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RESEARCH

A Study of the Structural, Optical and Antibacterial Properties of Green Synthesized Calcium Oxide Nanoparticles

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ABSTRACT

Green synthesis of nanoparticles has attracted great interest in recent years because it offers a cost effective and environment friendly method for the synthesis of nanoparticles. Calcium oxide (CaO) nanoparticles have potential applications in catalysis, wastewater treatment and biomedicine. In the present study, CaO nanoparticles are synthesized by an eco-friendly green synthesis via thermal decomposition of eggshells which is a common waste material from households and restaurants. The synthesized nanoparticles were subjected to characterization using X-ray diffractogram (XRD), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), Fourier transform infrared (FTIR) spectroscopy, photoluminescence (PL) spectroscopy and UV-vis spectroscopy. The structural studies confirmed the formation of cubic, crystalline and irregularly shaped nanoparticles. FTIR and EDX spectroscopy indicated intensive peaks attributed to the presence of calcium and oxygen atoms in the prepared samples. The PL emission spectra of CaO nanoparticles consisted of a broad peak for an excitation wavelength of 320 nm. The antibacterial activity of green synthesized CaO nanoparticles was investigated using gram-positive Staphylococcus aureus and gram-negative E.coli bacteria. The studies showed that the prepared nanoparticles exhibit antibacterial property.

KEYWORDS

Calcium oxide, green synthesis, thermal decomposition, antibacterial property.

INTRODUCTION

Metal oxide nanoparticles have wide range of applications in various fields. Calcium oxide, magnesium oxide, aluminium oxide, zinc oxide, manganese dioxide, titanium oxides, and iron oxide nanoparticles are the widely used nano-metal oxides[**1,2**]. Of these oxides, Calcium oxide (CaO) nanoparticle is non-toxic to human beings and have potential applications in catalysis, wastewater treatment and biomedicine. Several investigators have investigated the antimicrobial properties of CaO nanoparticles. The key advantages of using these inorganic nanoparticles as antimicrobial agents are their stability and greater effectiveness on resistant strains of microbial pathogens owing to their small size and large surface area. Investigations on the antibacterial properties of CaO nanoparticles have proved that it is a promising material as a bactericidal agent [**3,4**].

CaO nanoparticles have been synthesized by several methods like direct thermal decomposition [**3,5**], chemical co-precipitation [**6,7**] sol-gel synthesis [**8**], solution combustion [**9**] microwave synthesis [**10**], and green synthesis method [**11,12**]. Recently considerable interest has been shown by researchers in the green synthesis of nanoparticles which involves the synthesis of nanoparticles using bioactive agents such as plant materials, microorganisms, and various biowastes including vegetable waste, fruit peel waste, eggshell, agricultural waste etc. [**13**]. The use of natural bioactive agents greatly reduces the risk of environmental pollution and offers a cheap, low temperature method for the synthesis of nanoparticles. Egg shell is a common waste material from households and restaurants and its major component is calcium carbonate. Eggshells can be used as a major source of $CaCO_3$ for the synthesis of CaO nanoparticles and hence by proper management of waste eggshells, environment related

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problems can be minimised. Pasupathy et al. synthesized pure and biomodified CaO nanoparticles using the thermal decomposition of waste eggshells and analyzed the anti-bacterial and antimicrobial activity of the nanoparticles which established that CaO nanoparticles synthesized using waste chicken eggshells can be used as an antibiotic [**4**]. CaO nanoparticles which can be applied for future studies in the removal of heavy metals from industrial wastewaters have been synthesized by Habte et al. from eggshell through the sol-gel method [**8**]. Green synthesis of CaO nanoparticles using the leaf extract of Rhododendron arboretum, Papaya and Green Tea have been reported which resulted in particles showing sensitivity to both gram-negative and gram-positive bacteria [**11,12**].

In the present study, CaO nanoparticles are synthesized via an eco- friendly green synthesis method by the thermal decomposition of waste eggshells. Here powdered eggshell was thermally decomposed at two different temperatures, 400ºC and 600ºC for different durations of time. The synthesized nanoparticles have been characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FTIR), photoluminescence(PL) and UV-visible spectroscopy. Antibacterial activity of CaO nanoparticles was investigated by agar plate well diffusion method using gram-negative *E.coli* and gram- positive *Staphylococcus aureus* bacteria.

