Preparation and Characterization of Natural Nano Materials by Mechanical Grinding Method

Fatima J. Musaa
Hanaa J. Kadhim

Department of Polymer and Petrochemical Industries, College of Materials Engineering, University of Babylon, Babylon, IRAQ

Natural nanomaterial have gained great interest in their use in several fields, especially in the medical field. In this study, red eggshell powder of chickens was used. Mechanical grinding method was used with a steel mill for about 50 hours. Several tests were used to determine the structural and morphological properties of the material after dispersing it with an ultrasonic, including SEM and AFM test to determine the diameter of the nanoparticles and the topography of the particle surface, zeta function analysis to determine the stability of the material, FTIR to know the type of bonds and their groups, and XRD analysis to clarify the phases of the particles. these results show the size of nanoparticles approximately 900 nm, and by increasing the number of grinding hours and filtering the material with a 100 nm filter syringe, a nanomaterial was obtained with a size of 100 nm and less than.

Keywords: Size reduction; Zeta potential; Mechanical grinding; Ultrasonic dispersion
Received: 27 January 2025; **Revised:** 13 April 2025; **Accepted:** 20 April 2025

1. Introduction

Nanomaterials have gained a lot of attention recently because of their Nano size, enhanced biocompatibility, better disease targeting, and—most importantly—effective therapies for a wide range of ailments. Although nanomaterials are employed in almost every field, the biological sciences are where they show the greatest promise. The nanoparticles are produced utilizing a range of methods and can originate from both natural and artificial sources. These nanoparticles' remarkable capacity to be conjugated, linked, and encapsulated with other materials, chemicals, medications, and molecules makes them very attractive for a range of biological applications [1]. Natural source nanomaterials are gaining a lot of interest in both the scientific and industrial domains due to the growing environmental and sustainability concerns. Many different types of nanomaterials made from natural products are promising materials for coatings, packaging, medical, construction, electronics, filtration, transportation, and other fields because they have remarkable strength characteristics, are lightweight, transparent, and have excellent biocompatibility [2]. One of the materials used in this paper is chicken eggshells that have been mechanically treated to obtain Nano-sized particles. Eggshell is an osteoconductive, biocompatible, and bioresorbable biomaterial [3,4]. About 98.2% calcium carbonate, 0.9% phosphorus, and 0.9% magnesium make up an eggshell [5]. The eggshell-based supplement Membrell'sÒ BONEhealth Plus D3 & K2, which is recommended to support bone mineral density, was assessed as a natural bone transplant material in 2019 by researchers Rania, Dalia, and Mohamed. The results showed significantly higher amounts of osteoid, newly-formed bone, in the craniofacial deformities filled with the nano-sized eggshell powder "Membrell's Ò BONE health TM Plus D3 & K2." [6]. In 2018, E.O. Ajala and others determined the chemical composition of chicken eggshell to find practical uses for it. Subsequent investigation showed that the eggshell contains the proper amount of carbon and oxygen, making it a suitable substitute adsorbent for the removal of dyes and heavy metals from aqueous solutions. The study's conclusions suggest that chicken eggshells may be a natural source of nutrients rather than becoming an environmental annoyance [7]. In vitro chondrocyte differentiation and cartilage development can be induced by CESP. In postmenopausal women and women with senile osteoporosis, CESP lessens discomfort and stereopticon. When used as an oral supplement for a full year, it also improves the patients' bone mineral density, making it appropriate for the prevention and treatment of osteoporosis [8,9] due to of its mineral composition, which is 95% CaCO3, which is similar to coral, avian eggshell has been used in maxillofacial reconstructive surgery for a while now [10]. In 2015, Yuki Ohshima and colleagues discovered that heated eggshell powder (HESP) had antibacterial action against bacterial spores, fungi, and vegetative cells. Additionally, the Bacillus subtilis spores were killed by HESP [11]. K. Naemchan and associates investigated the impact of temperature on the shell of chicken eggs. The findings demonstrate that calcium oxide (CaO) derived from chicken egg shells can be applied commercially as a moisture absorber [12]. This paper aims to prepare and characterize a natural nanomaterial from eggshells for use in the manufacture of a composite material used in medical applications.

2. Experimental procedures

The basic material used in this work is red chicken eggshells. Distilled water is the dispersant used in all samples prepared for laboratory tests. Different equipment such as electronic balance (type: ABS120-4, No: AWB1000176), Ball Miller (No:030580), 8411 electric sieve shaker (type: 8411), electric blast dry box (model:wg43, Tianjin Taisite Instrument Co), and ultrasonic cell crusher (sjia-1200w, MTI co) were utilized for various measurement.

