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Encapsulation of coconut shell liquid smoke in chitosan-maltodextrin based nanoparticles

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Abstract

The study investigated the characteristics of chitosan-maltodextrin (CS-MD) nanoparticles incorporating coconut shell liquid smoke at various formulations. Chitosan-maltodextrin nanoparticles were prepared with the addition of 1.0% sodium tripolyphosphate (TPP) into a solution of liquid smoke. Sample consisting of CS-MD nanoparticles in 1.0% acetic acid without liquid smoke was used as a control. CS-MD nanoparticles were also evaluated at elevated temperatures (40 and 50°C) for 15 min. The CS-MD nanoparticles with the liquid smoke resulted in the range of pH from 2.41 to 3.02; viscosity 10.83 cP - 11.77 cP; particle size 1.3 nm - 12.7 nm and the zeta potential (-6.53) mV – (+3.12) mV. While, the control CS-MD nanoparicles without coconut shell liquid smoke showed pH 3.09; viscosity 11.17 cP; particle size 343.86 nm and the zeta potential (+5.17) mV.

Introduction

The utilization of nanoparticles as delivery system for bioactive food components has gained wide attention in recent years due to its ability to enhance bioavailability of active ingredients, revolutionize controlled release, confer protection to the bioactive compounds against environmental stress, and improve sensory aspects (Sanguansri and Augustin 2006). The distinct functional properties of the nanoparticles as excellent delivery vehicles may be related to its abilities in conferring high stability, high carrier capacity, feasibility in entrapping both hydrophilic and hydrophobic compounds, and its multiple routes of administration capability (Ghosh *et al*., 2009; Zimet and Livney, 2009; Fang and Bhandari, 2010). Delivery system is defined as one in which a bioactive material is entrapped in a carrier to control the rate of bioactive release. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10 - 1000 nm (Mohanraj and Chen, 2006; Hartig *et al*., 2007).

Various biocompatible and degradable natural polymers can be employed in the formation of nanoparticles via self-assembly method. In the present study, chitosan (CS) and maltodextrin (MD) have been chosen as the polycationic and anionic compounds. CS, being cationic in nature, has high affinity to cross-link with counter polyanions. CS is a derivative of chitin, a biopolymer found in the shells of crustaceans, cell wall of fungi and other microbials (Pranoto *et al*., 2005; Anal, 2010). Chitin is a polymer of N-acetylglucosamine and has espescially after deacetylation into the polyglucosamine, chitosan shows the interesting chemical reactivity as polycationic compound as well as its physiological features. CS has attracted much attention due to its biocompatibility, biodegradability, nontoxic nature, low immunogenicity, antibacterial properties, mucoadhesivity (Darmadji and Izumimoto, 1994; Anal *et al*., 2006), and its ability to act as an absorption enhancer (van der Lubben *et al*., 2001; Vila *et al*., 2002).

CS is normally insoluble in water above pH 6 due to its rigid crystalline structure and requires acids to be protonated (Lee *et al*., 2007). With an estimated pKa value of 6.2 to 7, CS is positively charged in acidic medium due to the protonation of its amino groups (NH_3^+). The positive NH_3^+ in the CS polymer chain can bind negatively charged surfaces via hydrogen or

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electrostatic bonding (Shahidi and Abuzaytoun, 2005; Anal and Stevens, 2005). Recently, CS has been used as wall material for encapsulation of sensitive core ingredients such as lipophilic drugs (Ribeiro *et al*., 1999), vitamin D_2 (Shi and Tan, 2002), astaxanthin (Higuera-Ciapara *et al*., 2004), ampicillin (Anal *et al*., 2006), and olive oil extract (Kosaraju *et al*., 2006).

Maltodextrin is one of the most commonly used materials for encapsulation of bioactive materials. Maltodextrin is water-soluble material and able to protect encapsulated ingredient from oxidation. Some studies have explored the use of carrier agents such as maltodextrin to protect sensitive compounds like vitamin C in fruit juice and to increase product stability in acerola powder (Desobry *et al*., 1997; Tax *et al*., 2003; Righetto and Netto, 2005). A recent study is found that maltodextrin can enhance the phenolic and anthocyanin content during processing of purple sweet potato flour (Ahmed *et al*., 2009).

The liquid smoke is a dispersion of smoke in water (Tranggono *et al*., 1999), and a mixture of steam and smoke from the colloidal dispersions in water obtained by pyrolysis of wood (Maga, 1987; Pszczola 1995). Wood smoke consists of light-scattering tarry droplets suspended in a medium of air and invisible vapours. The size of the droplets is dependent on the conditions of smoke generation, the average radius of fresh smoke being about 0.1 µm (Foster and Simpson, 1961).

