



**DIVERSE METHOD TO SYNTHESIZE AND CHARACTERIZE CALCIUM OXIDE  
(CAO) NANOPARTICLES AND THEIR POTENTIAL *IN VIVO* APPLICATIONS –  
REVIEW**

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**ABSTRACT**

Recently CaO NPs have attracted more consideration because of their unique intrinsic surface area, distinctive structure, optical and electronic properties, great basicity, nontoxic catalysts, and antimicrobial properties. This review overviews the studies done on CaO in detail. Here, we have discussed different synthesis methods of CaO NPs, like physical method (ball milling method), chemical method (sol-gel, co-precipitation, microwave irradiation, mechanochemical, impregnation, direct precipitation method), and biological method (plant-mediated synthesis) their characterization and the *in vivo* applications of CaO has been detailed. CaO NPs exhibit great potential application in agriculture, biomedicine, and industries. It also acts as an antimicrobial agent, anti-cancer agent, and potential drug delivery system. Therefore, it is important to study the nature and various applications of CaO NPs. This review focus on different synthesis methods, characterization process, and their potential use in a biomedical system *in vivo*.

**KEYWORDS:** sol-gel, co-precipitation, microwave irradiation, mechanochemical, impregnation.

**1. INTRODUCTION**

Nanotechnology is a branch of science that explores how atoms and molecules behave at nanoscales (1to100 nm). Those nanomaterials, which are smaller, lighter, and stronger, have a large surface area and specific physical and chemical properties. One of the most important uses of nanotechnology in chemistry is the synthesis of metal and metal oxide NPs.<sup>[1]</sup> Calcium mineral is abundantly found in the human body and vital for the creation and regulation of the bones, teeth, and diverse cellular functions. Calcium it also commonly used as a dietary supplement. Nanotechnology is intensely useful in fields, such as medical, agriculture, and manufacturing.<sup>[2]</sup> Calcium is important for cellular physiology, immune response, secretion of a hormone, activation of the enzymes, and blood clotting system.<sup>[3]</sup> Under acidic conditions, the calcium mineral is well absorbed.<sup>[35]</sup> Calcium oxide is a white caustic alkaline solid at room temperature that forms a crystalline solid. BURNT LIME or QUICK LIME are some other names for it.<sup>[4]</sup> The bandgap of bulk CaO is 7.1 eV<sup>[5]</sup>, while the dielectric constant is 11.8. CaO NPs are a non-toxic, inexpensive, and easily accessible raw material.<sup>[6]</sup> CaO is used to neutralize acidic gases<sup>[26]</sup> and also acts as a heterogeneous base catalyst in biodiesel synthesis.<sup>[26]</sup> This is referred to as flue gas desulphurization.<sup>[27]</sup> Due to

its intrinsic surface area and catalytic characteristics, this has played a critical role in the degradation of chemical hazards. CaO NPs have also been used as a pollution control agent and purifying harsh gases due to their CO<sub>2</sub> collecting properties.<sup>[29]</sup> CaO NPs have considerable antibacterial characteristics and unique structural and optical properties; they are safe for all living creatures in the environment. CaO NPs are used as prospective drug delivery agents in photothermal, photodynamic therapy and synaptic administration due to their unique structural and optical features. CaO NPs are employed in various fields, including electronics, environmental cleanup, sensors and catalysis.<sup>[6]</sup> They also have potential biomedical applications. CaO NPs are an effective adsorbent in the removal of Cr(VI) from solutions<sup>[31]</sup>, transesterification of sunflower<sup>[32]</sup> as well as palm oil.<sup>[33]</sup> It's also utilized in manufacturing steel as a dehydrating agent, water softener, possible hydrogen regulator for waste-water, and fertilizers.<sup>[36]</sup> CaO NPs can be synthesized using a) electrochemical reduction, b) vapour deposition, c) radiolysis reduction, d) thermal decomposition, e) chemical reduction of calcium metal salt, f) room temperature synthesis using hydrazine hydrate and starch, g) Sol-gel method<sup>[12]</sup>, h) Co precipitation<sup>[13]</sup>, i) Microwave irradiation method<sup>[19]</sup>, J)

