

Xanthan gum/Lysozyme nanoparticles (XG/Ly NPs) formulation and its application in stabilizing pickering emulsions

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ABSTRACT

This study investigated the formulation of nano xanthan gum (XG) through a hydrolysis reaction catalyzed by the enzyme lysozyme (Ly). Different reaction conditions of the xanthan gum cleavage process were studied including reaction times of 15, 30, and 45 min; pH levels of 8, 10, and 12; and reaction temperatures of 60, 80, and 100°C. Reaction efficiency was evaluated based on dextrose equivalent (DE) and XG/Ly particle size. Field emission scanning electron microscopy (FE-SEM) results showed that nano XG/Ly particles could be formulated with an average particle size of 500 nm when the reaction was operated at a pH of 12 and a temperature of 80°C for 15 min. Moreover, these nano polysaccharide particles were successfully applied in preparing pickering emulsions and improved their stability.

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1. Introduction

Xanthan gum (XG) is an anionic extracellular polysaccharide produced by the microorganism *Xanthomonas bacteria* (Cai et al., 2020). The molecular weight of xanthan gum is approximately two million g/mol and the main structure of this polysaccharide is quite similar to cellulose (Zhang et al., 2019; Cai et al., 2020). The backbone of xanthan gum is mainly linear bonds composing 1,4-linked β -D-glucose residues. Xanthan gum is a common food additive and has a long history of application in the food and pharmaceutical industry. Although this

polysaccharide was mainly applied as a strong thickening agent, recent studies suggested that nano xanthan gum particles were also potential materials for stabilizing advanced colloidal dispersions (Xu et al., 2015).

Among various pathways of nano polysaccharides preparations, enzymatic hydrolysis using lysozyme is an appropriate choice. Lysozyme, also known as muramidase or N-acetylmuramic hydrolase, is a small monomeric protein stabilized by four disulfide bonds among the eight cysteine residues of its polypeptide chain (Vu et al., 2019). This enzyme

is often found in large quantities in human and plant organs, tissues, and secretions (spleen, placenta, skin milk, tears, saliva, serum, etc.). Some studies successfully applied lysozyme to hydrolyze polysaccharides such as xanthan gum but reaction conditions for this process have not yet been optimized. Besides, the applications of nano xanthan gum particles are also very limited.

Currently, the emulsion system is an important step in the pesticide industry, because it helps improve the homogenization of solvent systems, additives, and biologically active ingredients. Among advanced colloidal dispersions, emulsions stabilized by solid particles (commonly known as pickering emulsions) are the choice of many recent researchers due to their low toxicity compared to synthetic surfactants adding tiny solid particles to the emulsions, they could be adsorbed at the oil/water interface and provide an effective barrier to limit the unwanted coalescence between droplets (Chen et al., 2023). The solid particles commonly used in pickering emulsions are polysaccharides, proteins, and lipids (Phan et al., 2015; Tu & Ha, 2015; Do et al., 2016).

In this study, xanthan gum will be hydrolyzed with the enzyme Lysozyme to form XG/Ly solid particles. Then, these novel solid nanoparticles will be utilized for preparing pickering emulsion systems. Various advanced technologies including FE-SEM and static light scattering will be applied to evaluate the effectiveness of hydrolysis reaction as well as the stability of pickering emulsions.

2. Materials and Methods

2.1. Materials

Chemical substances for silver nanoparticle

synthesis and analysis included: xanthan gum (XG, Mw = 1.3×10^7 Da), Lysozyme (CAS#12650-88-3), sunflower oil (Rossianka, Russia), 3,5 - Dinitrosalicylic acid (DNS) (Himedia, India), $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ (Xilong Scientific, China), D-Glucose (Xilong Scientific, China). All chemical reagents were in the analytical grade.

