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Functionality of xanthan and almond gum in colloidal shellac nanoparticles containing cinnamon

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Abstract. Instability of shellac nanoparticles at acidic pH is the main challenge of its use as an oral delivery system. This study aims to investigate the functionality of xanthan and almond gum in shellac nanoparticles containing cinnamon prepared by anti-solvent precipitation. The gums were added into the anti-solvent phase while the cinnamon extract was incorporated into the solvent phase. The results show that the minimum concentration of xanthan and almond gum to prevent shellac aggregation at simulated gastric pH (1.2) was 0.3% and 0.6%, respectively. Higher concentration of gums resulted in a bigger particle size and more negative ζ-potential, regardless the type of the gums. Cinnamon loading in the shellac-xanthan gum complex resulted in nanoscale sized particles, while the complex system consisted of shellac, almond gum 0.6% and cinnamon extract created a network entrapping the individual particle. In conclusion, xanthan gum incorporation was better than almond gum to stabilise shellac nanoparticle containing cinnamon in simulated gastric pH. This study confirms that shellac-xanthan gum complex can be potentially used for an oral delivery system of bioactive compounds.

1. Introduction

Nanoscience and nanotechnology are the new frontiers of the century and are considered as the "next big thing" in many industries, including in food manufactures [1]. Nanoparticulate delivery systems (e.g. nanoparticles, liposomes, nanocapsules and nanospheres) are some of the examples of nanotechnology applications in food industry [2]. Nanotechnology has been successfully applied to mask undesirable odours and flavours. In addition, nanotechnology can be used to improve bioavailability as well as to control the release of bioactive compounds [3, 4, 5]. Shellac, a nontoxic and physiologically harmless material secreted by lac insect (*Kerrialacca*), can be potentially used as a nanocapsule wall-material. It has tremendous film forming and protecting properties [6, 7]. However, the use of shellac as an oral delivery system of bioactive compounds in a nanoparticulate form has a limitation as it forms aggregate at gastric pH [8]. Nanoparticulate delivery system of bioactive compounds targeting the colon has to be stable in the harsh acidic conditions of the stomach [9, 10]. Therefore, it is important to create a system which can prevent the aggregation of shellac at acidic pH. Various natural polymers has been reported to have a great potency as a stabiliser in many systems; therefore, it might be an alternative to stabilise shellac colloidal particle in the gastric pH condition.

In our preliminary study, guar gum, K-carrageenan, Na-alginate, xanthan gum and almond gum were used (data not shown). Only the last two polymers showed a positive effect in preventing shellac aggregation at acidic pH. Thus, in this study, xanthan gum and almond gum were further investigated to prepare stable shellac nanoparticles. Xanthan gum is a natural biopolymer produced by fermentation by bacteria *Xanthomonas campestris* [11]. Xanthan gum has been extensively used as a stabilising agent in many products. It has pseudo plastic rheological properties, temperature stability and compatibility with many food ingredients [12]. Almond gum, a biopolymer derived from the tree *Amygdalus communis* L., is another type of gum which has a high potency as a stabilising agent in food. Almond gum is classified as arabinogalactan polysaccharide with arabinose, galactose, xylose, and uronic acid as its major constituents [13].

Cinnamon extract was used as the model of the core material of the nanoparticles in this study. This is based on the fact that synthesis of nanoparticles containing spice extract are still scarce until to date. Cinnamon extract contains a wide range of bioactive compounds acknowledged to have benefits for human health, such as an antioxidant, anti-inflammatory, antitumor, anticancer, antidiabetic, and anti-hypertriglyceridemia agent due to its phenolic and volatile constituents [14]. The long list of beneficial effect of cinnamon for health as well as its high antioxidant activity indicates that cinnamon extract can be potentially used in pharmaceutical and food industry [15]. For its application, nano-sized particles may have significant impact to improve its compatibility to food or beverage matrix. In this study, anti-solvent precipitation method was selected to fabricate the nanoparticles due to its low cost and easy operation. Thus, this study aims to investigate the functionality of xanthan and almond gum in stabilising shellac nanoparticles containing cinnamon extract.