The main purpose of liquid smoke application in proteinaceous food products is not only to act as a coloring and flavoring agent but also possess antibacterial and antioxidative properties (Darmadji and Izumimoto, 1994; Coronado *et al.*, 2002). Various phenolic compounds present in liquid smoke lowers the pH and destroys the walls of bacterial cells (Pszczola, 1995). Coconut shell liquid smoke has been reported to contain bioactive compounds such as phenols, carbonyls and organic acids. Therefore, the Coconut shell liquid smoke is potential in increasing shelf life of proteinaceous food products (Tranggono *et al*., 1999; Darmadji *et al.*, 2009; Zuraida *et al*., 2011).

The bioactive compounds of liquid smoke needs to be protected against deterioration during the process by mean of encapsulation. However, there is no study reporting the encapsulation of liquid smoke components. In addition, a little is known on combination CS-MD as encapsulates in different concentration into liquid smoke solution. A previous study demonstrated the ability of CS assembled into nanoparticles of 400 - 600 nm (Grenha, 2010). Preparing nanoparticles in this size range is facilitated by the use of adequate cross-linking agents. TPP is a non-toxic polyanion known for its capacity to cross-link CS, a reaction mediated by electrostatic forces, resulting in the formation of ionic cross-linked networks (Janes *et al*., 2001; Mi *et al*., 2003; Rodrigues *et al*., 2012).

The objective of this study was to produce CS-MD nanoparticles loaded with coconut shell liquid smoke and to evaluate the influence of concentration CS-MD and heat treatment on the characteristic of the nanoparticles.

Materials and Methods

Materials

Raw coconut shell liquid smoke used in this study was obtained from Tropica Nucifera Industry, Yogyakarta, Indonesia. This material was purified using redistilation method in the laboratory. Chitosan (CS) was purchased from Biotech Surindo, Indonesia (deacetylation degree 91.5%, moisture 10.43%, ash 0.71%). Maltodextrin (MD) with Dextrose Equivalent (DE) 10.8% was from Grain Processing Corp. (Iowa, USA), Sodium tripolyphosphate (TPP) and glacial acetic acid (HOAc) were supplied by Sigma Chemicals Ltd. (Munich, Germany). The other chemicals used for analysis were of analytical grade.

Analysis of coconut shell liquid smoke

Phenol content was determined following the procedure established by Senter *et al*. (1989) and carbonyl was quantified according to Lappin and Clark (1951). Total acidity was determined by using titration, weigh about 1 ml of sample in a 250 ml beaker diluted with about 100 ml of water. Titrated with 0.1N sodium hydroxide solution to an equivalence point of pH 8.15, as determined using a pH meter. Acidity was calculated as percent by weight of acetic acid using the factor: 1 ml of 0.1N sodium hydroxide is equivalent to 60.05 mg acetic acid. Total soluble solid content was assessed by hand-held refractometer (Atago N1, Tokyo, Japan) at 20^{o}C, and expressed as percentage (^{o}Brix).

Nanoparticles preparation

CS (0.5% w/v) and MD (9.5% w/v) were dispersed in an aqueous solution of glacial acetic acid (1.0% v/v). CS-MD nanoparticles were prepared with a slight modification of previously described methodology (Grenha *et al*., 2010), based on the polyelectrolyte complexation of CS with MD and additional ionic gelation of chitosan with TPP anions. Briefly, CS and MD were dissolved in coconut shell liquid smoke based on the various formulation CS : MD, i.e. 0% : 10%; 0.5% : 9.5%; 1.0% : 9.0% and

1.5% : 8.5%. Then, Pre-determined volume (1 mg/ml) TPP was added in these formula and agitated using a magnetic stirrer at 200 rpm for 30 min at room temperature. Nanoparticles were concentrated by centrifugation (Centrifuge Damon/IEC Division, Connecticut, USA) at 3000 rpm into 50 ml conical tube for 30 min at room temperature. The supernatant was discarded and nanoparticles were vacuum filtered (Gast, USA) using Whatman # 2. The solution of nanoparticles was heated at 40°C and 50°C into waterbath for 15 min and was homogenized using a rotor–stator homogenizer (Ultraturrax T50 Basic IKA Werke, Germany) at 4000 rpm for 2.5 min. CS-MD nanoparticles without heat treatment (room temperature) were used as a control.

