

Chemogenic Synthesis of Chromium Oxide Nanoparticles using Sol-Gel Method and its Potential on Pancreatic Lipase Inhibition

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Abstract

Obesity is a chronic disease which has been linked with several metabolic disorders. Till now, there is no ideal pharmacological treatment has been developed for obesity treatment. A nanoparticle or ultrafine particle has attracted the attention of researchers due to its unique physiochemical characteristics as a subject tool for physics, chemistry, and biology studies. One of the promising trace elements, chromium, has played a crucial role in the regulation of blood sugar levels and carbohydrates and fats degradation metabolism. Within our project, chromium has been synthesized into chromium oxide nanoparticles (Cr_2O_3 NPs) using sol-gel method, which has a higher surface area and an oxidizing potential. Chemogenic synthesized Cr_2O_3 NPs were characterized using UV-Vis Spectrophotometer (UV-Vis), Fourier Transform Infrared Spectroscopy (Ft-IR), Scanning Electron Microscope (SEM), and Energy Dispersive X-Ray Spectroscopy (EDX) confirm the formation and morphology of nanoparticles. Cr_2O_3 NPs were in spherical shape with an average size of 67.9 nm under SEM analysis and chromium (Cr) (56.7%) and oxygen (O) (43.53%) were analyzed using EDX. Two peaks (272 nm, 368 nm) were shown in UV-Vis characterization and two sharp peaks, 574 cm^{-1} and 640 cm^{-1} were present in Ft-IR analysis. Lastly, the pancreatic lipase inhibitory assay, which reduces the absorption of fats into the gastrointestinal system, was appraised on Cr_2O_3 NPs with 89.48% of inhibition, with half-maximal inhibitory concentration (IC_{50}) at 31.70 ± 2.14 ($\mu\text{g/mL}$) \pm SE.

Keywords

Obesity, Nanotechnology, Chromium oxide nanoparticles, Antioxidants, Lipase inhibition

Introduction

Mental pressures and excessive consumption of junk food led to the development of overweight and obesity that have become a worldwide issue among the young generations. In the year of 2019, 19.7% of adults were obese and 30.4% were overweight, among 50.1% of Malaysian (Benama, 2020). This is due to excessive calorie intake, and low energy expenditure (Kennett & Clifton, 2010). Overweight and obesity have been linked with other metabolic disorders such as hypertension, cardiovascular diseases (CVDs), hypercholesterolemia, and diabetes (Cao, 2007; Christiaens & Lijnen, 2010). In the other way, oxidative stress has played a role in inducing those metabolic disorders (Morry, Ngamcherdtrakul, & Yantasee, 2017). One of the

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crucial enzymes, which is pancreatic lipase, breaks down triacylglycerol (TAG) in ingested food into glycerol and fatty acids, which are then being absorbed by digestive system (Liu, Liu, Chen, & Shi, 2020). Orlistat, liraglutide, mazindol, and phentermine are few examples of commercial lipase inhibitors which have proven to have positive effect in weight loss by binding themselves to the active site of lipase. However, the drawbacks (side effects such as oily stools, nausea, dizziness, etc.) have overshadowed the advantages (Giordano, Frontini, & Cinti, 2016; Saunders, Umashanker, Igel, & Kumar, 2018). Hence, more promising approaches are required to be developed.