2.2. Methods

2.2.1. Synthesis of XG/Ly

Xanthan gum and lysozyme solutions (25 mg/mL) were stirred with distilled water at room temperature for 6 and 2 h, respectively. Xanthan gum/lysozyme mixture with a mass ratio of 1:1 was prepared after the pH was adjusted to 12. The mixture was further heated at 80°C for 15 min and cooled naturally to room temperature for 24 h. Then the pH of the xanthan gum/lysozyme nanoparticle solution was adjusted to 7 using 1 M HCl. Impurities were removed by the filtration using an 80 μm membrane, yielding a solution containing XG/Ly nanoparticles. Finally, XG/Ly powder was obtained by freeze-drying for 72 h.

The effect of pH solution on XG/Ly particle morphology: changing reaction solution pH at various including 8, 10, and 12. Reaction temperature and time were kept at 80°C and 15 min, respectively. Then, based on the maximum dextrose equivalent (DE_{max}) of samples, choose the most suitable treatment for the next experiments.

The effect of reaction temperature on XG/Ly particle morphology: The best pH result from the previous experiment was kept at different temperatures of 60, 80, and 100°C using a water bath (Mettler, Germany for 15 min). Then the most appropriate reaction temperature was selected based on the DE_{max} of samples.

The effect of reaction time on XG/Ly particle morphology: Similar to previous experiments, the pH solution and the reaction temperature were fixed. Reaction time was evaluated in a range of 15 - 45 min. Then the most appropriate reaction time was selected based on the DE_{max} of samples.

2.2.2. The determination of dextrose (DE, dextrose equivalent)

The reducing sugar equivalent DE of commercial RMD and maltodextrin (MD) was determined according to the Miller (1959) method with some modifications. The reducing sugar of the sample produces color with dinitrosalicylic acid (DNS) reagent. Based on the standard curve for glucose, the reduced sugar content in the analyzed sample is calculated.

Determination of DE: Take 25 mL sample, then add 15 mL of DNS and dilute to 50 mL with distilled water, cover, shake well, boil for 45 min, take out, cool to room temperature, and then measure color at wavelength 540 nm. The blank sample is 15 mL of DNS, titrated with distilled water to the 50 mL mark. The amount of reducing sugar in the sample is determined by the glucose standard curve at the ABS value measured at wavelength 540 nm after subtracting the ABS value of the blank sample.

DE (Dextrose equivalent) is determined by the formula:

$$DE = \frac{C \times V}{m \times 100}$$

C: reducing sugar concentration in the sample (mg/mL)

V: rated volume (50 mL)

m: mass of powder sample (mg)

2.2.3. Characterization of XG/Ly

The microstructure of XG particles was observed using a polarized light microscope (DM2500P, Leica, Germany) and FE-SEM (SU8000, Hitachi, Japan).

2.2.4. Preparation of pickering emulsion

The XG NP solutions (0.5%, w/w) and sunflower oil (10%, w/w) were dispersed into the aqueous phase. Oil-in-water (O/W) pickering emulsions (10% oil/water, w/w) were prepared by dispersing sunflower oil into XG NPs solutions using an ultrasound machine (Biologics - VT150, USA) with a frequency of 20 kHz for 1 min. The oil droplet sizes of the emulsions were determined using the Static Light Scattering (SLS) method (Mastersizer 3000, UK).

2.2.5. Statistical analysis

All experiments were conducted in triplication. One way, ANOVA was applied to evaluate the effect of reaction conditions on the formation of silver nanoparticles. Statistical analyses were conducted on the statistical software (Statgraphic, IBM, USA) with a 95% confidence interval.

3. Results and Discussion

3.1. Optimizing the synthesis of XG/Ly

For optimizing enzymatic reactions, pH is one of the most important factors. When changing the pH of the solution during the chain-cutting reaction, the DE equivalent of the product XG/Ly also changed and the difference between samples was significant ($P < 0.05$). Obtained results also revealed that the DE of samples XG/Ly showed an upward tendency to the increase pH solution (Figure 1a). Among the investigated pH (8, 10,

and 12), pH 12 showed the highest DE value and this parameter was applied for further tests.

