Colegio de
Postgraduados

Synthesis of silica chitosan oligosaccharides nanoparticles

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ABSTRACT

Objective: To obtain chitosan oligosaccharides (COS) and evaluate COS uses for the obtention of nanosystem based on silica as vehicle and compare the COS-silica nanosystem with the chitosan (Chi) precursor system as Chitosan-silica nanosystems.

Design/methodology/approach: A combination of hydrolysis chemical and mechanical (microwave assisted) were used to obtain COS with the oxidative action of hydrogen peroxide. Sol-Gel adapted method was used to synthetize silica nanoparticles (SiNPs) from sodium metasilicate and the electrostatic interactions between SiNPs and Chi/COS were used to functionalize the SiNPs surface with Chi/COS.

Results: Nanosystem composed from COS and SiNPs were obtained successful as A COS-SiNPs and C COS-SiNPs with particle size of 139.35 nm and 251.8 nm and zeta potential of 30.40 mV and 34.67 mV respectively with antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*.

Limitations on study/implications: Stabilize the systems compound of chitosan-silica nanoparticles due to the molecular weight of chitosan which loss the stabilized the SiNPs suspension and due the incompatibility of both systems pH.

Findings/conclusions: COS and COS-SiNPs stable systems were obtained with an improvement of the antimicrobial activity of the system in contrast of Chi-SiNPs systems.

Keywords: chitosan, silicon, Sol-Gel. Chemical hydrolysis, mechanical hydrolysis, microwave assisted.

INTRODUCTION

Chitosan is considered as the second most abundant polysaccharide followed by cellulose; chitosan is a biodegradable biopolymer derived from chitin by a deacetylation process. Chitin/Chitosan can be obtained from crustaceous exoskeleton, insects, algae, and from the cell wall of some fungus. Chitin is deacetylated and depolymerized to obtain chitosan in alkaline conditions where acetyl groups are delinked from the saccharide chain, thus reducing the affinity of the polysaccharide to stay attached making chitosan more water soluble and less viscous [1]. Chitin and chitosan are compounded from the same saccharide monomers whit the main difference in the proportion of the units in the polysaccharide structure, chitin is mostly compound with N-acetyl-D-glucosamine (GlcNAc) with a portion of D-glucosamine units (GlcN) and the saccharide units are joined by a β 1-4 glycosidic bond. In contrast, chitosan is mainly compound by GlcN units with a about 10% content of GlcNAc, the ration between GlcN and GlcNAc units defines the deacetylated grade of chitosan [2-4].

Furthermore, chitosan biodegradability other properties have been studied the last decades, as chitosan biocompatibility, low toxicity, antimicrobial effect due their amine groups ($-NH_2$), also chitosan have been studied in agricultural fields due their activity as elicitor in plant defense mechanisms hence chitosan can act as antimicrobial biopolymer coating which activates some defense mechanisms in plants as ethylene and salicylic acid pathways, two of the most important signaling events involving in the resistance of some plant-pathogen interactions as coffea species with *Hemileia vastatrix* (biotrophy pathogen cause coffee leaf rust disease) [5, 6].

The main properties who define the behave and applicability of chitosan are the deacetyl grade, the molecular weight, the availability of amine groups (NH_2) and the zeta potential due the electrostatic charge difference between the biopolymer and the pathogens cell wall [2, 3]. The chitosan can be categorized in function of their molecular weight (Mw) as low molecular weight when Mw<100 kDa, medium molecular weight when Mw<100-1000 kDa and high molecular weight when >1,000 kDa [4].

Besides the widely chitosan applications have inconvenient to scale and improve applications in nanosystems, chitosan solution usually shows high viscosity values and low water solubility in absent of acid environments which reduce their viability to design nanosystems with chitosan, especially if the vehicle particle has more stability in alkaline environments as silica nanoparticles obtained from sodium metasilicate [4, 7].

In contrast chitosan oligosaccharides (COS) have low viscosity values related to their low molecular weight lower than 3.9 kDa and their low polymerization grade, as the COS are obtain from Chitosan by the glycosidic bond hydrolysis, the COS conserve the saccharide units GlcN and GlcNAc which conform chitosan chain but in less length chain and with more functional groups available who are related to COS more water solubility in neutral pH solution with less viscosity, the hydrolysis of glycosidic bond results saccharide fragments of the original chain, at the breaking sites one fragment results in hydroxyl group and the other in aldehyde group [4, 8].

COS can be obtained by chemical, mechanical or enzymatic hydrolysis. Chemical hydrolysis can be divided into acid and oxidative hydrolysis. In acid hydrolysis high concentrations of acids is used where the break of glycosidic bond starts in the protonation of oxygen in glycosidic bonds followed by the group reduction by the addition of water molecule which results in the decomposition of the glycosidic [4, 9, 10]. The oxidative hydrolysis is bases in the uses of hydrogen peroxide to produced hydroxyl radicals due H_2O_2 high instability and attack the glycosidic linkages of chitosan [4, 11].