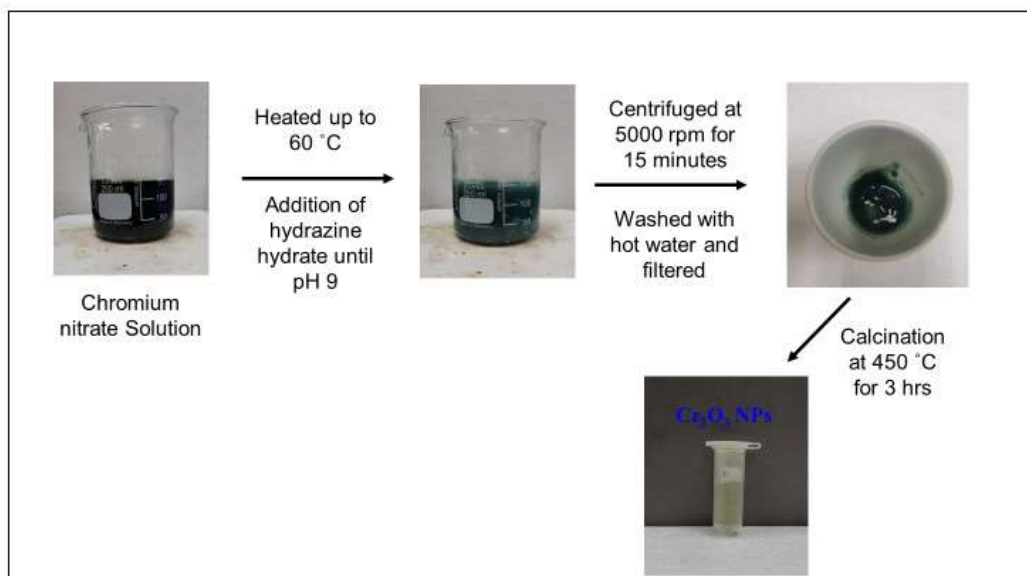
Nanotechnology encompasses the understanding of fundamental sciences, which are physics, chemistry, and biology. It is the production of materials on the scale of atoms and has been utilized in different field (Hulla, Sahu, & Hayes, 2015). The nanoparticle, which has the size of 1-100 nm, possesses unique physical and chemical characteristic accredited to great surface area to volume ratio. It varies from zero dimensional (0D) up to three dimensional (3D) (Zhang, Liu, & Gurunathan, 2016). Nanoparticles can be synthesized through several techniques such as co-precipitation, sol-gel, hydrothermal, solvothermal, green synthesis and many more. The presence of precursor, stabilizing and reducing agent fulfill the requirement in synthesizing of nanoparticles (Habtemariam & Kereta, 2020; Iqbal, Tufail, & Ghazal, 2017).

Metal oxide nanoparticle is a promising approach due to its lipase inhibitory property and may use as drug delivery system to enhance the efficiency of lipase inhibition (Tsou, et al., 2019). Trivalent chromium (Cr^{3+}) is a trace element which involved in several essential metabolisms such as lipid, nucleic acid, carbohydrates (CHO), and protein. It regulates the lipid pathway by reducing the rate of fatty acid synthase or hormone-sensitive pancreatic lipase (Han et al., 2020). In this study, Cr_2O_3 NPs have been chemically synthesized using sol-gel method which is cost-effective and easy to handle (Iqbal, Tufail, & Ghazal, 2017). Cr_2O_3 NPs have semiconducting ability, high chemical resistance, and physically hardness. It has been reported to possess antidiabetic, anti-inflammatory, antioxidant, and antimicrobial properties (Iqbal et al., 2020). In this study, lipase inhibition proficiency of Cr_2O_3 NPs were appraised.

Methodology

Chromium (III) nitrate salt, Folin & Ciocalteu's Phenol Reagent, sodium hydroxide, sodium nitrite and n-Heptane were purchased from R&M Chemicals, whereas for hydrazine hydrate was provided by Sigma-Aldrich. Quercetin hydrate was purchased from Acros Organics, sodium carbonate was purchased from Bendosen, tris hydrochloride was purchased from Fisher, Ethanol was purchased from Chemical Solutions, and 4-Nitrophenyl palmitate was purchased from Alfa Aesar.

Scheme 1 has shown the chemical synthesis of Cr_2O_3 NPs using chromium nitrate salt and hydrazine hydrate. Firstly, 100 mL of 0.1 mol of chromium nitrate salt was prepared and stirred for 30 minutes. Then, the solution was heated at 60 °C and the pH was adjusted to pH 9 by adding hydrazine hydrate in dropwise manner. The mixture was heated under stirring conditions for 1 hour. Subsequently, the dark green gel formed was centrifugated at 5000 rpm for 10 minutes for three rounds and washed using 50 °C hot water. After centrifugation, calcination was carried out at 450 °C for three hours to obtain the green powder.



Scheme 1. Synthesis of Cr_2O_3 NPs using chromium nitrate salt and hydrazine hydrate.

UV-vis spectrophotometer (Thermo Scientific GENESYS 10S) was used to analyze the absorption spectra of Cr_2O_3 NPs under 200 to 800 nm. The presence of functional groups was determined by using Ft-IR spectrophotometer (Perkin Elmer RX1) in the region of 4000 to 400 cm^{-1} . The morphology and the component in Cr_2O_3 NPs were evaluated using FESEM (JEOL JSM-6710F, Japan), combining with EDX (X-max, 150 Oxford Instruments).