Ball milling method<sup>[18]</sup> etc. Recently, studies have attracted of CaO NPs via green method.<sup>[37]</sup>

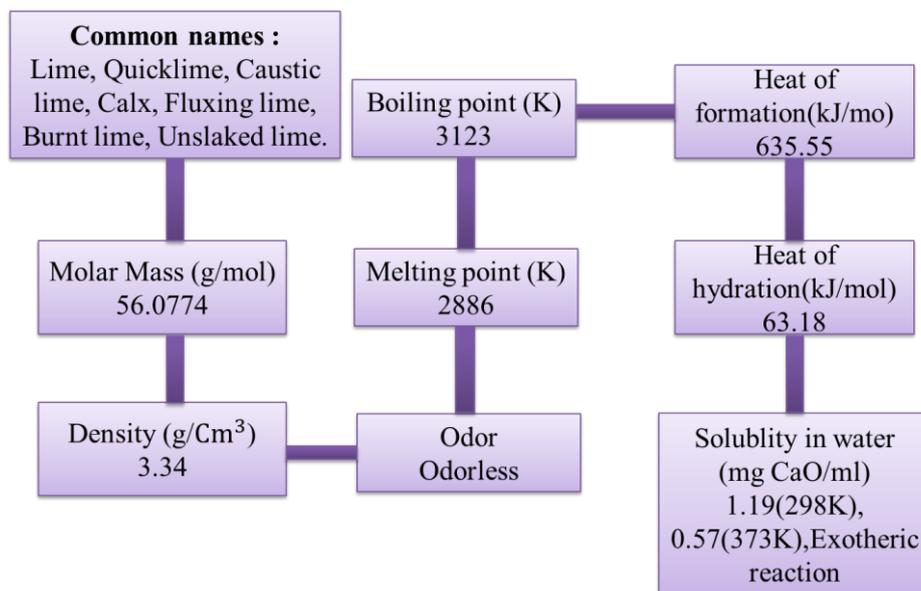


Fig 1: Physicochemical properties of Calcium oxide.

## 2. Ways to NPs synthesis

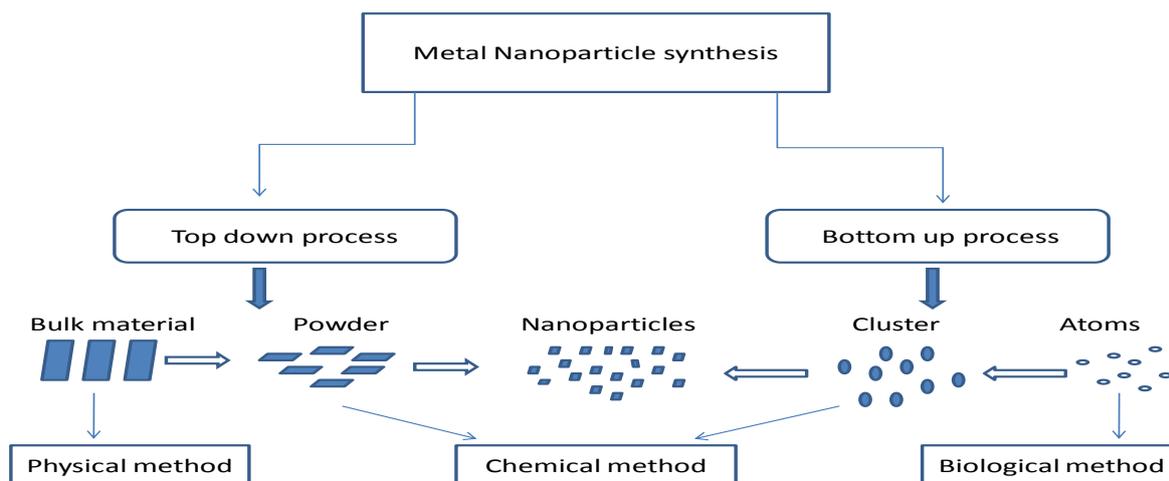


Fig 2: Approches to synthesis of nanoparticles.