Transmission electron microscopy(TEM)

The morphological property of the CS–MD nanoparticle was characterized using TEM (JEM-1400, JEOL, Japan) operated at an accelerating voltage of 120 kV. The TEM sample was prepared as follows, the nanoparticle suspension was dripped onto a 400-mesh copper grid precoated with Formvar and stained by 2% w/v phosphotungstic acid (Grenha *et al*., 2007). The sample was air-dried at room temperature for more than 2 h before analyzing them on the TEM.

Measurement of particle size and zeta potential of nanoparticles

Particle size and zeta potential were determined by a Delsa™ Nano C Zeta (Beckman Coulter Inc., Fullerton, CA, USA), using photon correlation spectroscopy (PCS). In this technique, the particle size was determined by measuring the rate of fluctuations in 30 mW dual laser light intensity scattered by particles as they diffuse through a fluid. The nanoparticles (5 ml) dispersed in deionized water were added to a cell holder and counting was performed (70 accumulation times). Each experiment was performed in a triplicate. The zeta potential was determined by measuring the electrophoretic movement of charged particles under an applied electric field. The Delsa instrument used a zeta potential module equipped with a ± 100 mV two laser diodes (658 nm). Scattered light was detected at 160° and at temperature of 25°C. About 2 ml of a suspension of charged particles in water was used for the measurements.

pH and viscosity of of the solution containing nanoparticles

A pH-meter (Schott, Deutschland, Germany) was used to determine the pH of the solution of nanoparticles at 26°C. Viscosity was measured for the 250 ml of nanoparticle dispersed solutions placed in 500 ml graduated cylinder using a viscometer Brookfield RVT Type (Middleboro, USA) at 25°C, spindle # 1 with 50 rpm for 30 sec.

Statistical analysis

The differences between the mean values of multiple groups were analyzed by one-way analysis of variance (ANOVA) with Tukey Methods range tests. ANOVA data with a $P < 0.05$ was classified as statistically significant. MINITABS 16.0 software, Origin 75 and Microsoft Excel 2007 program were used to analyze and report the data. Mean values from the experiments were done in triplicates report.

Results and Discussion

The approximate composition of coconut shell liquid smoke used in this study i.e. 2.08% phenols, 10.83% carbonyls, 9.97% acidy, and 8.4% total soluble solid. The pH of the original coconut shell liquid smoke was 2.54. Organic acids such as acetic acid and formic acid as antioxidant agent together with phenolic compound. Acids contained in coconut shell liquid smoke influence the food stuffs in their flavor, pH and shelf life. Phenolic compound provides aromatic and antioxidant effect as well as enhancing the shelf-life of the product. Carbonyls generally contribute to the color of the product once reacted with amino groups. Tranggono *et al.* (1999) and Darmadji *et al.* (2009) mentioned that bioactive compounds in liquid smoke gave not only preservative and antioxidative effect but also color, flavor and taste to the product.

Transmission electron microscopy

The preparation of CS-MD nanoparticles was based on an ionic gelation interaction between positively charged CS and negatively charged MD and TPP. Briefly, when the three components were mixed, an electrostatic interaction was established between the positively charged amino groups of CS and the negatively charged hydroxyl and phosphate groups of MD and TPP, respectively, leading to the nanoparticle formation in a process derived from inter and intramolecular linkages mediated by the anionic molecules (Janes *et al*., 2001). TPP affords a further intensive interaction, as it provides a cross-linking effect. Figure 1 displays the TEM microphotograph of representative 0.5% CS-MD nanoparticles in liquid smoke without heat treatment. The nanoparticles without heat effect were found to be spherical shapes with diameters 48.83 nm. This result was similar to the earlier reports (Calvo *et al*.,1997; Rodriguez *et*

al., 2012). They obtained the chitosan nanoparticles with quasi spherical nanoparticles and about 200 nm size by formation of a spontaneous complex between chitosan and polyanions such as tripolyphosphate.