EXPERIMENTAL

Material synthesis

In the present work, green synthesis of calcium oxide nanoparticle was carried out by the thermal decomposition of chicken eggshells. Collected eggshells were washed with distilled water and dried in hot air oven at 100ºC for 2hr. After that the eggshell was crushed with blender by physical method. The crushed powder formed was transferred into an alumina crucible. Then it is heated in a muffle furnace under air atmosphere at two different temperatures 400^0C and 600^0 C for different duration of time. After heating the samples for the required time, the alumina crucible is taken out of the muffle furnace and cooled down. The samples are then grinded well using an agate pestle and mortar to obtain the CaO nanoparticles. Eggshells contain $CaCO_3$ which on heating decomposes to CO_2 and CaO. Gaseous CO_2 formed gets evaporated resulting in pure and stable CaO nanoparticles .

Characterization

The XRD measurements were performed using Aeris benchtop X-ray diffractometer by using Cu-Kα lines (λ=1.5406 Å). Samples were analysed with a vertical goniometer with 2θ scan from 10^0-80^0 at a maximum scan speed of 2.17^0/sec. The diffraction peaks obtained are compared with those of the ICDD patterns to identify the crystalline phases. The surface morphology and atomic composition of the nanoparticles were investigated using a JEOL 6390LA/ Oxford XMXN model SEM-EDAX machine having a tungsten filament and accelerating voltage 0.5 to 30 kV. A Thermo Nicolet FTIR spectrometer with resolution 0.2 cm^{-1} and S/N ratio 55,000:1 was employed to record the Fourier transform infrared (FTIR) spectrum of the prepared nanoparticles in the range 4000-500 cm^{-1}. To record the PL emission spectra of the samples a Flouromax4C, Horiba spectrofluorometer having an ozone-free Xenon lamp as excitation source was used. The diffuse reflectance spectra (DRS) were recorded using a UV-Vis-NIR spectrophotometer (Perkin Elmer Lambda 365) having an external diffuse reflectance accessory with a 50 mm diameter integrating sphere and angle of incidence 8^0. Antibacterial activity test was performed against gram positive *Staphylococcus aureus* and gram-negative *E. coli* bacterial strains by agar well diffusion method.

RESULTS AND DISCUSSION

XRD analysis

The XRD pattern of various samples of CaO nanoparticles synthesized at 400^0 C and 600^0 C for different heating time was recorded. XRD spectra of all the samples were similar and in good agreement with ICDD card no. 00-017-0912 for cubic CaO. Characteristic peaks were found to have high intensity and narrow spectral width which confirms the crystalline nature of the synthesized CaO nanoparticles. Average crystallite size (D) of all the prepared samples is calculated by Scherrer's equation, which is given by,

$$D = \frac{0.9\lambda}{\beta \cos\theta} \quad (1)$$

where λ is the wavelength of the X-ray radiation (1.54060Å), β is the full width at half maximum of the diffraction peak (in radians) [**14**]. Here the value of 2θ corresponding to the diffraction peak having the maximum intensity in the XRD pattern was used in the calculation of D. The calculated D values for various samples synthesized at 400^0C and 600^0 C for different time duration are shown in **Table 1**. From the table it is found that as the synthesis temperature increases there is a slight increase in the crystallite size. However, for a particular synthesis temperature, the variation of crystallite size with heating time followed an irregular pattern.

Table 1. The average crystallite size of CaO nanoparticles synthesized at 400^0C and 600^0C.

CaO nanoparticles synthesized at	2θ (Degrees)	Crystallite size (nm)
400ºC for 2hr	29.42	40
400ºC for 3hr	29.60	44
400ºC for 4hr	29.51	43
600ºC for 3hr	29.50	46
600ºC for 6hr	29.48	44