2. Experimental

A traditional method described by Muhammad et al [16] was followed to prepare the cinnamon extract. In brief, 5 grams of cinnamon powder was extracted in 50 ml ethanol for 48 hours at 20°C. Afterwards, the cinnamon residue was separated using a vacuum filter and then the cinnamon extract was collected (Laboport, KNF Neuberger, Inc, USA)

Raw almond gum (containing both water soluble and insoluble substances) was obtained from Sepahan Nano-Food Co. (Isfahan, Iran). For the separation of the water-soluble from the insoluble part, the almond gum was firstly pulverised using a ball mill (Retsch, PM400, UK) for 30 min at 14 x g. Then, the powdered almond gum was mixed with distilled water in the concentration of 4% and left on stirring for 4 hours at room temperature (20°C). The mixture was kept at 4°C overnight for complete hydration. To separate the water-soluble part, the mixture was centrifuged at 20000 rpm for 30 min at 25°C, and then the clear solution on top was gently collected. The dry matter concentration of this solution was determined from the mass obtained by placing 10 ml at 105°C overnight [17].

The shellac colloidal nanoparticles were fabricated using an anti-solvent precipitation method [8]. The first solution (solvent phase) was made by solubilising 2% (w/w) g of shellac fine powder (SSB 55 Astra FP, SSB Stroever GmbH & Co. KG (Bremen, Germany)) in ethanol. The second solution (anti-solvent phase) was made by solubilising xanthan gum (Satiaxane CX 931, Cargill France SAS) in distilled water in different concentration. The first solution was injected by using a syringe to the anti-solvent phase with the ratio of 1:3 (w/w) in order to obtain homogeneous mixture. Afterwards, a vacuum rotary evaporator was used to remove ethanol from the mixture; thereby, the colloidal shellac nanoparticles were formed. A freeze-dryer (Vaco 5-D Zirbus technology GmbH) was used to obtain dry particles. To prepare shellac nanoparticles containing cinnamon, the cinnamon extract was subjected to replace the ethanol in the first solution in multilevel proportion (12.5% to 37.5% (w/w)).

Photon Correlation Spectroscope (PCS100M, Malvern Instrument Ltd, UK) and Zetasizer IIC (Malvern Instrument Ltd, UK), with the temperature setting of 25°C, were used to measure the particle size and the surface charge of the shellac nanoparticles, respectively. Before the measurement, a few drops of the colloidal shellac nanoparticles was appropriately diluted in distilled water and KCl 0.01 M for the particle size determination and the surface charge analysis, respectively.

To observe the shape of the nanoparticles, a JEOL JSM 7100F SEM equipped with a PP3010T Cryo-SEM Preparation System (Oxford Instruments) was used. The microstructural observations were conducted in the dried nanoparticles form and rehydrated nanoparticles form. After placed on the cryo-

specimen holder the sample was then cryo-fixed in slush nitrogen (–210°C) and transferred to the cryo-unit in the vitrified state. Subsequently, the sample was fractured well, sublimated for 20 min at -140°C and sputter coated with platinum (4 min, 0.5 mbar). The microstructural properties of the nanoparticles were observed in the microscope with the temperature setting of 140°C.

The measurement of apparent viscosity was conducted by an AR2000-ex rheometer (TA Instruments) equipped with concentric DIN cylinder (cylinder: 42.00 mm; rotor outer radius: 14.00 mm; stator inner radius: 15.00 mm; geometry gap: 5920 mm). Approximately, 20 g of samples was placed at the cylinder and then the analysis was performed at shear rate level 50 s^{-1}. A cross-hatched 40 mm stainless-steel plate was used for the determination of yield stress of shellac-gum colloidal system loaded with cinnamon extract. The measurement was conducted by increasing the shear stress from 0.01 to 50 Pa. All the tests were carried out at 20°C.

SPSS Statistics 23 was used to perform analysis of variance (one way ANOVA), Duncan's Multiple Range Test (DMRT) and T-test. The differences were considered significant at $p<0.05$. In addition, Pearson's test was also carried out to determine the correlation coefficient between gums concentration and the physicochemical characteristic of the nanoparticles.