Figure 1b depicted the effect of reaction temperature (60, 80, and 100°C) on the quality of XG/Ly particles at pH 12 with a reaction time of 15 mins. As hypothesized, the DE equivalent of products achieved the maximum value at the temperature of 60°C. No significant difference was recognized by ANOVA analysis between samples reacted at higher temperatures ($P > 0.05$). Because enzymes are very sensitive to thermal treatment, a too-high reaction temperature can lead to unwanted protein denaturation resulting in decreasing the enzyme activities.

The effect of reaction time (15, 30, and 45 min) on the hydrolysis reaction was summarized

in Figure 1c. The combination of obtained results of DE values and ANOVA analysis showed that the heating time had a very limited effect on the formulation of nano XG/Ly ($P > 0.05$). Because the maximum value was quickly recognized after 15 min, further prolonging the reaction was not the appropriate choice in considering heating costs.

In general, the optimization conditions for the xanthan gum hydrolysis reaction catalyzed by Lysozyme were the pH solution of 12 and the reaction temperature of 60°C for 15 min. Obtained nano XG/Ly particles achieved a DE value of 12,371 (two times higher than native XG granules).

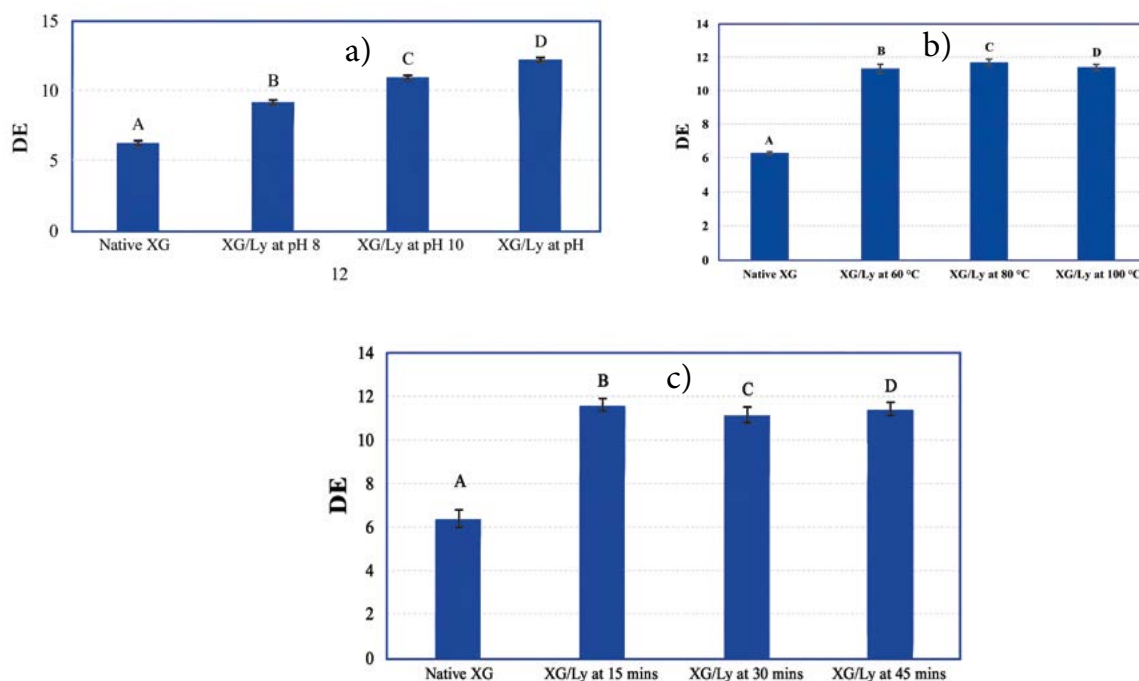


Figure 1. The dextrose equivalent of the xanthan gum/lysozyme (XG/Ly) sample when changing pH (a), temperature (b), and reaction time (c). Values within the different letters (A, B, C, and D) are significantly different ($P < 0.05$) between means of samples.