## 3. Biogenic Synthesis of CaO NPs

To eliminate the harmful effects of chemicals the conventional chemical synthesis has been replaced with green synthesis method. Some of the studies that support this have been given below in detailed.

### 3.1 Mechanism of CaO NPs synthesis using a plant extract

Flavonoids, tannins, saponins, coumarins, phenols, steroids, amino acids, anthocyanins, and carbohydrates are required for biogenic NP production. Therefore, mechanistically these phytochemicals play an important role in acting as a reducing /oxidizing agent during the

NPs synthesis. For example, the flavonoid quercetin contained in the leaf extract acts as a reducing agent, and its aromatic hydroxyl groups bind to calcium metal ions to produce a stable calcium and quercetin complex.<sup>[63]</sup> This is also conventionally available general mechanism in the formation of green NPs.

### 3.2 Synthesis of CaO NPs plant extracts

In green synthesis method, the precursor for calcium is usually calcium nitrate or calcined egg shell. The precursor is subjected to oxidation/ reduction via green plant extract to form a calcium precipitate, which is further processed to obtained the CaO NPs.

**Table 1: Green method available for synthesis of CaO NPs.**

S.NO	PRECURSOR USED	OXIDIZING/REDUCING AGENT	REFERENCE
1	Ca(NO <sub>3</sub> ) <sub>2</sub>	<i>Papaya and tea</i>	[24]
2	Calcined egg shell	<i>Ocimum sanctum(Tulsi)</i>	[2]
3	Ca(NO <sub>3</sub> ) <sub>2</sub>	<i>Rhododendron arboretum</i>	[30]
4	CaCl <sub>2</sub>	<i>Koenigii</i>	[7]
5	CaCO <sub>3</sub>	<i>Trigona sp. Honey</i>	[8]
6	CaCl <sub>2</sub>	<i>Ocimum tenuiflorum</i>	[9]
7	CaCl <sub>2</sub>	<i>Boccoli</i>	[10]
8	Ca(NO <sub>3</sub> ) <sub>2</sub>	<i>Piper betel</i>	[11]

#### 4. Chemical synthesis of NPs

**Table 2: Chemical method available for synthesis of CaO NPs.**

S.NO	NAME OF THE METHOD	PRECURSOR USED	OXIDIZING /REDUCING AGENT	REFERENCE
1	Sol gel method	CaCl <sub>2</sub>	NaOH	[12]
2	Co precipitation method	CaCl <sub>2</sub>	NaOH	[13]
3	Thermal decomposition method	CaCO <sub>3</sub> (Molluscan sheels)	-	[14]
4	Chemical reduction method	CaCl <sub>2</sub> .2H <sub>2</sub> O	NaOH	[15]
5	Direct precipitation method	CaCl <sub>2</sub>	NaOH	[16]
6	Urea as a fuel solution combustion method	Ca(NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O and NH <sub>2</sub> CONH <sub>2</sub>	-	[17]

#### 5. Physical Method for Synthesis of CaO NPs

##### 5.1. Ball milling methods

The eggshells were separated from the shell membranes and cleaned with water, with the dirty parts brushed away. In addition, in the ball milling reactor, 385 grams of duck eggshells were milled at 100 rpm. Eight milling balls were used, each with an average diameter of 18 mm. Every 2 hours, a sample of milled powder was taken to examine the effect of milling time. To obtain CaO NPs, one gram of duck eggshell powder from samples A, C, and D (each milled for 2, 10, and 20 hours, respectively) was calcined at 700°C for 7 hours.<sup>[18]</sup>

#### 6. Characterizations of CaO NPs

##### 6.1 UV – Visible spectroscopy

UV spectra with wavelengths ranging from 260 to 410 nm. At 265 and 350 nm, broad peaks have obtained, indicating the formation of CaO NPs in these wavelengths.<sup>[6]</sup>

