CHAPTER 2

Material and Experimental

In this research, investigation of a productive method to synthesize high purity nanoparticle CaO_2 using novel precursor as an alternative and effective precursor under mild condition is reported. The effect of some additive e.g. ethanol, ascorbic acid, triton-x was also validated. The synthesized CaO_2 powders were characterized by XRD, SEM, TEM and BET techniques.

2.1 Materials and chemicals

Chemicals included ACS grade

1) Calcium nitrate tetrahydrate; $Ca(NO_3)_2.4H_2O$ (SIGMAALDRICH, 99%)

MW = 236.15 g/mol

2) Hydrogen peroxide; H_2O_2 (Merck, 30%) MW = 34.01 g/mol

3) Ammonium hydroxide; NH_4OH (J.T. BAKER 28.0–30.0%) MW = 35.04 g/mol

4) Calcium hydroxide; $Ca(OH)_2$ (SIGMA-ALDRICH, 97%) MW = 74.09 g/mol

5) Calcium chloride; $CaCl_2$ (SIGMA-ALDRICH, 99%) MW = 110.98 g/mol

6) Calcium carbonate; $CaCO_3$ (SIGMA-ALDRICH, 99%) MW = 100.09 g/mol

7) Sodium hydroxide; NaOH (SIGMA-ALDRICH) MW = 40.00 g/mol

8) Nitric acid; HNO_3 (EMSURE,65%) MW = 63.01 g/mol

9) Ascorbic acid; $C_6H_8O_6$ (SIGMA-ALDRICH) MW = 176.12 g/mol

10) Triton-X; $C_{14}H_{22}O(C_2H_4O)_n$ (n = 9-10) MW = 647.00 g/mol

11) Ethanol; C_2H_6O solution MW = 46.07 g/mol

2.2 Instruments

1) Scanning Electron Microscope (SEM); (JEOL JSM-6335F)

2) Transmission electron microscope (TEM); (JEOL JEM-2010)

3) X-Ray Diffraction (XRD); (Rigaku Mini Flex II X-ray diffractometer)

4) Brunauer–Emmett–Teller(BET);(Micromeritics TriStar II)

5) UV–visible spectrophotometer; (UV–vis 2450, Shimadzu)

2.3 Preparation of CaO_2 powders

2.3.1 $Ca(NO_3)_2.4H_2O$ as a precursor

$Ca(NO_3)_2.4H_2O$ 48 grams were dissolved in 50 mL distilled water. Next, 30 mL of 30% H_2O_2 was added rapidly at room temperature. The solution was controlled pH using NH_4OH or NaOH to a pH of 10. Finally, the precipitated was separated from the solution by filtering through Buchner funnel and washing powders several times with distilled water or NH_4OH. The powders then dried at 80°C, 150°C or 200°C for 2 h or 6 h.

2.3.2 $Ca(NO_3)_2.4H_2O$ as a precursor with ethanol as additive

$Ca(NO_3)_2.4H_2O$ 48 grams were dissolved in 50 mL distilled water, then 30 mL of 30% H_2O_2 and 5 mL of ethanol were added while stirring rapidly at room temperature. The solution was controlled pH using NH_4OH to a pH of 10. Finally, the precipitated was separated from the solution by filtering through Buchner funnel and washing powders several times with distilled water or NH_4OH. The powders then dried at 80°C for 2 h.

2.3.3 $Ca(NO_3)_2.4H_2O$ as a precursor with ascorbic acid as additive

Ca(NO$_3$)$_2$.4H$_2$O 48 grams were dissolved in 50 mL distilled water, then 30 mL of 30% H_2O_2 and 20 mL of ascorbic acid were added while stirring rapidly at room temperature. The solution was controlled pH using NH_4OH to a pH of 10. Finally, the precipitated was separated from the solution by filtering through Buchner funnel and washing powders several times with distilled water or NH_4OH. The powders then dried at 80°C for 2 h.

2.3.4 $Ca(NO_3)_2.4H_2O$ as a precursor with Triton-X as additive

$Ca(NO_3)_2.4H_2O$ 48 grams were dissolved in 50 mL distilled water, then 30 mL of 30% H_2O_2 and 5 mL Triton-X were added while stirring rapidly at room temperature. The solution was controlled pH using NH_4OH to a pH of 10. Finally, the precipitated was separated from the solution by filtering through Buchner funnel and washing powders several times with distilled water or NH_4OH. The powders then dried at 80°C for 2 h.