The red eggshells used daily in homes were recycled in this work after washing and cleaning them well from all impurities and drying them in the open air. The initial grinding process was in the kitchen grinder for 2 minutes, after which the particle size was measured in the laboratory using a laser particle size analyzer, and was 35 micrometers. In the next stage, a standard electric grinder was used to grind ceramic materials for different periods, taking into account the continuous drying of the material in the drying ovens. After 33 hours of grinding, the particle size was examined, and it was approximately 300 nanometers. To obtain a smaller particle size, another mill with a smaller ball size and higher grinding speed was used for approximately 20 hours, with the material being dried before grinding. Several solutions of different weights of the ground material were prepared with distilled water for filtering them with a 100-nanometer filter syringe to separate 100-nanometer particles from the rest of the larger sizes. Then, a centrifuge was used to separate the distilled water from the nanomaterials settled at the bottom of the ampoules.

3. Results and Discussion

Figure (1) shows the XRD pattern of chicken eggshell. A sharp diffraction peak at 2Θ confirmed the crystalline form of eggshell = 23.056°, 29.401°, 39.412°, 47.505°, and 48.507° and the intensities 230, 2200, 470, 460 and 464 (a.u), respectively, indicate the presence of the peaks, an indication of the presence of the crystalline region. The eggshell is highly crystalline, which is in agreement with [13].

A scanning electron microscope image of eggshell powder at a different magnification scale is displayed in Fig. (2a) as standard electric grinder after 15 hours of grinding, and in Fig. (2b) as sieving with a 100 nm filter after 50 hours of grinding in a steel grinder. The microscopic structure of the particles was discovered by using SEM on eggshells that had been ground for more than 50 hours, filtered to remove large particles, and then examined only for nanoparticles. In Fig. (2a), it is noted that the particle size is not equal, with the shape differing from one particle to another. In Fig. (2b), the particles were nearly uniform in size and shape from 100 nm and below, which is in agreement with [7].

Figure (3), the EDX analysis of eggshell, shows that red eggshells consist of several elements in different proportions, such as calcium 54.52%, oxygen 36.71% the highest proportion, carbon 3.67%, antimony 2.65%, manganese 1.48% medium proportions, magnesium 0.73%, phosphorus 0.17%, and sulfur 0.05% small proportions. This is in agreement with Ajala [7].

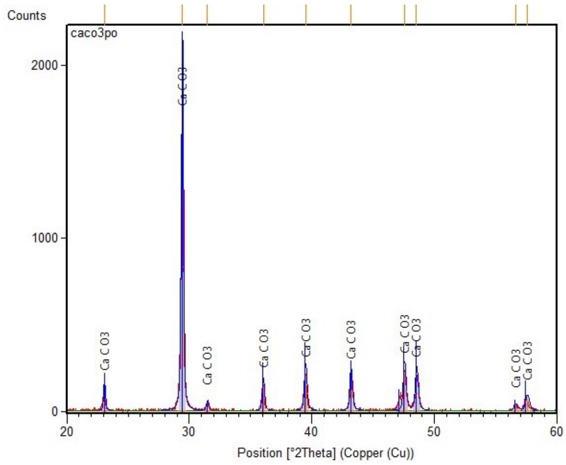
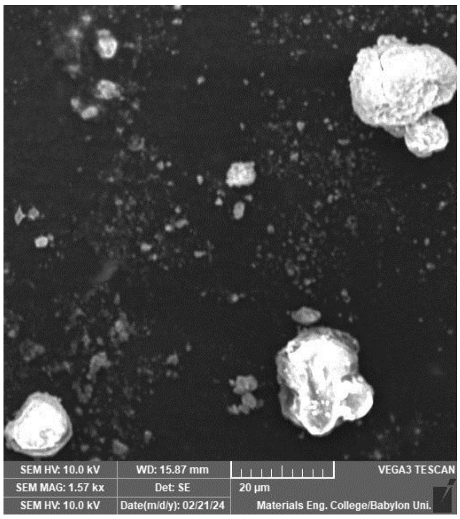
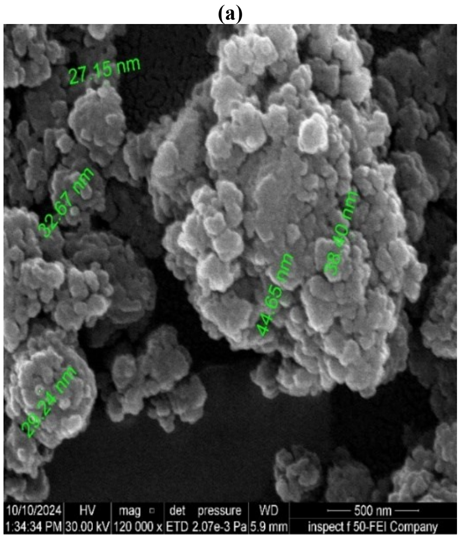