##### 6.2 UV Diffused Reflectance Spectroscopy (UV- DRS)

The Plancks equation was used to compute the band gap energy of CaO NPs. CaO has a band gap energy of 3.44 eV.<sup>[4]</sup>

##### 6.3 X- ray diffraction spectroscopy

XRD patterns of calcinated molluscan shells from “*Lima*”, “*Lottioidea*”, and “*Olivareticularis*” show the

existence of the CaO cubic phase. The strong diffraction peaks at 32°, 37°, 54°, 64°, 67°, and 80° correspond to the miller indices of the reflecting planes for (1 1 1), (2 0 0), (2 2 0), (3 1 1), (2 2 2), and (4 0 0) of CaO and are compatible with JCPDS File No. 37-1497.<sup>[23]</sup> The broadening of the peaks indicates nanometer-sized particles. Using the Debye Scherrer equation, the average crystallite diameters of CaO NPs from “Lima”, “Lottioidea,” and “Olivareticularis” were 49, 42, and 35 nm respectively.

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

Where is the wavelength of CuK radiation (= 1.5418), is the XRD peaks total width at half-maximum in radians, and  $2\theta$  is the diffraction angle.<sup>[14]</sup>

#### 6.4 Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy is recognize the functional group present in molecules. Furthermore, distinct absorption bands can be used to determine the properties of compounds. In the range of 400 to 4000  $\text{cm}^{-1}$ , FTIR spectra have been observed.<sup>[4]</sup>

**Table 3: Studies have shown FTIR peaks and the following wave number corresponding to the functional group.**

Band Assignments	Wave Number ( $\text{cm}^{-1}$ )	Show in	Class of compound
O-H stretching	3643.53	Medium, sharp	Alcohol
C-H stretching	2985.81	Medium	Alkane
O=C=O stretching	2357.01	Strong	Carbon-dioxide
C=O stretching	1803.44	Strong	Anhydride
O-H bending	1433.11	Medium	Alcohol
Ca-O stretching	875.68	Strong	Calcium oxide
Ca-O stretching	707.88	Strong	Calcium oxide

#### 6.5 Scanning electron microscopy (SEM)

The agglomerated form of CaO have been shown in SEM micrograph. The micrographs obtained have shown high resolution hexagonal morphology which corresponds to the synthesized CaO NPs.<sup>[4]</sup>

#### 6.6 Transmission electron microscopy (TEM)

TEM reveals that the NPs are spherical. The particle size ranged between 24 to 38nm, as predicted by the Debye-Scherrer equation. CaO NPs have smooth edges and a spherical shape. The selected area electron diffraction (SAED) pattern confirmed that CaO NPs are crystalline.<sup>[11]</sup>

#### 6.7 Energy dispersive X-ray spectroscopy (EDS)

The elemental composition was investigated using EDS analysis. The results unequivocally confirmed that the biosynthesized samples were primarily composed of Ca and O elements. The percentage elemental compositions of the NPs are as follows: O (47.86%), Ca (50.02%), C (2.12%); O (47.17%), Ca (50.63%), C (2.20%); O (47.01%), Ca (50.19%), C (2.80%) for C1, C2, and C3 respectively.<sup>[10]</sup>

#### 6.8 Atomic force microscopy (AFM)

AFM microscopy is a technique for measuring the height of nanomaterials with extreme precision. For example, CaO NPs have a three-dimensional topography with a constant thickness and an average size of 2.3 nm. Furthermore, the two-dimensional topographic pictures show that the biosynthesized CaO are usually non-uniformly dispersed.<sup>[19]</sup>

#### 6.9 High-resolution transmission electron microscopy (HRTEM)

HRTEM was used to perform morphological characterization, a direct method of visualizing particle size and estimating it precisely. As a result, HRTEM reveals cubic-shaped nanomaterial microstructures similar to those found in x-ray diffraction studies. For example, the average particle size of 18 nm is consistent with a D-value of 16 nm calculated from XRD peak broadening.<sup>[19]</sup>