2.3.5 $Ca(OH)_2$ as precursor

$Ca(OH)_2$ 3 grams were dissolved in 10 mL HNO_3. Then 30% H_2O_2 30 mL was added. The solution was controlled pH using NH_4OH to a pH of 10. Finally, the precipitated was separated from the solution by filtering through Buchner funnel and washing powders several times with distilled water or NH_4OH. The powders then dried at 80 °C for 2 h

2.3.6 $CaCO_3$ as a precursor

$CaCO_3$ 3 grams were dissolved in 10 mL HNO_3, and then 30 mL of 30% H_2O_2 was added while stirring at room temperature. The solution was controlled pH using NH_4OH to a pH of 10. Finally, the precipitated was separated from the solution by filtering through Buchner funnel and washing powders several times with distilled water or NH_4OH. The powders then dried at 80 °C for 2 h

2.3.7 $CaCl_2$ as precursor

$CaCl_2$ 3 grams were dissolved in 50 mL distilled water, and then 30 mL of 30% H_2O_2 was added while stirring at room temperature. The solution was controlled pH using NH_4OH to a pH of 10. Finally, the precipitated was separated from the solution by filtering through Buchner funnel and washing powders several times with distilled water or NH_4OH. The powders then dried at 80 °C for 2 h

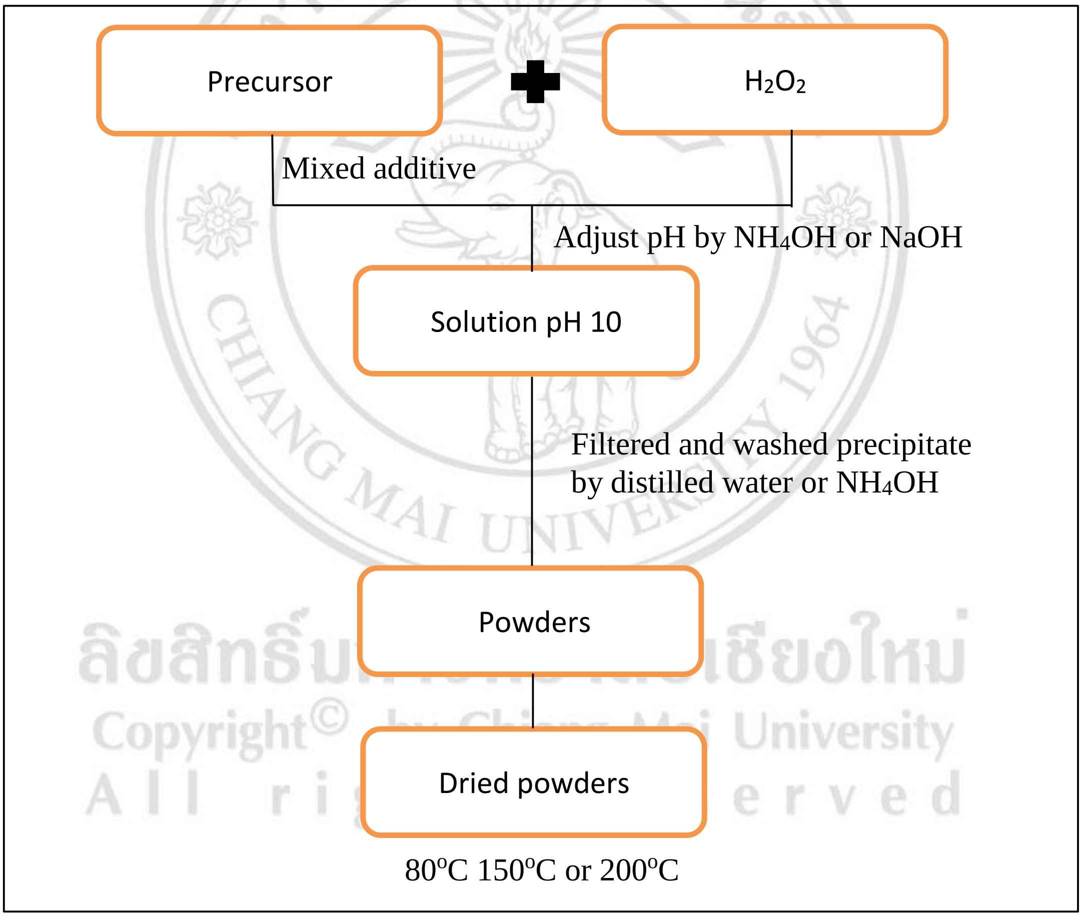

Figure 3. Schematic flow chart of synthesis of calcium peroxide varying precursors.