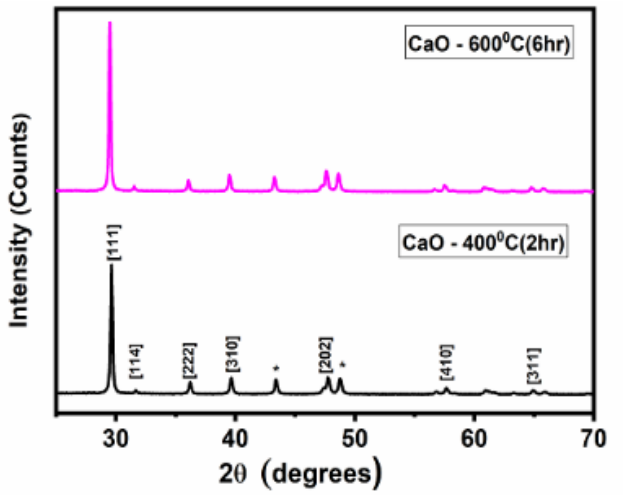

Fig. 1. XRD pattern of CaO nanoparticles synthesized at 400ºC for 2hr and at 600ºC for 6hr.

From the XRD analysis, it was observed that among the samples synthesized at 400ºC, the crystallite size was least for the sample heated for 2hr (40nm). For the samples synthesized at 600ºC, the one heated for 6hr was found to have the smallest crystallite size (44nm). Hence these two samples were taken for further studies. **Fig. 1** depicts the XRD pattern of the above-mentioned samples synthesized at 400^{0}C and 600^0 C. The various diffraction peaks were assigned to (111), (114), (222), (310), (202), (410) and (311) planes corresponding to the cubic structure of CaO associated with the standard spectrum (ICDD card no. 00-0017-0912).The starred peaks represent impurity peaks corresponding to unreacted $CaCO_3$ which is present in eggshell. The XRD pattern of the samples are similar to that obtained for CaO nanoparticles by earlier investigators [**6,15,16**].

SEM and EDX analysis

Surface morphology of the prepared CaO nanoparticles was analyzed using SEM. The SEM micrographs of green synthesized CaO nanoparticles prepared at 400ºC for 2hr at three different magnification scales (5 µm, 2 µm and 1 µm) are shown in **Fig. 2(a)-(c)**. The grown particles are observed to be closely packed, irregularly shaped and agglomerating to each other. However, the exact particle size could not be determined accurately due to agglomeration of the particles.

Energy Dispersive X-ray Spectroscopy (EDX) is used to determine the relative amounts of each atom in the sample. The distribution of the atoms in the samples can also be mapped using EDX analysis. From EDX spectra of CaO nanoparticles synthesized at 400^{0}C for 2 hr (**Fig. 2(d)**) it is found that the prepared samples contain only Ca and O with wt% 43.38 and 56.62 respectively. The peak around 2.2eV is due to gold used for sample preparation. Hence, from EDX analysis it is evident that no impurity atoms are found in the synthesized samples.

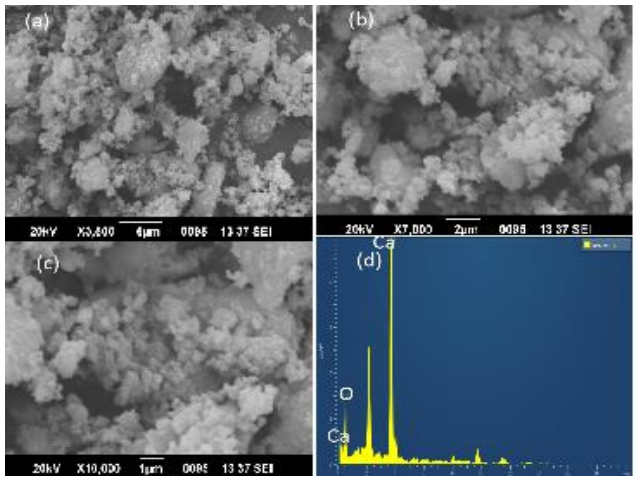

Fig. 2. (a)-(c) SEM images for different resolutions and (d) EDX spectrum of CaO nanoparticles synthesized at 400ºC for 2hr.

FTIR studies

FTIR analysis is performed to determine the various functional groups present in an organic or inorganic compound. **Fig. 3** illustrates the FTIR spectrum of CaO nanoparticles synthesized at 400ºC for 2hr and at 600ºC for 6hr. It consists of peaks at 718 cm^{-1}, 878 cm^{-1}, 1402 cm^{-1} and 1797 cm^{-1}. The peak at 718 cm^{-1} was due to Ca-O bonding. The peak at 878 cm^{-1} is due to the presence of Ca-O-Ca bond [**10,11**]. The broad band around 1402 cm^{-1} is due to the presence of C-O bond and the weak peak at 1797 cm^{-1} is due the presence of amines and amides present in the eggshell. Thus, the presence of various bonds in the CaO nanoparticles is confirmed by FTIR analysis.