#### 6.10 Thermogravimetry (TGA) and Differential scanning calorimetry (DSC) analysis

TGA-DSC patterns usually help us to analyze the thermal properties of the sample under investigation. In

many samples, the highly pure CaO cubic phase resulted in an endothermic peak of around 800°C. In addition, the TGA pattern demonstrated a significant weight loss (58%) for all seashell samples in the temperature range of 650–850°C, which could be attributable to CaCO<sub>3</sub> breakdown into CaO.<sup>[14]</sup>

#### 6.11 Photoluminescence studies

CaO NPs PL emission spectra at different calcination temperatures have been attributable to three distinct peaks significant emission peak at around 231 nm is observed during stimulation at 468<sup>o</sup> nm, which could be caused by excitonic recombination. Band edge emission could explain two peaks, one at 375 nm and the other at 400 nm. At 365 nm excitation, defect-related weak emission is detected between 480 and 500<sup>o</sup> nm. Oxygen vacancies could be the source of these defect-related green emissions. As the calcination temperature rises, the intensity of PL emission increases. This contradicts optical absorption spectral analyses (Aseel et al., 2018), which found that higher calcination temperatures resulted in higher absorbance.<sup>[25]</sup>

### 7. Application of CaO NPs *in vivo*

#### 7.1 Antibacterial activity test

The antibacterial activity of CaO NPs against *E.coli* and *V.cholerae* has been investigated in the study it is evident that CaO NPs and a concentration of 5, 10, 15, 20, 25mg/ml could act potentially against the bacteria. This antibacterial activity may be due to the disintegration of the bacterial membrane by the CaO NPs. The large surface area of CaO NPs has been said to be responsible for cell membrane disintegration.<sup>[16][20][21]</sup>

#### 7.2 Minimum Inhibitory concentration (MIC)

The MIC was determined using the broth microdilution method. CaO NPs experimental concentration range from 0.125mM to 32mM. The MIC value was determined by comparing the cell densities in the positive control Vs the test.

#### 7.3 Minimum Bactericidal Concentration (MBC)

MBC was determined via the Dilution plating method, and the colony-forming unit (CFU) was used to calculate the minimum bactericidal concentration (MBC). All values were determined in triplicate and at least four times to ensure accuracy (Arup Roy et al.,).<sup>[19]</sup>

**Table 4: Antimicrobial activity (mM).**

Name of organism	MIC	MBC
<i>P.aeruginosa</i>	4	8
<i>S.epidermidi</i>	2	4
<i>C.tropicalis</i>	8	8

#### 7.4 Time-kill assays

CaO-NPs were tested for their efficiency against the pathogens in time-kill assays. In this assay, all of the tested strains were given different concentrations of CaO-NPs ranging from 0.125 to 8 mM, and their growth was measured every 1 hour. For 4 hours, all of the microorganisms were exposed to CaO-NPs. The antimicrobial effect was dose-dependent and rapid for all of the tested strains. In the case of *Staphylococcus epidermidis*, the microbial reduction was significant and reached nearly 100% within 2 hours, and in the case of *Pseudomonas aeruginosa*, it took 3 hours. *Candida tropicalis* was reduced by 97% after 3 hours of incubation. The lowest most effective concentrations of CaO-NPs for *Staphylococcus epidermidis* were 4 and 8 mM. The most effective doses of CaO-NPs for

*Pseudomonas aeruginosa* were 8 mM at 3 h and 4 mM at 4 h. The most effective antimicrobial CaO-NPs concentration against *Candida tropicalis* was 8 mM after 4 hours (Arup Roy et al.,).<sup>[19]</sup>

### 8. Other application

#### 8.1 Antibacterial activity

The antibacterial activity of CaO-NPs prepared using different plant extracts have been listed in the table below.

Table 5: The antibacterial activity of CaO-NPs prepares using different precursors.