3. Results and discussions

The use of xanthan and almond gum was intended to prevent shellac aggregation at acidic pH. Interestingly, an instant aggregation of some parts of shellac was found in the step of injection of the solvent phase into the anti-solvent phase when the gums was absent. The instant aggregation can be prevented by the incorporation of either 0.1% of xanthan gum or 0.5% of almond gum. At the pH 1.2, the lowest concentration of xanthan and almond gum to inhibit shellac nanoparticles aggregation were 0.3% and 0.6%, respectively (Figure 1, Table 1). At the lower concentration of the gums, the repulsion was not adequate to prevent aggregation. Prevention of shellac aggregation at simulated gastric pH is by the electrosteric stabilisation [8]. The electrosteric stabilisation is gained by the adsorption of the gums on the surface of the colloidal particles. Hydrogen bonding between shellac and xanthan gum were reported by Patel et al. [18] on the contrary, until to date none of literature has discussed a possible interaction between shellac and almond gum [8]. There are some factors affecting the ability of the gums to stabilise shellac nanoparticles in addition to its concentration, such as composition, molecular weight and chain length [19]. This may be one of the reason why both gums have different stabilisation effect.

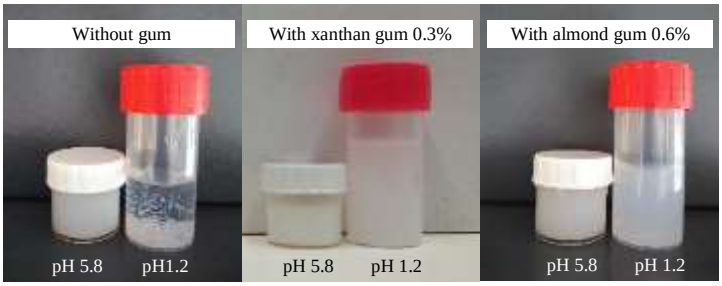

Figure 1. Influence of xanthan and almond gums incorporation on the visual appearance of the colloidal shellac nanoparticles

The gum concentration has a significant influence on the particle size of the nanoparticles when observed at pH 5.8 (the natural pH of the shellac colloidal system), regardless the type of the gum. It indicates that more gum molecules were adsorbed onto the shellac particles surface [8]. The higher concentration of gums can also increase the viscosity. A high viscosity can play a significant role to inhibit diffusion between solvent and anti-solvent, consequently, resulted in the formation of agglomeration [19]. Figure 2A confirms that a higher concentration of the gums led to a higher apparent viscosity. This manifestation is mainly because of the pseudoplastic effect of the gums which have been widely reported in previous studies [11]. Interestingly, it was observed that at the same

concentration (e.g. 0.3% and 0.5%), xanthan gum is more viscous than almond gum. Therefore, it can be understood that the particle size of colloidal particles made by the combination of shellac-xanthan gum were relatively bigger than that of shellac-almond gum at the same concentration of the gums.

Table 1. Particle size of shellac colloidal dispersion stabilised by xanthan gum and almond gum

Gum concentration	Xanthan gum		Almond gum	
	Stability at pH 1.2	Particle size (nm) at pH 5.8	Stability at pH 1.2	Particle size (nm) at pH 5.8
0.0%	aggregation•	66.5 ± 1.9[a]		
0.1%	aggregation	99.4 ± 1.6[b]		
0.2%	aggregation	127.0 ± 4.5[c]		
0.3%	stable	147.1 ± 3.9[d]	aggregation•	109.5 ± 0.9[a]
0.4%	stable	153.2 ± 1.2[e]	aggregation•	137.1 ± 3.2[b]
0.5%	stable	207.5 ± 4.2[e]	aggregation	195.7 ± 2.5[c]
0.6%			stable	381.4 ± 3.2[d]
0.7%			stable	428.4 ± 12.5[e]
0.8%			stable	625.8 ± 17.0[f]
Pearson's correlation coefficient with gum concentration	0.975**		0.967**	

Mean values with the same letter do not differ significantly ($p>0.05$) in the same column. Notation (•) indicates that the nanoparticle was formed, but aggregation of some parts of the particle was observed in the injection step. Notation (**) indicates significant at $p<0.01$.