2.4 Evaluation of photocatalytic activity

2.4.1 Photocatalytic oxidation

Methylene blue 1×10^{-5} M, was added to 0.100 g CaO_2, $Ca(OH)_2$, $CaCO_3$ powders or 1 mL H_2O_2 and continuous stirring with an aquarium air pump. Leave suspension for adsorption equilibrium in the dark for 30 mins. Next, switched on a visible or UV lamps and then collect 1 mL of the solution at interval of 30 mins. Change in methylene blue concentration was monitored using UV–Vis spectrophotometer at 660 nm.

2.4.2 Photocatalytic reduction

Photoreduction activity was carried out by observing color change of a resazurin (Rz) ink. First, pellets of CaO_2, $CaCO_3$, $Ca(OH)_2$ were prepared . Next, drop Rz onto pellets, including H2O2 solution before switch on visible or UV light. Photoactivity will be observed if color change from blue to pink

2.5 Antibacterial assay

S.aureus and *E.coli* were swabbed on an NA plate using a sterilized cotton bud. Next, a small portion of each powder was put on *E.coli* or *S.aureus* bacteria in NA plate. Then, incubated at room temperature for 24 h and find out a clear zone.

2.6 Oxygen releasing

Set up equipment as shown in Fig 4. Prepare 3 tubes of 0.010 grams of CaO_2 powders. Adjust pH of distilled water to 4, 5 or 6 by NH_4OH and HNO_3 solution. Transfer 50ml of each pH solution into the tubes. Close each tube with a lid that has a tube connector to release produced gas. Then, fill water to burette and upside down cover the tube connection. Record volume of released oxygen in burette.

Figure 4. Apparatus in the experiment of oxygen releasing.

2.7 Characterization

2.7.1 X-Ray Diffraction (XRD)

X-Ray Diffraction (XRD), which study of crystal structures and atomic spacing. It's use Bragg's Law ($n\lambda = 2d\sin\theta$) for analysis. This technique is used for the identification of unknown crystalline materials widely.

X-ray powder diffraction is used for the identification of unknown crystalline materials widely. Used studies in many part for example geology, environmental science, material science, engineering and biology etc.

2.7.2 The Brunauer–Emmett–Teller (BET)

The BET method is the most widely used procedure for the determination of the surface area of solid materials and involves the use of the BET equation.

$$v = \frac{v_m c p}{(p_0 - p)\{1 + (c-1)\left(\frac{p}{p_0}\right)\}}$$

The total pore volume and pore size were estimated by Barrett–Joyner–Halenda (BJH) method

2.7.3 Scanning Electron Microscopy (SEM)

A scanning electron microscope (SEM) is a one type of create images of a sample. Show image in 2D. You can found surface of sample, porous or physical property of sample. Preparing sample by disperse sample in ethanol solution. Next sonicated 15 mins and drop solution on SEM stubs. Finally, stubs were coated with gold particle by plasma sputtering under argon atmosphere to it has more conductivity.

2.7.4 Transmission electron microscopy (TEM)

Transmission Electron Microscopy (TEM) is an essential characterization tool for measures of particle nanomaterials directly. TEM image provides detail about size, size distribution, and morphology. Sample preparation can be done by disperse sample in ethanol solution and dropping solution on a copper grid that is coated with a thin layer of carbon has a relatively low electron density and drying for imaging.

2.7.5 UV-vis spectrophotometry

It is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Absorption measurements can be at a single wavelength or over an extended spectral range. UV-vis spectrophotometry used for analysis absorbance of sample. It can analytic both solid and liquid phase. The absorption or reflectance in the visible range (λ=400-800nm) directly affects the perceived color of the solution by Beer’s law.

$$\mathbf{A = \varepsilon bc}$$

ε is the molar absorptivity ($Lmol^{-1}cm^{-1}$)
b is the path length of the sample. Which, the path length of the cuvette measurement in 1cm.
c is the concentration of the compound in solution (M)