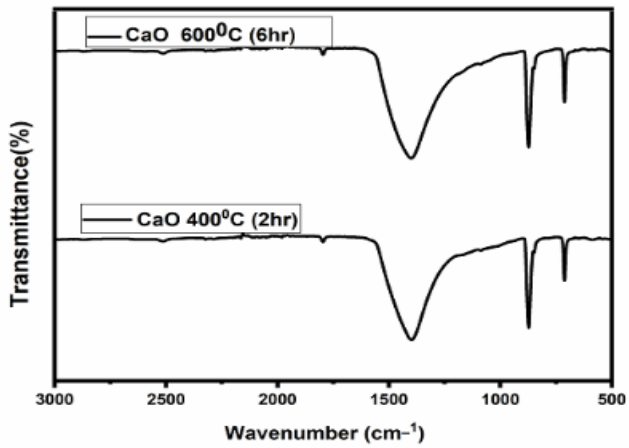

Fig. 3. FTIR spectra of CaO nanoparticles synthesized at 400ºC for 2hr and at 600ºC for 6hr.

PL studies

PL studies give information about the electronic structure and surface or deep defects in the material, depending on the wavelength of light used for excitation. The optical processes inside semiconductor samples can be studied extensively using PL spectral analysis. If the sample is

excited by light with energy greater than the band gap, then an electron-hole pair is created. These can recombine radiatively emitting a photon of energy hv which results in luminescence. **Fig. 4 (a)** represents the PL emission spectra of CaO nanoparticles synthesised at 400ºC for 2hr and at 600ºC for 6hr for an excitation wavelength of 320 nm.

It is seen that the PL intensity is greater for the sample synthesized at 400ºC for 2 hr and it consists of a broad peak centred at around 470nm. There are plenty of oxygen vacancies and other defects on the surface of CaO nanoparticles and they form defect levels between the conduction band and valence band of CaO. The excited electrons from the conduction band bottom undergoes non-radiative transitions to the various defect levels and the PL emission is attributed to the radiative transitions from these levels to the top of the valence band. Since particle size of samples synthesized at 400ºC for 2 hr is smaller than that synthesized at 600ºC for 6 hr the former exhibits more luminescence. The PL emission of the sample synthesized at 400ºC for 2 hr can be deconvoluted as shown in **Fig. 4 (b)**. The deconvoluted spectrum consists of intense peaks at 423, 487 and 528 nm (curve A, B and C) and weak peaks at 442 and 464 nm (curve D and E) which are due to point defects present in the CaO lattice.

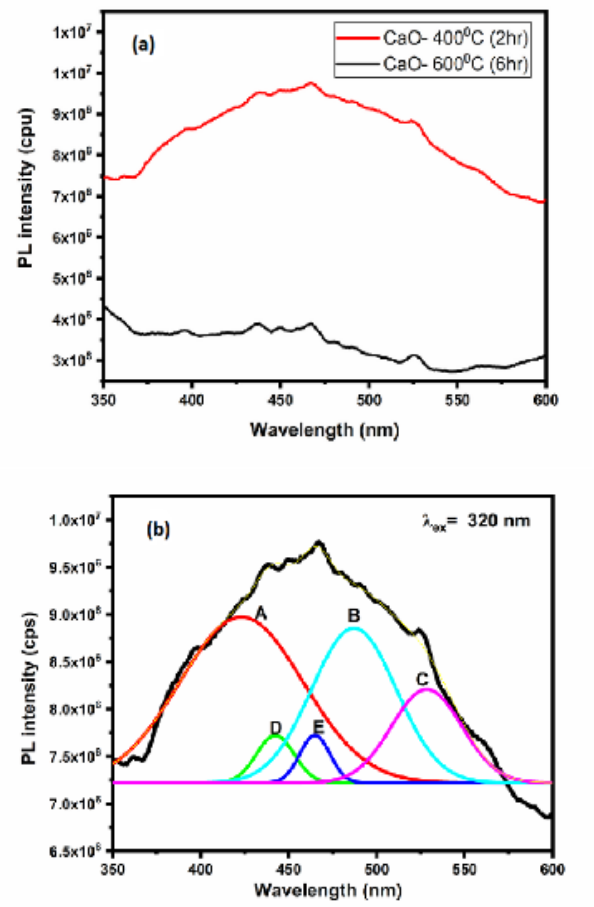

Fig. 4. (a) PL spectrum of CaO nanoparticles and (b) deconvoluted PL emission spectrum of CaO nanoparticles synthesized at 400ºC for 2 hr.