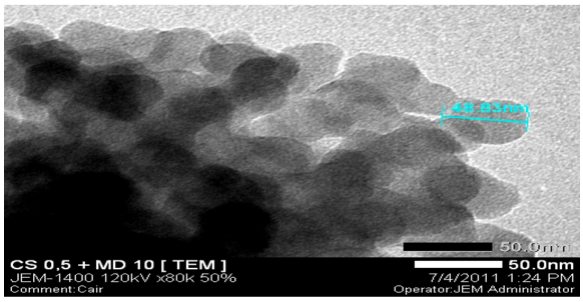

Figure 1. TEM microphotograph of representative 0.5% CS-MD nanoparticles in liquid smoke without heat treatment

Particle size

Table 1 shows the mean size of each CS-MD nanoparticles prepared with various formulations in w/v, i.e. 0% : 10%; 0.5% : 9.5%; 1.0% : 9.0% and 1.5% : 8.5%. It demonstrates that the size of nanoparticles decreased with increase in CS and decreased in MD concentration. This increase in size with concentration showed a linear relationship within the tested range. When CS-MD and TPP were mixed in acetic acid solution, they had maximum size (343.86 nm) and the minimum size (2.56 nm) of CS-MD nanoparticles was obtained for a CS concentration of 1.5% and a MD concentration of 8.5% in liquid smoke.

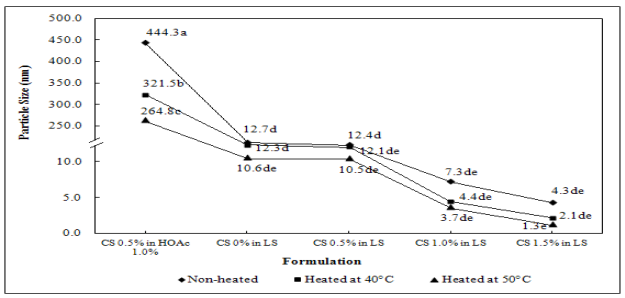

Figure 2. Particle size (nm) measurements of CS-MD nanoparticles in formulations at various temperatures. Figures in the graph followed by the same letter indicating no significant difference at $p < 0.05$

adsorbed into the surface and the cross-linking structure related to the concentration of CS-MD of the layer were affected by heat treatment. With further heat treatment, however, particle size was maintained within a regular range, because the matrix structure in which CS and MD were combined through ionic gelation inside particles was more stable than the particle surface.

The results were in agreement with the report by Jang *et al*. (2008) that showed the particle size of the Chitosan-Ascorbic acid nanoparticles decreased with increasing heat treatment. According to the Eötvös empirical equation, the general trend is that the surface tension decreases with increasing temperature, reaching a value of zero at a critical temperature (Brunel *et al*., 2008). This could explain why the particle size decreased when temperature

Table 1. Characteristics (particle size, ζ potential, pH and viscosity) of the different CS-MD nanoparticles formulations (mean ± SD, n = 3)

Formulations	Size (nm)	ζ Potential (mV)	pH	Viscocity (cP)
F1 = Chitosan 0.5% in HOAc 1.0%	343.86 ± 91.32 a	5.17 ± 3.80 a	3.09 ± 0.13 a	11.17 ± 0.19 c
F2 = Chitosan 0% in Liquid Smoke	11.87 ± 1.08 b	-4.38 ± 2.20 b	2.66 ± 0.22 b	11.04 ± 0.19 c
F3 = Chitosan 0.5% in Liquid Smoke	11.67 ± 1.03 b	-0.04 ± 1.33 c	2.70 ± 0.19 c	11.26 ± 0.19 c
F4 = Chitosan 1.0% in Liquid Smoke	5.16 ± 1.92 c	1.17 ± 0.66 d	2.75 ± 0.17 d	11.48 ± 0.10 a
F5 = Chitosan 1.5% in Liquid Smoke	2.56 ± 1.53 c	1.73 ± 1.00 e	2.79 ± 0.16 e	11.67 ± 0.12 b

^{a,b,c,d,e} Different superscripts within a column indicate significant differences among formulations ($p < 0.05$).

Figure 2 shows the change in size of CS-MD nanoparticles during heat treatment. In the case of size CS-MD nanoparticles at control, 40°C and 50°C were rapidly reduced in the first formulation and then were slowly reduced into a regular range. The average size of CS-MD nanoparticles was smaller with increasing temperature (data not shown), and the overall size of CS-MD nanoparticles in acetic acid was bigger than in liquid smoke.

These results indicated that the layer formed during absorption of bioactive compounds in liquid smoke (e.g. phenols, carbonyls and organic acids) into the surface of the particles was affected by the initial heat treatment. In other words, they were increased from 40°C to 50°C. Therefore, particle size is important to enhance the nanoparticles mediated bioactive compounds of liquid smoke, such as phenolic should be entrapped in the smallest CS-MD nanoparticles by using heat treatment at 50°C and concentration of CS 1.5%.