Fig. (1) XRD pattern for red eggshell powder of chicken

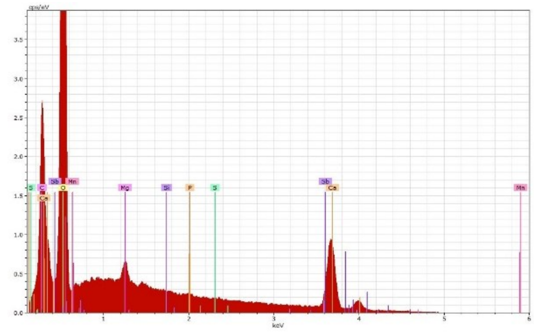


(a)



(b)

Fig. (2) SEM images for red eggshell powder of chicken (a) after 15 hours (b) after more than 50 hours of grinding



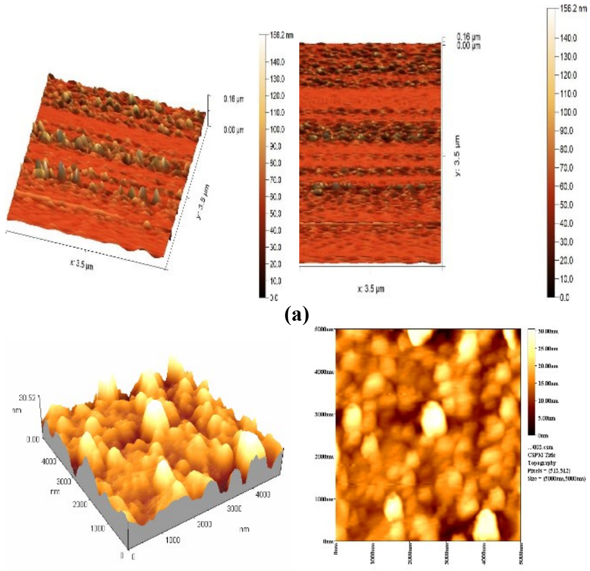
El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
Ca	20	L-series	54.52	54.52	33.62	7.58
O	8	K-series	36.71	36.71	56.70	4.20
C	6	K-series	3.67	3.67	7.55	0.52
Sb	51	M-series	2.65	2.65	0.54	0.63
Mn	25	L-series	1.48	1.48	0.67	0.33
Mg	12	K-series	0.73	0.73	0.75	0.07
P	15	K-series	0.17	0.17	0.14	0.04
S	16	K-series	0.05	0.05	0.04	0.03
Si	14	K-series	0.00	0.00	0.00	0.00
		Total:	100.00	100.00	100.00	

Fig. (3) EDX spectrum and elemental analysis for red eggshell powder of chicken

In addition, table (1) shows the atomic force microscopy analysis of ESP (Fig. 4a), which shows the analysis of the material after 15 hours of grinding, where the surface roughness of the micro-sized particles is large and clear with irregularity in shape and size. Figure (4b) shows the analysis of the material after more than 50 hours of grinding, where the surface roughness is less than b and the sizes of the nano-sized particles are more similar in shape and size due to the effect of increasing grinding hours on the material, which is in agreement with [14].

Table (1) Difference in parameters between A and B for ESP material

The property	A	B
Amplitude parameters:		
Roughness average Sa	6,1904 nm	3.27nm
Root mean square Sq	10.0916 nm	4.44nm
Surface skewness Ssk	0.589900	0.658
Surface kurtosis Sku	1.894	4.41
Peak-Peak Sy	296.3 nm	29.9 nm
Ten point height Sz	156.159 nm	29.3 nm
Hybrid parameters :		
Root mean square slope Sdq	1.336	0.0481 (1\nm)
Surface area ratio Sdr	41.19	0.114
Functional parameters:		
surface bearing index Sbi	Upper 0.6021-lower 81.18	0.593
Valley fluid retention Svi	11.41	0.108
Reduced summit height Spk	5.099nm	7.19 nm
Core roughness depth Sk	418.6nm	8.68 nm
Reduced valley depth Svk	60.104 nm	4.55 nm
Spatial parameters :		
density of summits Sds	17.38 nm	31.3 (1\um2)



(b)

Fig. (4) 2D and 3D AFM images for red eggshell powder of chicken (a) after 15 hours of grinding (b) after more than 50 hours of grinding