3.2. The results of XG/Ly

The morphology of XG before and after the enzymatic reaction was observed using polarized light microscopy and scanning electron microscopy and the images were depicted in Figures 2 and 3, respectively. Normally, the native XG particles are long polysaccharide fibers with a size of 100 - 500 μm and their morphology is quite

clumpy including the clusters of polysaccharides cluster together (Figure 2). However, their size was significantly reduced due to the hydrolysis process catalyzed by Lysozyme at 60°C and pH of 12 for 15 min. According to the FE-SEM image (Figure 3), XG particles after the reaction had a size of 200 - 500 nm and therefore they could be considered as solid nanoparticles.

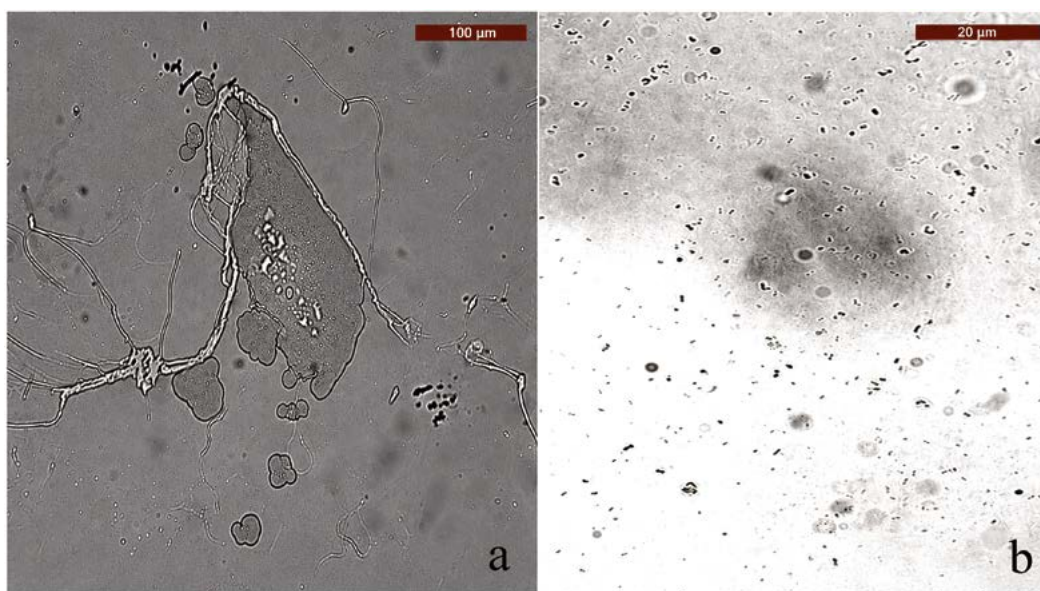


Figure 2. Image of native xanthan gum (a) and nano xanthan gum/lysozyme particles (b).