The Commission Internationale de l'Eclairage (CIE) coordinates (x,y) were determined from the PL emission data of the samples. By using CIE colour coordinates the emission colour from the sample can be represented on a two-dimensional plane to give the CIE chromaticity diagram. The CIE chromaticity diagram of CaO nanoparticles synthesized at 400ºC for 2hr and at 600ºC for 6hr is represented in **Fig. 5**. It is found that the CIE coordinates for the sample synthesized at 400^0C (0.29,0.35) lie in the near green region while that of 600^0 C (0.31,0.33) is nearer to the white region of the chromaticity diagram, which is in good agreement with the PL emission spectra. Hence, it can be concluded that the PL intensity and emission colour depends on the synthesis temperature of the nanoparticles.

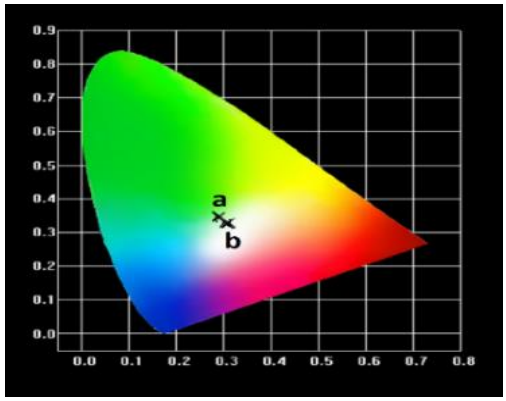

Fig. 5 CIE chromaticity diagram of CaO nanoparticles synthesized at 400ºC for 2hr (Point a) and at 600ºC for 6hr (Point b).

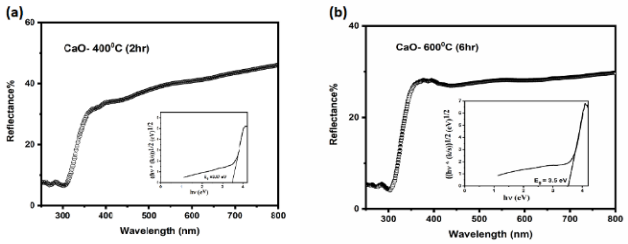

Fig. 6. DRS and inset $[(k/s)\ h\nu]^{1/2}$ vs hν curves of CaO nanoparticles synthesized (a)at 400ºC for 2hr and (b) at 600ºC for 6hr.

DRS studies

The DRS of CaO nanoparticles synthesised at 400ºC for 2hr and at 600ºC for 6hr is shown in **Fig. 6(a)** and **6(b)** respectively. The absorption of CaO nanoparticles lies in the UV region and there is only very low absorption in the visible region. From the DRS the bandgap of the samples can be determined from Kubelka- Munk equation given by,

$$F(R) = \frac{(1-R)^2}{2R} = \frac{k}{s} \qquad (2)$$

where F(R) is the Kubelka-Munk function, R is the diffuse reflectance, k is the absorption coefficient , and s is the scattering coefficient [**17**].

The optical band gap is related to the energy hν by the relation,

$$\alpha h\nu = C\left(h\nu - E_g\right)^n \quad (3)$$

where α is the absorption coefficient which is equal to k/s, 'C' is a constant, 'E_g' is the optical gap of the material and exponent 'n' is the nature of the transition. The estimated values of n = 1/ 2, 2 , 3/ 2, 3 are assigned to direct, indirect, forbidden direct and forbidden indirect transitions, respectively.