Prior to evaluate the pancreatic lipase inhibitory ability of Cr_2O_3 NPs, pancreatic lipase was dissolved in Tris- HCl buffer (1 mg/mL). Then, 900 μL of lipase solution was added into 250 μL Cr_2O_3 NPs solution (0-600 $\mu\text{g/mL}$). The mixture was incubated at 37 $^\circ\text{C}$ for 15 minutes. After that, 50 μL of 4-nitrophenyl palmitate (dissolved in n-Heptane) was added, following by incubation at 37 $^\circ\text{C}$ for 15 minutes. Lastly, the absorbance was measured at 405 nm with orlistat being used as positive control. The percentage of lipase inhibition was calculated using the equation that stated below (Jo et al., 2017):

$$\% \text{ of lipase inhibition: } \left(1 - \frac{A_1 - A_2}{A_3 - A_4} \right) \times 100 \%$$

where A_1 is the absorbance value of test sample with substrate, A_2 is the absorbance value of test sample without substrate, A_3 is the absorbance value of blank solution, and A_4 is the absorbance value of negative control.

Results and Discussion

The UV-Vis characterization was carried out using deionized water with the concentration of Cr_2O_3 NPs at the concentration of 1 mg/10mL at room temperature. It showed absorption peaks at 272 nm and 368 nm, which is similar with the reported result by (Iqbal, et al., 2020). This is due to surface plasmon resonance (SPR) because of collective conduction of oscillation of electrons because of electromagnetic radiations (Phang, et al., 2021). The absorption peak at around 390 nm indicated the transition of d^3 electron from chromium trivalent (Cr^{3+}) of Cr_2O_3 NPs (Khalaji, 2021). Ft-IR spectroscopy evaluation was conducted to determine the functional

groups present in Cr_2O_3 NPs. The sample was prepared with KBr pellet for IR measurement at the range of $4000\text{--}400\text{ cm}^{-1}$. The Ft-IR spectrum showed peaks at 574 cm^{-1} and 640 cm^{-1} indicated the present of chromium oxide (Cr-O) single bond (Salehi, 2015). The first two peaks at 411 cm^{-1} and 444 cm^{-1} expressing the bending mode of Cr-O (Henderson, 2010). Moreover, the bands at 1638 cm^{-1} due to C=C stretching, and 3412 cm^{-1} were due to OH group from the moisture of surrounding or Cr_2O_3 NPs. The present of phenolic group in Cr_2O_3 NPs was implied by the peak at 1408 cm^{-1} . Other weak peaks were observed at 879 cm^{-1} , 1048 cm^{-1} , and 2972 cm^{-1} .

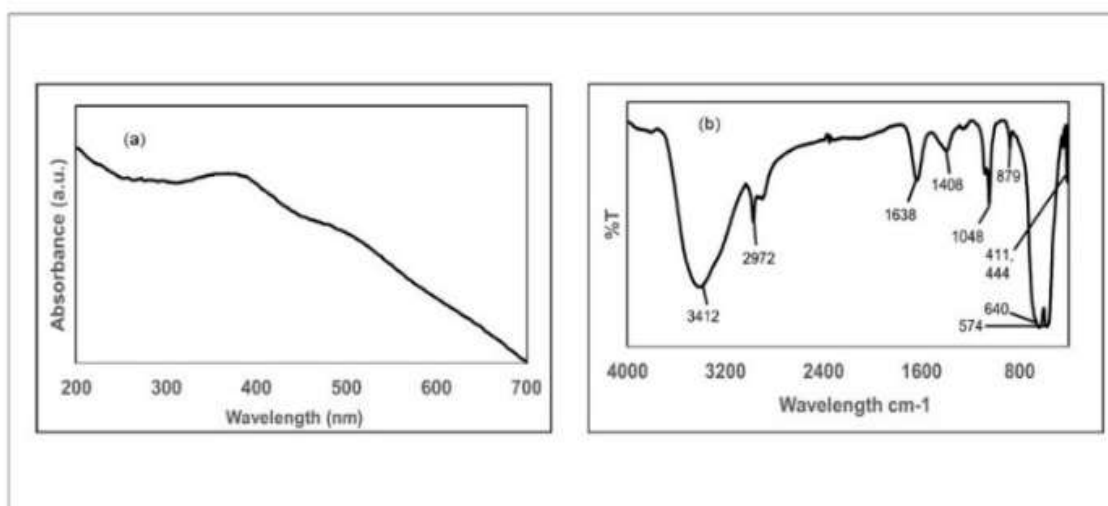


Figure 1. UV-Vis spectrum (a) and Ft-IR spectrum (b) of chemically synthesized Cr_2O_3 NPs

