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Research Note

Tailoring Chitosan as a Pickering Emulsion Stabilizer – A Preliminary Assessment

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Emulsion based systems are gaining widespread popularity because of their promising ability to function as delivery systems for biologically active molecules and hence is being used in various sectors such as nutraceutical, pharmaceutical and cosmetic industries (Zhu et al., 2019; Boonlao et al., 2022). However, these are thermodynamically unstable systems owing to various destabilization mechanisms such as Ostwald ripening, flocculation, sedimentation, coalescence etc. (Xu et al., 2022). Therefore, an important pre-requisite in designing emulsion-based delivery system is the judicious selection of emulsifiers. The most commonly used emulsifiers are tween, span or other biopolymers. In recent times, there is an increasing trend to use solid particles instead of the conventional emulsifiers/ surfactants for fabricating stable emulsion delivery systems. Emulsions stabilized by solid particles are referred as pickering emulsions, which is pioneered by S.U. Pickering. This process has received tremendous acceptance globally owing to its surfactant free nature, resistance to coalescence and safety aspects. In addition, in a recently published report, it was highlighted that pickering emulsions can be used for production of powders with high oil content and superior controlled release attributes (Li et al., 2022).

Among the wide range of biopolymers used for emulsification studies, chitosan has received tremendous acceptance especially in drug and nutrient delivery studies. Chitosan, a natural biopolymer, is mostly obtained by the partial deacetylation of chitin. Its biodegradability combined with non-toxicity, biocompatibility, bioadhesive nature etc. has favored it as an excellent candidate for nutrient/ drug delivery studies. It has been already reported that chitosan *per se* is not an ideal emulsion stabilizer owing to its hydrophilic properties (Lekshmi et al., 2017). Hence, systematic scientific efforts are being carried out to improve its emulsification attributes. The present study was carried out with an objective to widen the functionality of chitosan to perform as a pickering emulsion stabilizer via hydrophobic modification.

Hydrophobic modification of chitosan is being reported as an effective strategy to improve its functionality and thereby making it as an excellent pickering emulsifier. Among the different techniques of hydrophobic modification, self-assembly method is considered to be an easy and facile approach. In the present study, low and medium molecular weight chitosan (Sigma Aldrich, USA) having degree of deacetylation ≥75 % were used at a concentration of 1 % for preparation of self-assembled chitosan. Subsequently, the solutions were hydrophobically modified by changing the pH to 6.9 using 1 M NaOH. The particle size, zeta potential and rheological attributes of the modified chitosan solution were then determined to ascertain its physio-chemical attributes. For measuring the particle size and zeta potential, the solutions were appropriately diluted to reduce the light scattering effect.

The visual observation of the solution indicated that when the pH was increased to 6.9, a macroscopic precipitation has happened indicating the self-assembly formation (Fig. 1 c & d). The possible mechanism for self-assembly of chitosan can be because of the deprotonation of the hydrophilic -

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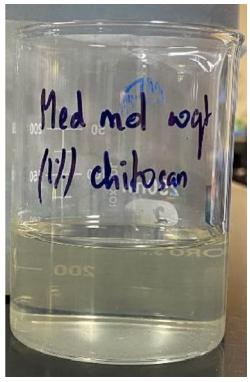
NH^{4+} groups, thereby increasing the hydrophobicity of the chitosan chain. Further, the coalescence of the particles thus produced could be the possible reason for the macroscopic precipitation.

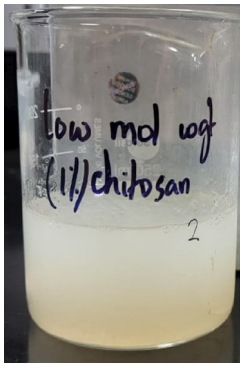
The size analysis of the hydrophobically modified chitosan was carried out to study the effect of self-assembly process. The size distribution analysis revealed that the particle size of the modified chitosan was 3078 and 3568 nm respectively for the medium and low molecular weight self-assembled chitosan. The results clearly indicate that the physical properties, specifically the size of the self-assembled chitosan seems to be affected by the molecular weight. Furthermore, the zeta potential, which is another indicator of the emulsion stability, was also compared. The zeta potential of the medium and low molecular weight self-assembled chitosan was found to be 12.03 and 6.76 mV respectively. Similar to particle size distribution, the zeta potential value was also found to be influenced by the molecular weight of chitosan.

Being an important parameter that influence the long-term emulsion stabilization, the rheological properties of the self-assembled chitosan was also evaluated. The viscosity of the medium and low molecular weight self-assembled chitosan was found to be 14.96 and 12.89 cp respectively. To further elucidate the flow behaviour of the self-assembled chitosan, they were subjected to detailed rheological analysis by increasing the shear rate from 40 to 200 rpm. It was observed that irrespective of the type of chitosan employed, the viscosity of medium and low molecular weight chitosan has decreased considerably from 14.96 to 2.72 and 12.89 to 2.43 cp respectively. This clearly indicates that the solutions had a clear shear thinning behaviour.