The chemical composition of eggshell particles was investigated using the FT-IR method. In Fig. (5), the main peaks in this figure are located at 712, 876, 2515, and 3417 cm^{-1}, with alternate peaks at 1427 cm^{-1}. These peaks are significantly linked to the presence of carbonate minerals in the eggshell matrix as shown at about 3757.33 cm^{-1} stretching bond belongs to the presence of the water molecule, a broad peak at about 3417.86 cm^{-1} belong to the stretching bond (O-H), peak at about 2515.18 cm^{-1} refers to the stretching bond of the C-H group, a broad peak at about 1427.32 cm^{-1} belong to the stretching (C-H), strong peak at about 876 cm^{-1}, and peak at about 712 cm^{-1} and refers to the bending bond of the group (N-H) [7].

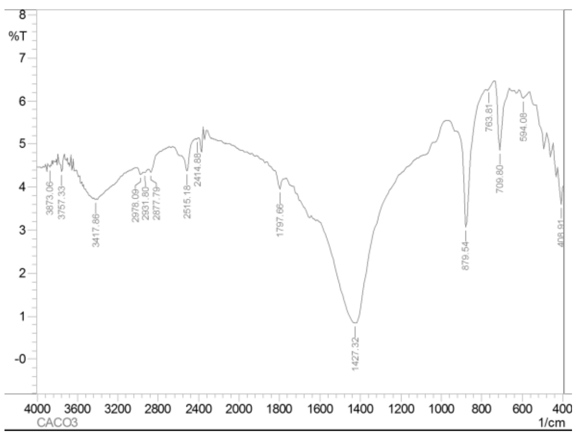
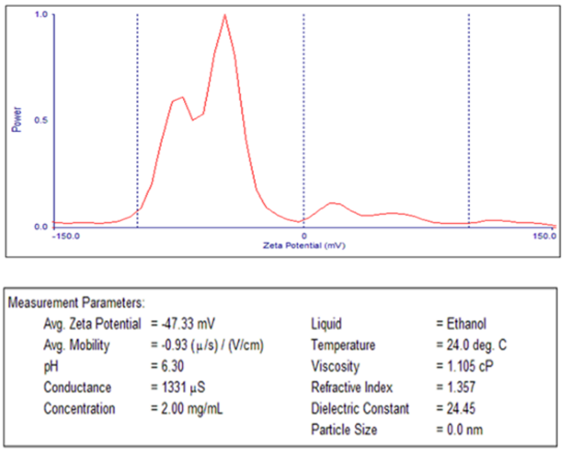


Fig. (5) FTIR spectrum for red eggshell powder of chicken

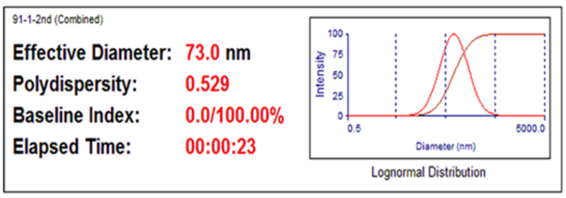
Figure (6a) shows a zeta potential scale, which represents the measure of the electrostatic potential at the electrical double layer surrounding a nanoparticle in solution. An essential and commonly used

Vol. 21, No. 3, July-September 2025, pp. 380-384

measurement for determining the stability of colloidal systems is the zeta potential. All particles in a colloidal system have a charge, either positively or negatively, because of ions' selective adsorption. Particles are characterized as relatively stable if their zeta potential is larger than 30 mV, as incipiently unstable if their zeta potential is between 10 and 15 mV, and as coagulated if their zeta potential is between 0 and 5 mV. Red eggshell is a strongly anionic substance because its zeta potential is -47.33 mV [15]. The mobility scale -0.93 (μ/s)/(V/cm) indicates the presence of free ions that lead to repulsion between charges. This is in agreement with Mohammad [16]. The stability of the nanoparticles is shown by the polydispersity index; if it ranged between 0 and 0.5, the nanoparticles were stable; if it was between 0.6 and 1, the nanoparticles were unstable [17,18]. Figure (6b) shows that the material has good stability according to the polydispersity scale (PDI=0.529).