The optical band gap E_g of CaO nanoparticles was calculated by plotting k/s vs photon energy (hν) from the Kubelka-Munk function F(R) which is related to the diffuse reflectance R by the above equation. The linear part of this curve is extrapolated to zero on the x- axis (hν) to get the value of optical band gap (inset of fig.6(a) & 6(b)). It is observed that CaO nanoparticles synthesised at 400ºC for 2hr has a bandgap of 3.47 eV while that synthesized at 600ºC for 6hr has a bandgap of 3.5 eV. The bandgap of the prepared samples is found to be lower than that of the reported results for CaO nanoparticles by earlier researchers [6,9,17-20].The decrease in the value of bandgap may be attributed to the band tailing effect, which is caused by structural disorders like crystal defects.

Antibacterial studies

Antibacterial activity of CaO nanoparticles was studied by agar well diffusion method using gram- negative *E.coli* (ATCC 25922) and gram- positive *Staphylococcus aureus* (ATCC 25923) bacteria. Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *E.coli* and *Staphylococcus aureus* (growth of culture adjusted according to McFarland Standard, 0.5%). Wells of about 10mm was bored using a well cutter and different concentrations of sample were added into it. After incubation of the plates at 37°C for 24 hr, the antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was employed as a positive control. CaO nanoparticles synthesized at 400ºC for 2hr (1000 µg concentration) showed a zone of inhibition of 14mm against *E.coli* (**Fig. 7(a)**) whereas no activity was observed against *Staphylococcus aureus* (**Fig.7(b)**). The results revealed that CaO nanoparticles is a promising candidate for the removal of E. coli present in drinking water, which is an important faecal indicator.

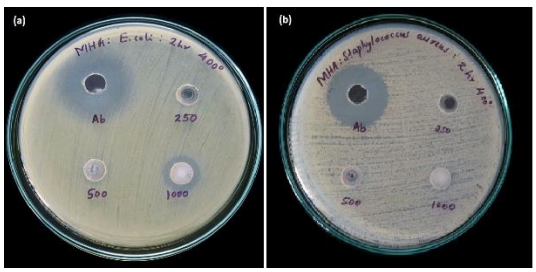

Fig. 7. Antibacterial activity of CaO nanoparticles on (a) E.coli and (b) Staphylococcus aureus.

CONCLUSIONS

In summary, CaO nanoparticles have been synthesized by thermal decomposition of waste eggshells. The XRD pattern confirmed the cubic crystalline nature of CaO nanoparticles. The SEM images of the CaO nanoparticles reveal the formation of irregularly shaped particles that are agglomerated to each other. The FTIR and EDX spectra of the samples confirmed the presence of calcium and oxygen atoms. The synthesized CaO nanoparticles exhibits considerable PL intensity which depends on the synthesis temperature. Antibacterial activity of CaO nanoparticles were studied by agar well diffusion method. The results revealed that CaO nanoparticles is a promising candidate for the removal of E. coli bacteria present in drinking water. Thus, utilisation of waste eggshells for the synthesis of CaO nanoparticles prevents environmental hazards. Moreover, utilizing waste materials as a precursor for the synthesis makes the whole process cheaper, green and sustainable.

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CONFLICTS OF INTEREST

There are no conflicts to declare.

SUPPORTING INFORMATION

Supporting informations are available online at journal website.

