Mandal D, Pramanik P and Roy S. Toxicity of cobalt oxide

nanoparticles to normal cells; an *in-vitro* and *in-vivo* study. Chemico-Biological Interactions. 2014;226:58-71.
5. Clogston, JD and Patri AK. Zeta potential measurement. In: Characterization of nanoparticles intended for drug delivery. Humana Press. 2011. p. 63-70.
6. Dieck H, Doring F, Roth HP, Daniel H. Changes in rat hepatic gene expression in response to zinc deficiency as assessed by DNA arrays. The Journal of nutrition 2003;133(4):1004-1010.
7. El-Hack ME, Alagawany M, Arif M, Chaudhry MT, Emam M Patra A. Organic or inorganic zinc in poultry nutrition: a review. World's Poultry Science Journal 2017;73(4):904-915.
8. Habib, S. Synthesis of ZnO nanoparticles using Ball milling method. Material Science Research India. 2009;6(1):79-82.
9. Jayarambabu N, Kumari BS, Rao KV and Prabhu YT. Germination and growth characteristics of mungbean seeds (*Vigna radiata* L.) affected by synthesized zinc oxide nanoparticles. International Journal Current Engineering and Technology. 2014;4(5):3411-3416.
10. Leeson S, Caston L. Using minimal supplements of trace minerals as a method of reducing trace mineral content of poultry manure. Animal Feed Science and Technology 2008;142(3-4):339-347.
11. Lu L, Guo X, Zhao J. A unified nonlocal strain gradient model for nanobeams and the importance of higher order terms. International Journal of Engineering Science. 2017;119:265-277.
12. Rajendran D, Thulasi A, Jash S, Selvaraju S and Rao SBN. Synthesis and application of nano minerals in livestock industry. Animal Nutrition and Reproductive Physiology (Recent Concepts). Satish Serial Publishing House, Delhi, 2013, 517-530.
13. Reda FM, El-Saadony MT, El-Rayes TK, Attia AI, El-Sayed SA, Ahmed SY, *et al*. Use of biological nano zinc as a feed additive in quail nutrition: biosynthesis, antimicrobial activity and its effect on growth, feed utilisation, blood metabolites and intestinal microbiota. Italian Journal of Animal Science 2021;20(1):324-335.
14. Sadraei R. A simple method for preparation of nano-sized ZnO. Research and Reviews: Journal of Chemistry. 2016;5(2):45-49.4
15. Salah N, Habib SS, Khan ZH, Memic A, Azam A, Alarfaj E, *et al*. High-energy ball milling technique for ZnO nanoparticles as antibacterial material. International journal of nanomedicine. 2011;6:863
16. Sandoval M, Henry PR, Ammerman CB, Miles RD and Littell RC. Relative bioavailability of supplemental inorganic zinc sources for chicks. Journal of Animal Science. 1997;75(12):3195-3205.
17. Talam S, Karumuri SR, Gunnam N. Synthesis, characterization, and spectroscopic properties of ZnO nanoparticles. International Scholarly Research Notices, 2012.
18. Theivasanthi T, Alagar M. X-Ray Diffraction Studies of Copper Nano powder. Archives of Physics Research 2010;1(2):112-117.
19. Xu M, Li J, Iwai H, Mei Q, Fujita D, Su H, *et al*. Formation of nano-bio-complex as nanomaterials dispersed in a biological solution for understanding nano biological interactions. Scientific reports. 2012;2(1):1-6.
20. Zhao CY, Tan SX, Xiao XY, Qiu XS, Pan JQ, Tang ZX. Effects of dietary zinc oxide nanoparticles on growth performance and antioxidative status in broilers. Biological trace element research. 2014;160:361-367.