Zeta potential of nanoparticles

Zeta potential is the surface charge that greatly influences the stability of the nanoparticles in suspension through the electrostatic repulsion between them. As illustrated in Table 1, when CS-MD and TPP were mixed in acetic acid solution, they spontaneously formed compact nanocomplexes with

an overall positive surface charge (+5.17 mV). In the liquid smoke, zeta potential of CS-MD nanoparticles increased linearly with increasing CS concentration. This simple linear relationship could be easily explored for modulating the density of particle surface charge to facilitate adhesion and transport properties of the nanoparticles.

The results of zeta potential of the nanoparticles in liquid smoke without CS presented negative value (-4.38 mV), probably due to liquid smoke consisting of some organic acids such as acetic acid that presents the negatively charged carboxylic acid group. The results were in accordance with those of the earlier report of Vargas *et al*. (2009) on Chitosan-Oleic acid films who sportted that the absence of chitosan particles was negatively charged.

Increasing the concentration of CS resulted in the increase in zeta potential. It indicated the presence of CS attachment onto the surface of the nanoparticles. The zeta potential of the nanoparticles was inverted from negative to positive values upon addition of increasing amounts of CS as shown in Table 1. In the formulation prepared with 0.5% (w/v) of CS, the zeta potential was neutralized, whereas at a CS concentration of 1.0% (w/v), an inversion of the zeta potential was noted. This positive value was further increased after incorporation of a CS at concentration of 1.5%. This trend of the zeta potential as a function of CS concentration should logically be attributed to the surface association of positively charged CS to the nanoparticles. This surface association process is supposed to be driven by the electrostatic interaction between the negatively charged MD solid cores and TPP cross lingking agent with the positively charged CS chains. The same phenomenon of surface attachment of CS to negatively charged cores has been previously observed by another researcher while developing CS-coated emulsions and CS-coated polymer nanoparticles (Calvo *et al*., 1997; Vila *et al*., 2002; De Campos *et al.*, 2003). As expected, the differences in the zeta potential values of the formulations were remarkable. The zeta potential of the CS-MD nanoparticles in liquid smoke showed a less negative value than that of the nanoparticles in acid acetic.

The trends of CS-MD nanoparticles in liquid smoke show that the increasing CS concentration tends to increase in zeta potential with values ranging (-4.4 mV) – (+1.7mV). It suggested that the positive charge amino groups of CS was more dominant on the surface of the particles. In Figure 3, heat treatment of CS-MD nanoparticles at 40°C, values of zeta potential is -1.3 mV and at 50°C zeta potential slight increases to +0.7 mV. Nevertheless, the value of zeta potential CS-MD nanoparticles in unheated treatment was highest than heated at 40°C and 50°C (data not shown). The results were supported with the findings of Jang and Lee (2008), and they found the slow reduction of zeta potential of CS-Ascorbic acid nanoparticles during heat treatment at 60, 80 and 100°C.

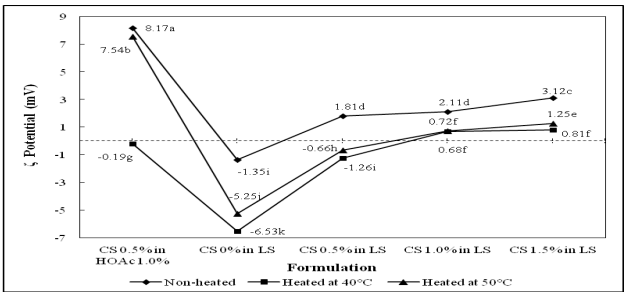

Figure 3. Zeta potential (mV) measurements of CS-MD nanoparticles in formulations at various temperatures. Figures in the graph followed by the same letter indicating no significant difference at $p < 0.05$.

pH of the nanoparticles

The pH of CS-MD nanoparticles was significantly different depending on the formulations and heat treatment as shown in Table 1. The pH of the CS-MD nanoparticles in acetic acid was significantly higher than other formulations. In general higher CS concentrations in liquid smoke lead to higher pH values. The formulation without CS in liquid smoke presented the lowest value, in comparison to the other CS concentrations due to initial pH value (2.54) of liquid smoke and has much acidic compounds such as acetic, propionic, butiric, and valeric acid. Our results are in agreement with Darmadji (2002) and Zuraida *et al*. (2011), who obtained similar values of pH in the coconut shell liquid smoke applied on fish ball product. In addition, reported that in US Patent 5637339, 1997-2015 (Moeller, 1997) the pH of liquid smoke was 2.3 - 2.5. Working with liquid smoke, Tranggono *et al.* (1999) found that acetic acid was dominant in coconut shell liquid smoke, while Kadir *et al*. (2012) reported that coconut shell liquid smoke consists mostly of acetic and propionic acids.