The overall morphology and elements compositions of Cr_2O_3 NPs were analyzed using SEM and EDX. Based on SEM spectrum, it shown Cr_2O_3 NPs with an average size of 67.9 nm. The particles were studied in the 20000x and 30000x magnification, shown in spherical shape and with slightly agglomeration. It is due to high surface tension of Cr_2O_3 NPs (Mello, Faria, Alves, & Scandian, 2020). Figure showed that only two elements, which are Chromium (Cr) and Oxygen (O) present with the percentage of 56.7% and 43.53% for EDX analysis. This indicated high purity of chemically synthesized Cr_2O_3 NPs.

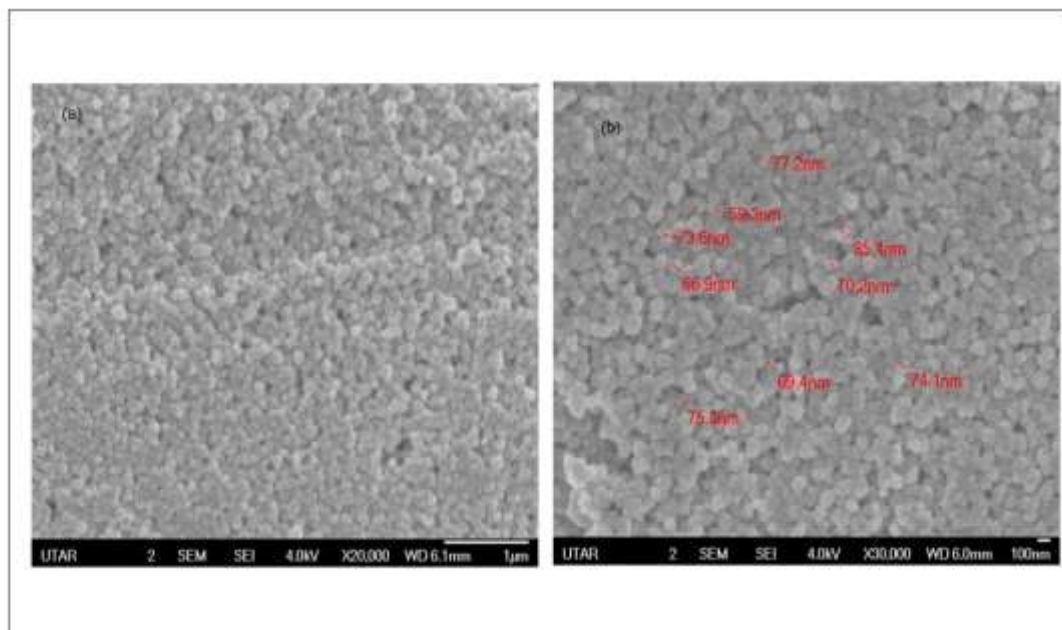


Figure 2. SEM images (20000x magnification) (a) and (30000x magnification) (b)