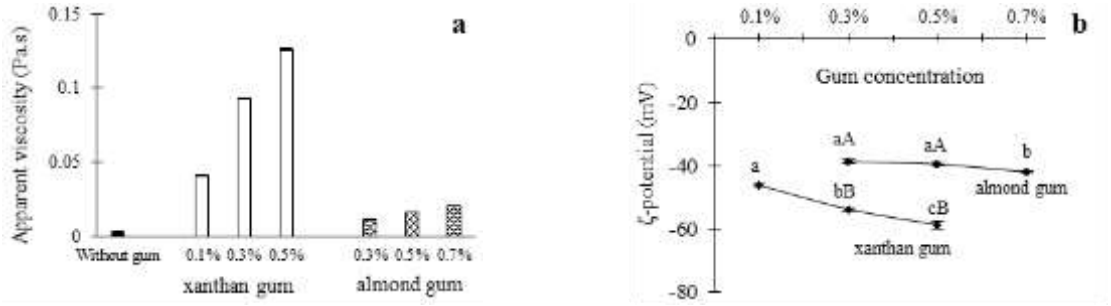

Figure 2. (a) Apparent viscosity of xanthan and almond gum in different concentration at the shear rate of 50 s^{-1}; (b) The effect of gum incorporation on the zeta potential of shellac colloidal dispersion. Mean values with the different lowercase and uppercase letter differ significantly ($p<0.05$) in the same type of gum and the same gum concentration, respectively.

Gums incorporation resulted in the more negative surface charge, regardless the type of the gums. Both gums have been reported as a negatively charged polymer in previous studies [8, 19]. However, when comparing both gums at the same concentration, for instances 0.3% and 0.5%, it was noticeably shown that the surface charge of xanthan gum had higher negative values than that of almond gum; and the statistically significant difference was observed between the samples (Figure 2B). The fact that the rise of almond gum concentration gave a limited effect on the surface charge of the nanoparticle may be because the almond gum has a lower number of carboxylate groups. As mentioned by Cerrutti et al [20], a mutual repulsion of electrical charges is the basis of electrostatic stabilisation. In the electrostatic stabilisation, the carboxylic group can act as a proton donor or acceptor in hydrogen bonds, while the carboxylate group can act as a proton acceptor. Since xanthan gum contributes with appreciably more negative charges than almond gum, the incorporation of xanthan gum induces more repulsive effect. It might be the reason why a less concentration of xanthan gum (0.1%) was required to prevent instant aggregation of shellac during injection step than the almond gum (0.5%).

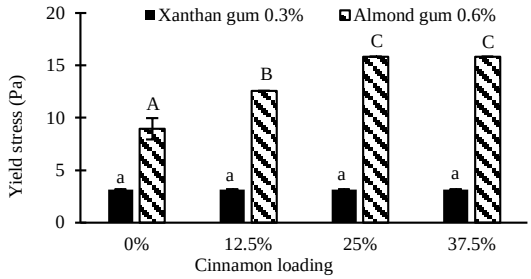

Figure 3. The effect of cinnamon extract loading on the yield stress of the shellac-gum complex. Statistical analysis was carried out separately for each type of gum. Mean values within each gum type with the same notation do not differ significantly ($p>0.05$).

The minimum concentration of xanthan and almond gum to stabilise shellac nanoparticles was found at the concentration of 0.3% and 0.6%, respectively. Therefore, the study was continued by incorporating cinnamon extract in the shellac nanocapsule templates. In Figure 3, it was shown that the blank nanoparticles made of shellac-almond gum had a higher yield stress than shellac-xanthan gum. The incorporation of cinnamon extract resulted in a more viscous colloidal system in the shellac-almond gum system, but not in the shellac-xanthan gum system. It is interesting since the apparent viscosity of almond gum was lower than that of xanthan gum before the nanoparticles fabrication as previously shown in Figure 2A. To get better understanding on the network formation of the colloids, the microstructural properties of the colloidal nanoparticles were then investigated.