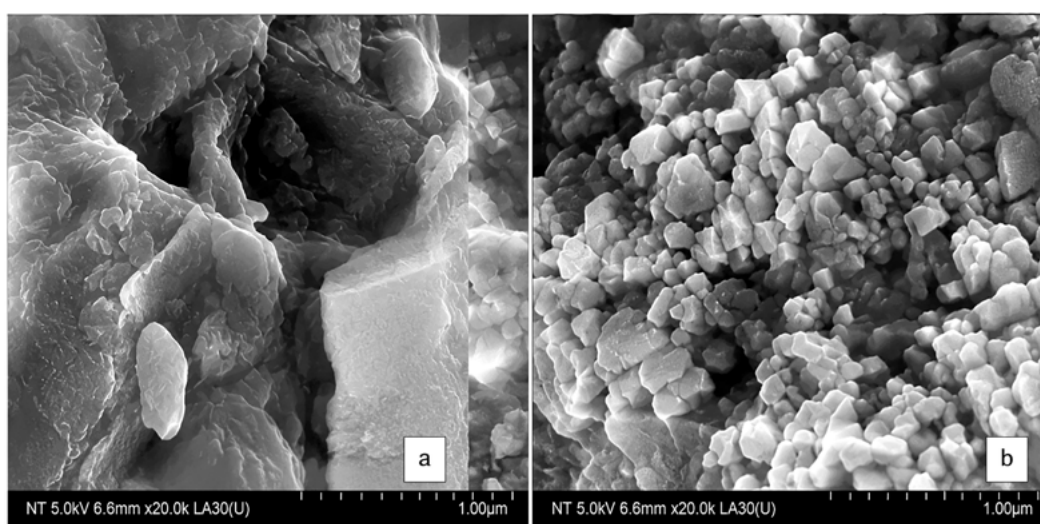


Figure 3. Field emission scanning electron microscopy image of native xanthan gum (a) and nano xanthan gum/lysozyme particles (b).

3.3 Pickering emulsions stabilizing by XG/Ly nanoparticles

The microstructure of pickering oil-in-water emulsion stabilized by XG/Ly nanoparticles was illustrated in Figure 4 while their droplet size distribution was displayed in Figure 5. Firstly, it can be seen that micro pickering emulsions with droplet sizes in a range of 10 - 100 μm successfully were produced. As expected, XG/Ly nanoparticles were located at the O/W interfaces,

forming a firm barrier protecting dispersed oil droplets. After 14 days of storage, these oil droplets tended to gather resulting in increasing the size of the emulsion particles. However, the dispersions still retained their color as well as the stable state, and no precipitation was observed (Figure 6). Therefore, it can be confirmed that the obtained XG/Ly particles after the hydrolysis reaction were small enough to form a highly stable pickering emulsion.

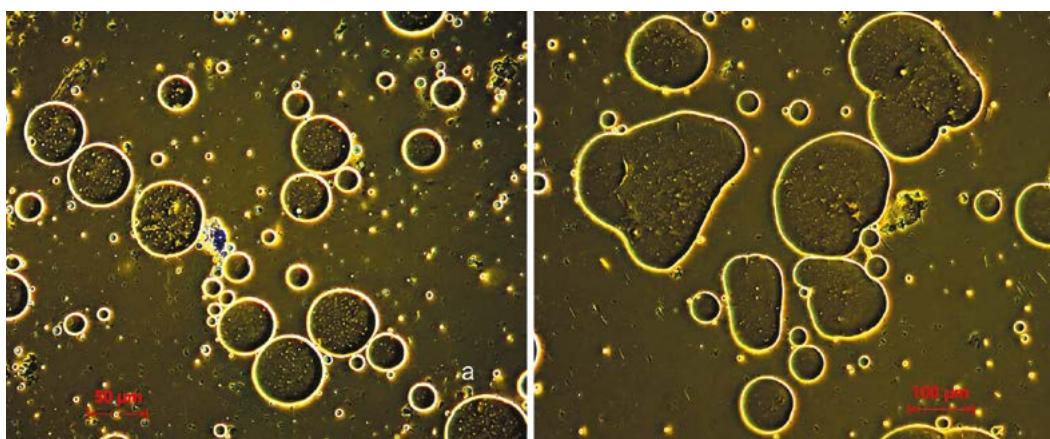


Figure 4. Light microscopic images of pickering emulsions with different concentrations of xanthan gum nanoparticles (a) after 1 day; (b) after 14 days.