REFERENCES

1. Chavali, M.S.; Nikolova, M.P.; *SN Appl. Sci.*, **2019,**1, 607.
2. Roy, A.; Gauri, S.S.; Bhattacharya, M.; Bhattacharya, J.; *J. Biomed. Nanotechnol.*, **2013**, 9, 1.
3. Pasupathy, S.; Rajamanickam,M.; *Int. J. Pharm. Sci. & Res.*, **2019**, 10(10), 4731.
4. Suja, A.; Sarathy, V.P.; *Int. J. Pharm. Sci. Rev. Res*., **2018,** 49(1), 121.
5. Tangboriboon, N.; Kunanuruksapong, R.; Sirivat, A.; *Mater. Sci.Pol*., **2012**, 30(4) 313.
6. Butt A.R.; Ejaz, S.; Baron, J.C.; Ikram, M.; Ali, S.; *Dig. J. Nanomater. Bios.*, **2015**, 10, 799.
8. Habte, L.; Shiferaw, N.; Mulatu, D.; Thenepalli, T.; Chilakala, R.; Ahn, J.W.; *Sustainability*., **2019**, 11(11), 3196.
9. Bhavya, C.; Yogendra, K.; Mahadevan. K.M.; *Indian J. Appl. Res*., **2015**, 5(6), 361.
10. Roy, A.; Bhattacharya, J.; *Int. J. Nanosci*., **2011**,10(3),413.
11. Ramola, B.; Joshi, N.C.; Ramola, M.; Chhabra, J.; Singh, A.; *Orient. J. Chem*., **2019**, 35(3), 1154.
12. Anantharaman, A.; Ramalakshmi, S.; George, M.; *Int. J. Eng. Res. Appl*., **2016**, 6, 27.
13. Kumar, R.P.; Bharthiraja, B.; Nanomaterials: Application in Biofuels and Bioenergy Production Systems; Academic Press, **2021**.
14. Cullity, B.D.; Stock, S.R.; Elements of X-ray Diffraction; Prentice Hall, New Jersey, **2001**.
15. Ikram, M.; Saeed, M.; Haider, J.; Haider, A.; Anwar Ul-Hamid; Shahzadi, A.; Nabgan, W.; Rafque, A.; Dilpazir, S.; Ali, S.; *App. Nanosci.*, **2022**, 12(9), 2657.
16. Butt, A.R.; Butt, I.A.; Nazir, A.; Ikram, M.; Sadiq, S.; Rashid, K.; Shujah, T.; Ali, S.; *The Nucleus*., **2015**, 52, 159.
17. Kubelka, P.; *J. Opt. Soc. Am*.,**1948**, 38, 448.
18. Kumar, S.; Sharma, V.; Kumar, J.; Pradhan, Sharma, S.K.; Singh, P.; Sharma, J.K.; *Nano Biomed. Eng.*, **2021**, 13(2), 172.
19. Tran, F.; Blaha, P.; *J. Phys. Chem. A.*, **2017**, 121,3318.
20. Yang, X.; Wang, Y.; Yan, H.; Chen, Y.; *Comp. Mater. Sci*., **2016**, 121,61.

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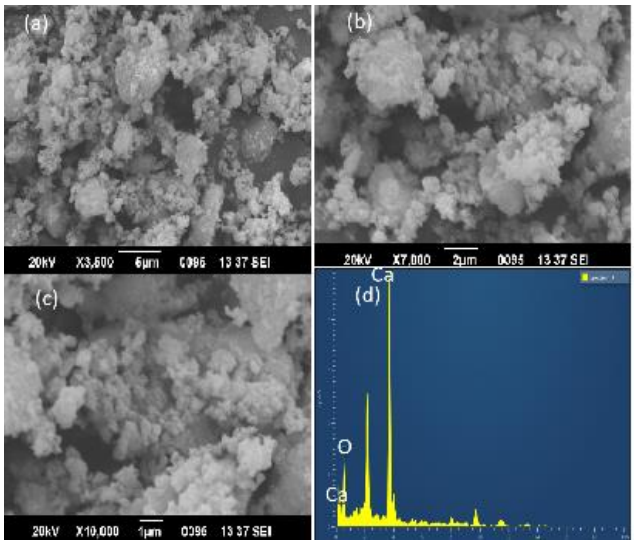
Dr. Rekha S is working as Assistant Professor, in the Department of Physics, Maharaja's College, Ernakulam. Her research interest includes synthesis and characterization of luminescent materials, nanomaterials for optoelectronic and biomedical applications. She has published several papers in various international journals.

GRAPHICAL ABSTRACT

CaO nanoparticles were synthesized via thermal decomposition of waste eggshells and SEM/EDAX analysis of the samples is given below. The SEM images (a)-(c) of the CaO nanoparticles revealed the formation of irregularly shaped particles that are agglomerated to each other. EDX spectra confirmed the presence of major elements Ca and O.



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SUPPORTING INFORMATION

Table 1. Atomic composition of CaO nanoparticles synthesized at 400ºC for 2hr.

Element	Wt %	Atomic %
O	56.62	76.58
Ca	43.38	23.42
Total	100	100

Table 2. CIE coordinates and bandgap of CaO nanoparticles synthesized at 400ºC for 2hr and at 600ºC for 6hr.

CaO nanoparticles synthesized at	CIE coordinates		Emission colour	Band gap (eV)
	x	y		
400ºC for 2hr	0.29	0.35	Near green	3.47
600ºC for 6hr	0.31	0.33	Near white	3.5