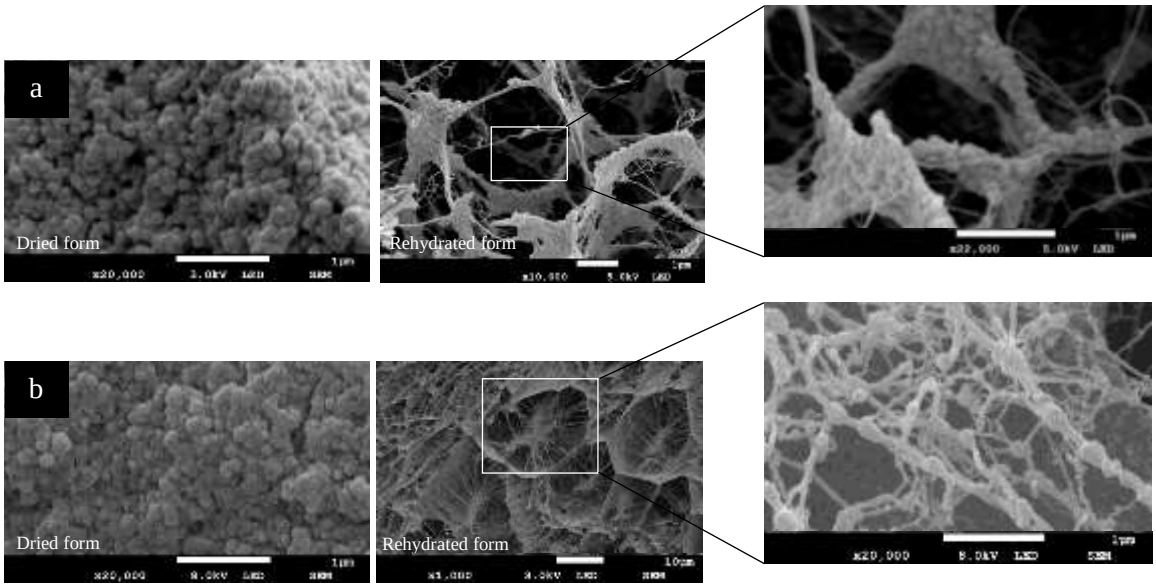

Figure 4. Typical morphology of shellac nanoparticle incorporated with xanthan gum 0.3% (a) and almond gum 0.6% (b) loaded with cinnamon extract 12.5%.

Figure 4 shows that the individual nanoparticle has spherical shape with a smooth outer surface agreeing a report by Joye & McClements [19]. The difference on the microstructure of shellac colloidal nanoparticles loaded with cinnamon stabilised using xanthan gum and almond gum was observed. As shown, shellac-xanthan gum nanoparticles containing cinnamon were in the nanoscale size with spherical shape. PCS measurement confirmed that the particle size of the shellac-xanthan gum nanoparticles containing cinnamon was in the range of 100-200 nm. Shellac-almond gum system loaded with cinnamon also have spherical shape when observed in dry form. It was also perceived that the individual particle has nanoscale size. However, by the PCS measurement, it was found that the

particle size of shellac-almond gum incorporated with varied level cinnamon extract were surprisingly in the range of 3 to 9 μm. When observed in the rehydrated form, it was discovered that the complex system consisted of shellac, almond gum 0.6% and cinnamon extract created a network entrapping the individual particle. The nanoparticles were flocculated forming a bigger particle and even formed a gel-like matrix when observed visually.

4. Conclusions

In conclusion, xanthan and almond gum have an ability to stabilise shellac colloidal nanoparticles through electro-steric mechanism. However, almond gum needs a higher concentration than xanthan gum to prevent shellac nanoparticles aggregation at acidic pH. The shellac-xanthan gum complex was more suitable for cinnamon extract carrier than the shellac-almond gum complex since the gel-like structure of the shellac-almond gum complex were formed after the incorporation of the cinnamon. Further study is required to investigate the encapsulation efficiency and particle loading of the nanoparticles made from shellac-xanthan gum-cinnamon complex.

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