S.No	Name of the plant	Antibacterial activity	Ref
1	<i>Azadirachta indica</i>	<i>Bacillus bacteria</i>	[77]
2	<i>Cissus quadrangularis</i> (stem extract)	<i>Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, B. cereus, Salmonella typhi, Shigella dysenteriae, vibrio cholera, klebsiella pneumoniae.</i>	[76]
3	<i>Cissus quadrangularis, Acalypha indica, Phyllanthus niruri</i> (leaves)	<i>Bacillus subtilis, staphylococcus aureus, E. coli.</i>	[56]
4	<i>Mentha piperata</i>	Antibacterial activity	[78]
5	<i>Oscimum sanctum</i>	Antibacterial activity	[79]
6	<i>Rhododendron arboreum</i>	<i>E. coli, Staphylococcus mutans, Proteus vulgaris.</i>	[30]
7	Papaya and tea	<i>Pseudomonas aeruginosa, E. coli, Staphylococcus aureus.</i>	[24]
8	<i>Ocimum tenuiflorum</i>	<i>Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Bacillus subtilis.</i>	[9]
9	Piper betel leaf	<i>Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus mutans.</i>	[11]

### 8.2 Cytotoxicity of CaONPs

The CaO NPs exposure of MRC 5 cell viability and also VERO cell viability. CaO NPs have a deleterious impact on ROS. Anticancer potential of CaO NPs have been observed in stomach cancer A-549 cell causing apoptosis and thereby assessing the cell cycle.<sup>[38][11]</sup>

### 8.3 Application in agriculture

The production of root hairs, which are important for the uptake of immobile nutrients like phosphorus, was aided by CaO NPs. CaO NPs assisted seedling growth. During seedling growth, CaO caused an increase in shoot and root length. Plants and animals absorb calcium carbonate in calcium hydrogen carbonate ( $\text{HCO}_3$ )<sub>2</sub>, which is dissolved in most cases and used to make skeletons and shells. The use of soluble calcium aids plant growth. Calcium increases seedling fresh biomass and dry weight by enhancing photosynthesis and expanding the growth of plant components. It also improves nitrogen utilization efficiency, resulting in lower production costs and less nitrogen pollution. Seedling development rose as CaO concentrations increased, whereas toxicity levels decreased. The specific leaf area (SLA), specified leaf weight (SLW), and leaf area duration (LAD) of Bengal gram (*Cicerarietinum* L) seedlings were calculated, and CaO NPs demonstrated higher growth with increasing concentration and crop growth periods.<sup>[9]</sup>

All biochemical parameters are total carbohydrates, proteins, photosynthetic pigments, and POD enzyme

activity. As the quantity of CaO NPs increased, all biochemical content increased.<sup>[42]</sup> The biological production of NPs and their impact on seedling growth have been the subject of several research. So far, only a few studies on the biological production of CaO NPs and their potential effects on plants have been published. CaO NPs can be employed as an inorganic fertilizer in Bengal gram fields.<sup>[9]</sup>

Photosynthesis is stimulated by CaO, which increases the size of plant components. Low  $\text{Ca}^{2+}$  levels in plant roots, on the other hand, induced the development of the mitotic apparatus and the cell plate, resulting in blossom end root, unpleasant pits, leaf necrosis, poor fruit storage, and water soaking.<sup>[52]</sup>

### 8.4 Removal of heavy metals

It is essential to remove excess chromium, mercury, and other heavy metals from the water system before it is discharged into the water bodies. Mercury causes substantial long-term injury to various body parts. The methods like ion exchange, osmosis, and electrolytic precipitation already have a drawback because they are expensive for daily usage. This drawback has been replaced effectively by using CaO. CaO has a significant capacity and is an efficient adsorbent for some dangerous chemicals. Using NPs of CaO to remove chromium (II) and mercury (II) ions from aqueous solutions provides speed, simplicity, and high sensitivity.<sup>[43-51]</sup>