In Figure 4, pH of unheated treatment of CS-MD nanoparticles showed higher value than heated at 40°C and 50°C. The pH value CS-MD nanoparticles increased with increasing CS concentrations in liquid smoke. This phenomenon was probably caused by initial pH of CS, MD and TPP were higher than pH of liquid smoke i. e. around pH neutral 7.

Viscosity of the solution containing nanoparticles

As shown in Table 1, the increase in CS concentration in the liquid smoke tended to increase the viscosity. This indicates that the rheological

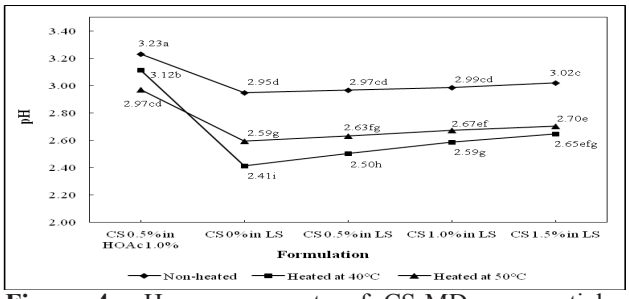

Figure 4. pH measurements of CS-MD nanoparticles in formulations at various temperatures. Figures in the graph followed by the same letter indicating no significant difference at $p < 0.05$.

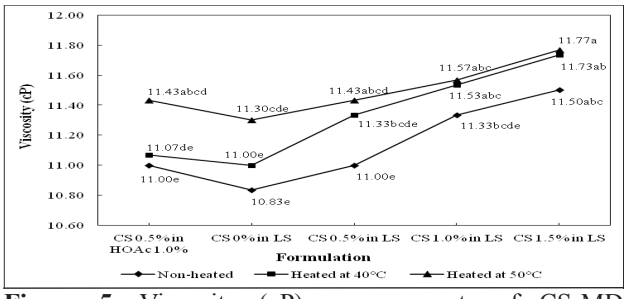

Figure 5. Viscosity (cP) measurements of CS-MD nanoparticles in formulations at various temperatures. Figures in the graph followed by the same letter indicating no significant difference at $p < 0.05$

properties of CD-MD nanoparticle were also notably influenced by other factors, apart from the disperse phase volume fraction, such as the electrical net charge of the particles. The results were in accordance with those of the earlier report of Mucha (1997), that the shear stress and viscosity of chitosan solutions increased with increasing chitosan concentrations, corresponding to a progressive increase of entanglements between the macromolecular chains. The lower electrical charge (lower zeta potential) measured in 0.5% CH could explain the reported decrease in its apparent viscosity in comparison with 1.5% CH.

The inreased viscosity with increasing zeta potential and it becomes highest for formulation 1.5% CS as illustrated in Figure 5. The observed decrease in viscosity of the liquid smoke solution without CS addition, in consequence, the lower hydrodynamic size of macromolecules. The reduced viscosity for the system containing acetic acid is consistent with the data obtained for the solvents containing stoichiometric amount of acetic acid in relation to chitosan and TPP. The maximal concentration of 1.5% CS combined with MD showed the highest stability at all heat treatment temperatures used i.e 40 and 50^{o}C. MD is used in acetic acid and liquid smoke solution as texture modifiers to modify viscosity or gelation of the formulations. Moreover, for this work, MD with DE 10.8 was used, which has a higher percentage of long oligosaccharide chains that can form strong network structures within the continuous phase to hold the particle in place. Therefor, it can combine with CS which affects viscosity to result in higher solution stability. The viscosity of the suspension containing CS-MD nanoparticles was increased after heat treatment, coherently with the particle size reduction and due to the increase in the chitosan adsorption on the surface, in agreement with the growth of the interfacial area in the system.

Conclusion

In summary, CS-MD nanoparticles in liquid smoke have been synthesized and characterized in the present study. The nanoparticles obtained in the present study showed small particle size, spherical shape, and surface charges ranging from negative to positive, which may improve their stability in the presence of chitosan as a biological cation. The heat treatment at various temperatures in liquid smoke demonstrated increasing value with the increase of temperature for all parameters, except the particle size of the smallest yield.

Acknowledgements

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