A. power zeta potential diagram



B. particle size eggshell powder

Fig. (6) Zeta potential for red eggshell powder of chicken (A) power zeta potential diagram (B) particle size eggshell powder

4. Conclusions

In this research paper, red chicken eggshell nanoparticles were prepared by mechanical grinding. The prepared eggshell nanoparticles exhibited a highly crystalline morphology. The eggshell nanoparticles were sized between 27 and 100 nm after grinding for more than 50 hours. High presence of calcium (54.52%) was confirmed in addition to other elements such as O, C, Sb, Mn, Mg, P, and S. The material is highly anionic and that the repulsion between charges is high, as the zeta potential was measured at 47.33 mV and the pdi=0.529, which means that the material is relatively stable. From these results, it became clear that it is possible to prepare nanoparticles from red eggshells using a simple and economical mechanical grinding method for use in manufacturing a composite material for medical purposes.

References

[1] F.A. Khan and F. Khan, **"Applications of nanomaterials in human health"**, Springer (2020).

[2] R.M. Alradha, J. Hanaa and A.A. Al-Zubiedy, "Preparation and characterization of polyvinyl alcohol/starch/bio oil extraction blends coating for food packaging using electrospinning technique", *Egypt. J. Chem.*, 66(2) (2023) 141–149.

[3] E. Durmuş et al., "Evaluation of the biocompatibility and osteoproductive activity of ostrich eggshell powder in experimentally induced calvarial defects in rabbits", *J. Biomed. Mater. Res. Part B Appl. Biomater.,* 86(1) (2008) 82–89.

[4] S.J. Lee and S.H. Oh, "Fabrication of calcium phosphate bioceramics by using eggshell and phosphoric acid", *Mater. Lett.*, 57(29) (2003) 4570-4574.

[5] E.C.Y. Li-Chan, W.D. Powrie and S. Nakai, "The chemistry of eggs and egg products", in **Egg Science and Technology**, CRC Press (2017), pp. 105–175.

[6] R. Salama, M. Khashaba and D. El Rouby, "Histomorphometric evaluation of a nano-sized eggshell-containing supplement as a natural alloplast: An animal study", *Saudi Dent. J.*, 31(3) (2019) 375-381.

[7] E.O. Ajala et al., "Characterization and evaluation of chicken eggshell for use as a bio-resource", *Arid Zo. J. Eng. Technol. Environ.*, 14(1) (2018) 26-40.

[8] A. Schaafsma et al., "Positive effects of a chicken eggshell powder-enriched vitamin–mineral supplement on femoral neck bone mineral density in healthy late post-menopausal Dutch women", *Brazilian J. Nutr.*, 87(3) (2002) 267-275.

[9] J. Rovenský et al., "Eggshell calcium in the prevention and treatment of osteoporosis", *Int. J. Clin. Pharmacol. Res.*, 23(2-3) (2003) 83-92.

[10] L. Dupoirieux, D. Pourquier and F. Souyris, "Powdered eggshell: a pilot study on a new bone substitute for use in maxillofacial surgery", *J. Cranio-maxillofacial Surg.*, 23(3) (1995) 187-194.

[11] Y. Ohshima et al., "Antimicrobial characteristics of heated eggshell powder", *Biocontrol Sci.*, 20(4) (2015) 239-246.

[12] K. Naemchanthara et al., "Temperature effect on chicken egg shell investigated by XRD, TGA and

FTIR", *Adv. Mater. Res.*, 55 (2008) 333-336.
[13] N. Shiferaw et al., "Effect of eggshell powder on the hydration of cement paste", *Materials (Basel)*, 12(15) (2019) 2483.
[14] A. Qasem, D. Hussain and A.H. Ali, "Antibacterial activity with eggshell nano-particles activated by microwave plasma", *J. Optoelectron. Biomed. Mater.,* 13(4) (2021) 177-182.
[15] D.K. Naser, A.K. Abbas and K.A. Aadim, "Zeta potential of Ag, Cu, ZnO, CdO and Sn nanoparticles prepared by pulse laser ablation in liquid environment", *Iraqi J. Sci.*, 61(10) (2020) 2570-2581.
[16] M. Al Mijan, D.-H. Kim and H.-S. Kwak, "Physicochemical properties of nanopowdered eggshell", *Int. J. Food Sci. Technol.*, 49(7) (2014) 1751-1757.
[17] R.M. Darweesh, B.M. Al-dabbag and H.J. Kadhim, "Preparation and characterization of nanocomposite textile filter for separation of the oil/water mixture using PA-PVA blends reinforced with carbon nanoparticles", *Baghdad Sci. J.*, 21(11) (2024) 3570-3582.
[18] R.M. Darwish, H.J. Kadhim and B. Al-dabbagh, "Thermal and morphological properties of electro spun polyamide–poly vinyl alcohol/CNPs nanofibers for filtration process", *Fullerenes, Nanotub. Carbon Nanostruct.*, 31(11) (2023) 1019-1029.