### 8.5 Transesterification reaction catalysts

Nano-crystallized CaO is an effective catalyst for the transesterification reaction due to its large surface area, small crystallite sizes, and low defect count. CaO NPs appear to be a significant factor in increasing their catalytic activity. CaO is a heterogeneous catalyst that can prevent unwanted soap formation in transesterification reactions by preventing reactions with free fatty acids. However, as demonstrated by Xin (2009)<sup>[53]</sup> and Mahreni and Sulistyawati (2011)<sup>[54]</sup>, the CaO compound used as a catalyst in the transesterification reaction is still micro-sized. As a result, the researchers plan to use calcium oxide NPs as a transesterification catalyst. Mahreni and Sulistyawati (2011) used a calcium oxide catalyst made from calcined eggshells to produce biodiesel at 200 to 900°C temperatures. The catalyst is then used in the transesterification reaction, with different reaction times.

### 8.6 Food additives

Because of their bioavailability, CaO and calcium acetate are allowed as food additives. In addition, preservative, pH regulator, dough conditioner, and calcium oxide FCC food grade also prevents food components from aggregating and increases bioavailability.<sup>[56]</sup>

### 8.7 Drug delivery

NPs are being employed as a medication delivery system, and this technology has the potential to revolutionize medical needs. CaO NPs are also being studied as a potential medicine delivery vehicle. CaP and CaCO<sub>3</sub> are calcium-based ceramic NPs with a lot of potential as bioactive carriers for drug delivery systems in the human body, at a nanoscale.<sup>[41]</sup>

Photothermal, photodynamic therapy, synaptic delivery<sup>[57]</sup>, and chemotherapeutic agents use CaO NPs as potential drug delivery agents.

### 8.8 Photocatalytic degradation

The photocatalytic activity of the CaO NPs was examined by Onwudiwe *et al.*, (2018), who used broccoli leaves extract to make them. The synthesized CaO-NPs were cubic, spherical, and hexagonal, with diameters ranging from 32 to 45 nm. When exposed to UV light, acting as a photocatalyst and degraded bromocresol green dye up to 57.63 %.<sup>[75]</sup>

### 8.9 Other important applications

CaO NPs have been employed as bactericides and toxic chemical adsorbents. Drugs have progressed from medicinal substances to growth factors, and their quantification has increased. Several nanoparticle-based therapies have improved medication efficacy while reducing toxicity.<sup>[56]</sup> CaO NPs synthesized from papaya leaves are effective photocatalysts for degrading dyes used in the textile industry. Electronics, environmental remediation, and sensor applications use CaO NPs.<sup>[58]</sup> CaO, for example, plays a role in electronics as a desiccant in LED.<sup>[60]</sup> CaO is utilized to neutralize acidic

gases and remove SO<sub>2</sub> from flue gases.<sup>[59]</sup> Flue gas desulfurization is the term for this process. CaO NPs are important in breaking down chemical threats because of their intrinsic surface area and catalytic capabilities. They're known as destructive adsorbents. Because of their CO<sub>2</sub> collecting characteristics, CaO NPs have also been used as a pollution control agent and in purifying hard gases.<sup>[29]</sup> NPs can be used to treat surface water purification, desalination, disinfection, and other impurities such as heavy metals, pathogens, and organic contamination. The NPs can also be utilized to remediate wastewater from municipal and industrial sources and sludge. NPs are a better alternative to chemicals since they are less expensive, have higher competency, and require less treatment time. Nano filtration is a relatively recent membrane filtering method used in the food and dairy industries to purify water.<sup>[28]</sup> CaO purifies substances like citric acid, glucose, and certain dyes before further refinement.<sup>[60]</sup> In steel manufacturing, it is used as an absorbent. Also act as water softener, waste water hydrogen regulator and fertilizer for agriculture purpose.<sup>[61]</sup>

## 9. CONCLUSION

The Synthesis of CaO NPs utilizing diverse methods and their characterization were established in this review. We have also observed that some authors have conducted various studies with these NPs establishments of various other applications in many fields that could be a boon to human and healthcare industries.

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