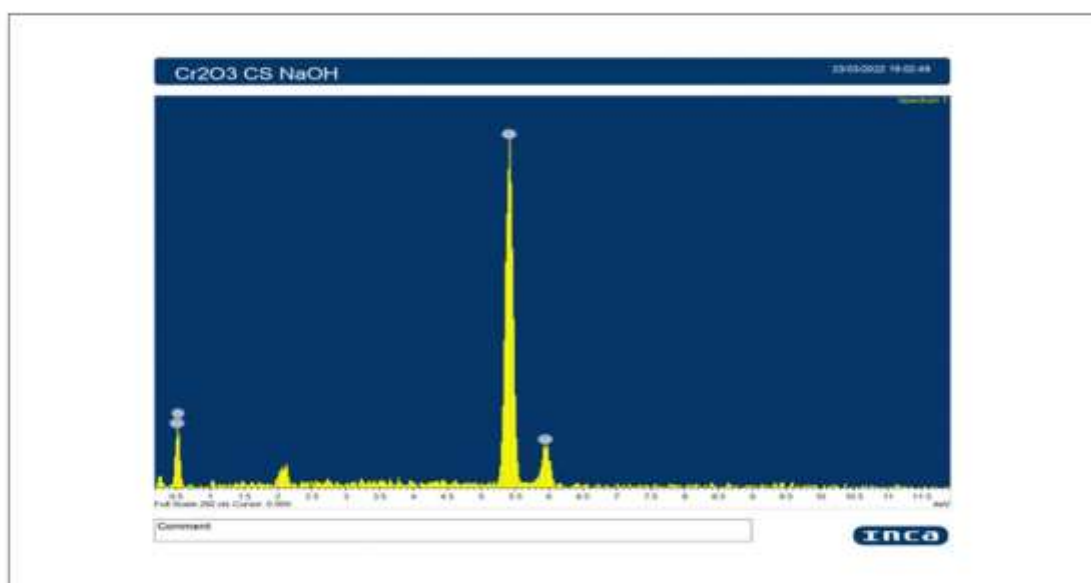


Figure 3. EDX spectrum of chemically synthesized Cr_2O_3 NPs

Pancreatic lipase is an enzyme which responsible for the breakdown and absorption of triacylglycerol (TAG) from dietary fats. The lipase inhibitor could reduce fats being absorbed into digestive system and expel through faeces. It works by binding to the active site and altered the structural confirmation of the enzyme lipase (Liu, Liu, Chen, & Shi, 2020). Cr_2O_3 NPs demonstrated lipase inhibition at 81.74 %, with IC_{50} at 312.5 ± 0.87 ($\mu\text{g/mL}$) \pm SE determined at (0-600 $\mu\text{g/mL}$), whereas for orlistat, it inhibited lipase enzyme at 89.48 %, with IC_{50} at 31.70 ± 2.14 ($\mu\text{g/mL}$) \pm SE determined at (0-60 $\mu\text{g/mL}$).

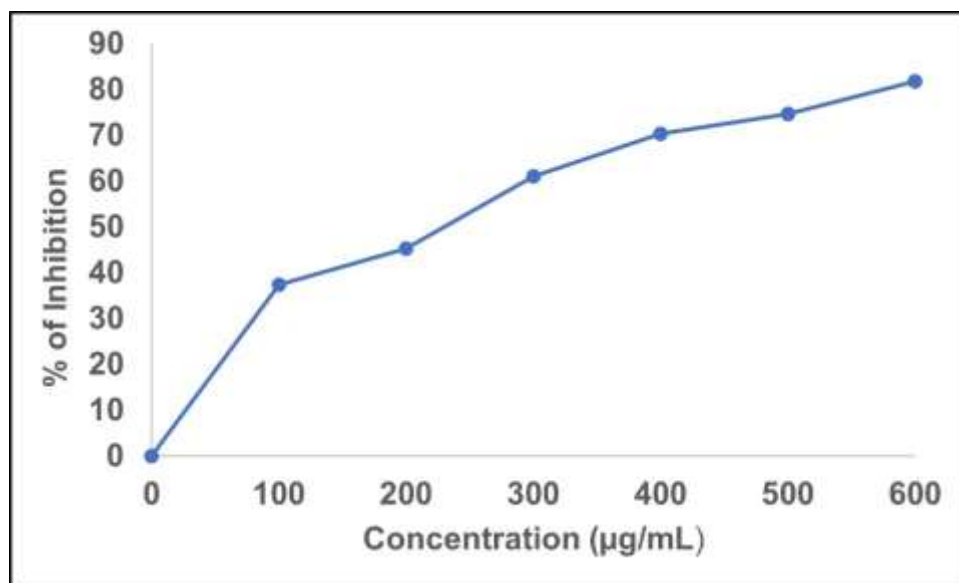


Figure 4. Lipase inhibition assay of chemically synthesized Cr₂O₃ NPs

Conclusion

In conclusion Cr₂O₃ NPs has been successfully synthesized using chemogenic sol-gel method with chromium (III) nitrate salt as precursor and hydrazine hydrate as reducing and stabilizing agent. This method is easy to perform, and the Cr₂O₃ NPs are pure and spherical in shape, determined through several characterizations. The lipase inhibition properties of Cr₂O₃ NPs have been evaluated. The results showed that Cr₂O₃ NPs have significant pancreatic lipase inhibitory performance.

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