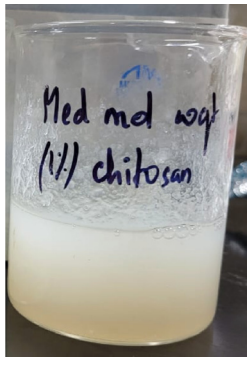
a. Native chitosan (low molecular weight)



b. Native chitosan (Medium molecular weight)



c. Self-assembled chitosan (low molecular weight



d. Self-assembled chitosan (medium molecular weight)

Fig. 1. a, b shows the visual appearance of native chitosan of low and medium molecular weight respectively; c, d shows the visual appearance of the self-assembled chitosan of low and medium molecular weight

Subsequently, the hydrophobically modified chitosan was used for development of pickering emulsions using squalene as the oil phase. Briefly, to 100 ml of the modified chitosan solution, squalene was added at the rate of 30 % of the total wall material concentration. This was then subjected to high speed homogenization at the rate of 12,000 rpm for 10 minutes. Further, to investigate the effect of ultrasound on modulating the emulsion stability, the homogenized emulsions were given an additional ultrasonic treatment with a standard voltage of 60 V with a pulse time of 2 seconds for a total time of 10 minutes. Accordingly, four different treatments were prepared which are given in Table 1. The emulsions thus prepared were transferred to graduated test tubes for further emulsion stability studies.

The emulsion stability analysis has shown that there was no phase separation in any treatment irrespective of the type of chitosan employed and the ultrasonication treatment. The particle size, zeta potential and the rheological properties of the different emulsion were studied. The particle size of emulsions prepared from self-assembled low and medium molecular weight chitosan was found to be 8405 and 7271.33 nm, respectively. However, when the effect of ultrasonication treatments on emulsions were analysed, it was clear that the particle size has decreased drastically from 8405 to 3495.33 nm in case of self-assembled low molecular weight chitosan, whereas for self-assembled medium molecular weight chitosan the particle size has reduced from 7271.33 to 4834.33 nm. During sonication, the larger particles of the emulsion may get dispersed to smaller droplets by virtue of the high shear forces and cavitation. However, the polydispersity index (PDI) of all the treatments remained same, 1, indicating their polydisperse distribution. A small particle size is always advantageous from an emulsion stability point of view, indicating that an ultrasonication can be an additional step preceded or succeeded by the conventional emulsification process.

In case of the emulsions prepared without ultrasound treatment, zeta potential values were found to be 7.13 and 7.28 mV, respectively. Interestingly, it was noticed that when the same emulsions were subjected to ultrasonication process for 10 min, the zeta potential has increased from 7.28 to 14.37mV in case of medium molecular weight based self-assembled chitosan. However, the values remained almost the same for low molecular weight based self-assembled chitosan with a marginal increase from 7.13 to 7.77 mV. The electrophoretic mobility of the different treatments was also studied and the values were in the range of 0.55 and 0.57 for medium and low molecular weight based self-assembled chitosan respectively. Similar to zeta potential value, there was a significant change in the electrophoretic mobility too, with the value increasing from 0.57 to 1.13, whereas for low molecular weight-based

Table 1. Particle size (PS), zeta potential (ZP), mobility and polydispersity index (PDI) of various treatments

Treatment	PS (nm)	ZP (mV)	Mobility (μmcm/Vs)	PDI
MMW CS	8793.00 ± 413.63	67.31 ± 9.27	5.12 ± 0.50	0.72 ± 0.08
LMW CS	6987.00 ± 149.62	63.00 ± 4.43	4.94 ± 0.35	0.27 ± 0.03
MMW SAC	3078.00 ± 343.00	6.76 ± 0.65	0.94 ± 0.01	1.00 ± 0.00
LMW SAC	3568.00 ± 540.78	12.03 ± 0.15	0.53 ± 0.05	1.00 ± 0.00
MMW Emul	7271.33 ± 1575.01	7.13 ± 0.18	0.55 ± 0.01	1.00 ± 0.00
LMW Emul	8405.00 ± 1561.00	7.28 ± 1.08	0.57 ± 0.08	1.00 ± 0.00
MMW SAC UAE	4834.33 ± 250.58	14.37 ± 0.97	1.13 ± 0.07	1.00 ± 0.00
LMW SAC UAE	3495.33 ± 2700.92	7.77 ± 0.88	0.61 ± 0.07	1.00 ± 0.00

(All data are given as mean ± SD, n=3)

MMW CS: Medium molecular weight chitosan; LMW CS: low molecular weight chitosan; MMW SAC: Medium molecular weight self-assembled chitosan; LMW SAC: low molecular weight self-assembled chitosan; MMW Emul: Medium molecular weight emulsion; LMW Emul: Low molecular weight emulsion; MMW SAC UAE: Medium molecular weight self-assembled chitosan with ultrasonic assisted emulsion; LMW SAC UAE: Low molecular weight self-assembled chitosan with ultrasonic assisted emulsion.

chitosan, the change was marginal with the value changing from 0.55 to 0.61 (Table 1). These significant findings point out the significance of effect of molecular weight in modulating the physio-chemical properties of chitosan.

The inefficacy of native chitosan to perform as an effective stabilizer for conventional oil-in-water emulsions has been reported in our earlier study (Lekshmi et al., 2017). To broaden the emulsification attributes, a general practice is to use chitosan in conjunction with other wall materials such as protein, gum, etc. (Lekshmi et al., 2019). In this context, the present study offers a feasible strategy to broaden the emulsification attribute of chitosan via hydrophobic modification. By adjusting the pH of native chitosan solution to 6.9, self-assembled chitosan of superior emulsification ability can be fabricated which is found to be quite an easy process. The physio-chemical attributes of the self-assembled chitosan thus prepared was found to be influenced by the molecular weight. Self-assembled chitosan fabricated from medium molecular weight chitosan exhibited better stability attributes than the low molecular weight-based ones. The study further demonstrated that by employing an additional ultrasonication step in the pickering emulsion process, stability parameters can also be improved.

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