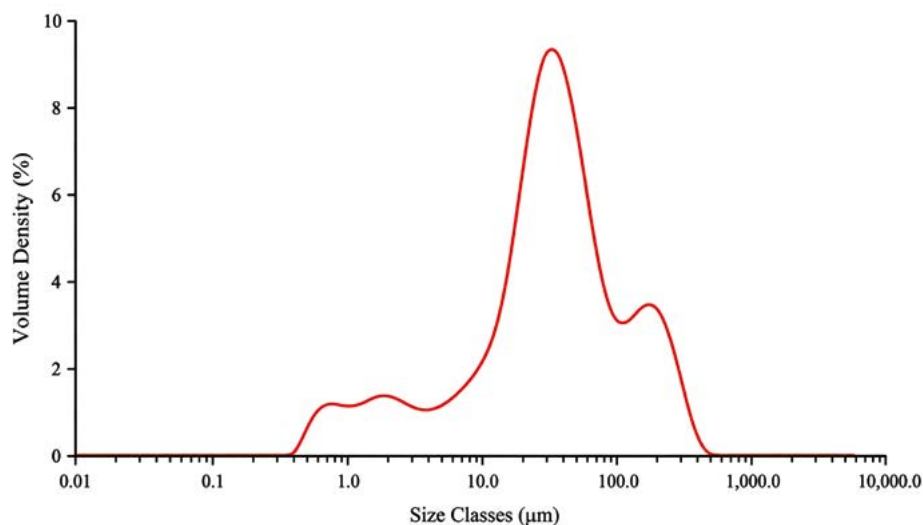


Figure 5. Droplet size distributions of xanthan gum nanoparticles pickering emulsions.

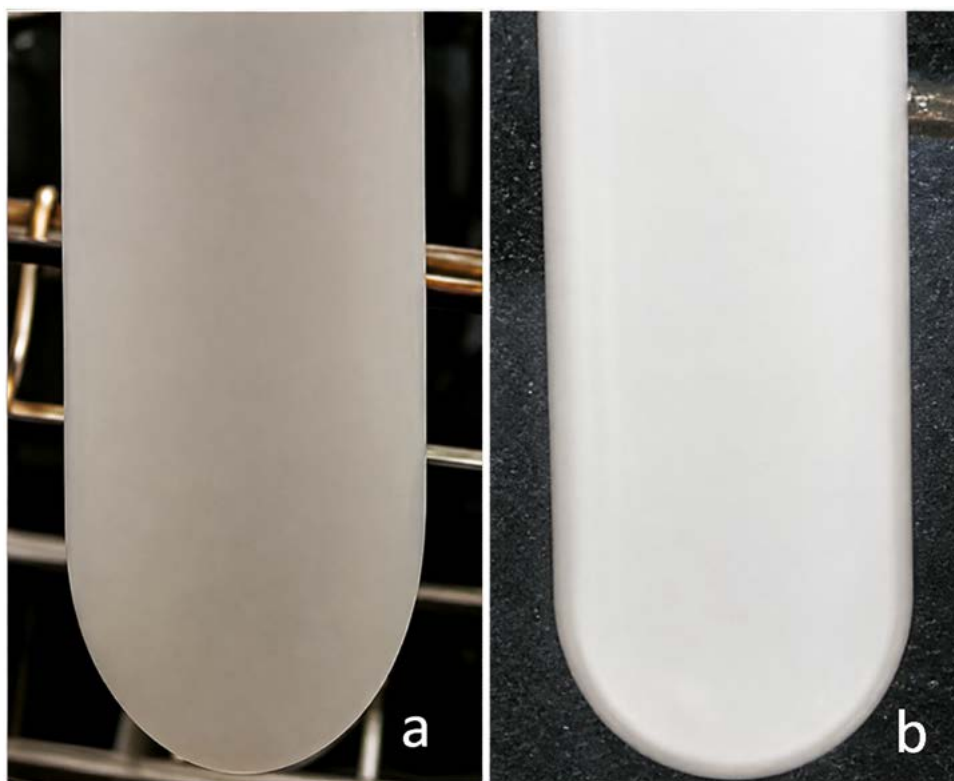


Figure 6. Image of pickering emulsion after 1 day (a) and after 14 days (b).

4. Conclusions

The study prepared XG/Ly NPs with sizes of 200 to 500 nm using the biological cutting method. The result shows that nanoparticles contributed to forming a barrier on the surface of oil resulting in improving their stability. Further research can focus on optimizing the XG circuit-cutting process and investigating factors affecting the pickering emulsification process.

Conflict of interest

The authors have no conflicts of interest to declare.

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