Mechanical hydrolysis can be combined with acid or oxidative hydrolyses to accelerate the reaction. Mechanical methods can be found microwave and gamma radiation assisted methods where the microwave assisted hydrolysis is less efficient in contrast to gamma radiation but significantly reduces their cost operation, environment impact and scalability. Microwave assisted operates in two ways can generate shear stress and thermal degradation of the polysaccharide [4, 9, 12].

Nanomaterials has gained research attention recently due their wide application in several industries due their behavior and activity change at nanoscale in contrast of their bulk material properties and due it versatility to design more nanomaterials to

obtain different morphologies, arrangements or surface modification [13-15], especially in agronomy has ben used to control plant pathogens as insects *e.g.* to control *Tribolium castaneum* with copper nanoparticles (CuNPs) [16-18], and other diseases as Coffee Leaf Disease (CLF) caused by *Hemilea vastatrix* [5], additionally, nanomaterials has been used un food packaging to improve food quality [19], and to improve seed germination [20-22].

SiNPs has special attention due its versatility to obtained in different shape, arrangement and surface chemistry, SiNPs can be obtain from several methods and silicon sources, the mainly used methods are based on Sol-Gel synthesis as the main bottom-up method used due its facilities to control the surface particle chemistry, shape, surface charge and particle size and usually silicon metasilicate or tetraethyl orthosilicate are used as silicon source [7, 13, 19, 23, 24].

MATERIALS AND METHODS.
Silica nanoparticles synthesis

Silica nanoparticles (SiNPs) were synthetized as we report previously by Sol-Gel method [7] sodium metasilicate 0.3M solution distilled water (Metso pentabead 20[®], commercial grade) was used as silicon source, the sodium metasilicate solution was filtered with ion-exchange resin to decrease the sodium ions in the solution and reduce the pH from 14 to 10, followed 5 ml of ethanol (Fermont[®], analytic grade) was added to catalyzed the condensation of siloxane bonds and leave 15 min in low stirring. The previous solution was homogenized in 2,000 rpm for 30 min with PEGlynated silicon surfactant with methoxy terminal groups (Silwet[®] L-77, Momentive) to improve micelle formation, finally the obtained suspension was treated in 105 °C reflux for 30 min to improve stability to the suspension.

Silica-chitosan nanoparticles synthesis

Low molecular weight chitosan (Sigma-aldrich[®]) was dissolved at 3 wt% in previously prepared acetic acid (Fermont[®], analytic grade) 1% in distilled water at 70° in vigorous stirring until the chitosan was completely dissolved. To synthetize the silica-chitosan nanoparticles (SiChiNPs) the previously chitosan solution was added into the homogenization step described above with the treated sodium metasilicate and PEGlynated silicon surfactant.

Silica-chitosan oligosaccharides nanoparticles synthesis

To synthetize silica-chitosan oligosaccharides nanoparticles (SiCOSNPs) the previously synthetized SiNPs was dispersed in distilled water at 0.5% with slow stirring, followed obtained COS was added in two different final concentrations 0.5% and 1% and let in agitation for 1 h, the obtained suspensions were labeled as ASiCOSNPs and CSiCOSNPs respectively.

Characterization

The particle size and zeta potential were obtained in particle size analyzer Litesizer 500 (Anton Paar[®]). The IR spectrum was obtained in ATR-FTIR 4300 (Agilent[®]), the samples were dried above the ATR to avoid the water molecules interference. The viscosity

was obtained at 25 °C by viscosimeter VISCO QC 100 (Anton Paar®). The inhibitory effect of obtained nanoparticles was analyzed by antibiogram method in disposable Petri dishes with previously prepared Mueller-Hinton agar (BD Bioxon®) against *Escherichia coli* ATCC® 25922 and *Staphylococcus aureus* ATCC® 25923.

RESULTS AND DISCUSSION

Viscosity

The viscosity measurements (Table 1) show a significative decrement from the original chitosan solution when the hydrogen peroxide (Table 1. COS H_2O_2) was added from 41.60 P to 3.2 P and more decrement after the microwave treatment (Table 1. COS H_2O_{2-m}) to 32.39 cP. The viscosity decrement can be related to the reduction of molecular weight of the chitosan and the results shows after the microwave treatment confirm the synergic degradation by the combination of chemical and mechanical hydrolysis to obtain chitosan oligosaccharides [8, 10-12].

Fourier-transform infrared spectroscopy (FTIR)

The IR spectrum show similar behave between the dried chitosan spectrum (chi s) and the dried chitosan oligosaccharide spectrum (cos s) with the main difference of the cos s spectrum shows more definition in it peaks spectrum, which according with [25] this can be related to the increase of available functional groups due the glycosidic bond break. Both spectrums start at 650 cm^{-1} regions with stretching of C-O-C and C-O-H bonds at 656.7 cm^{-1} and 652.2 cm^{-1} for chi s and cos s respectively which can be related to the breaking of glycosidic bonds and protonation of the CO$^-$ groups [25].

Both spectrums show well define peaks at 887.1 cm^{-1} (chi s) and 890.8 cm^{-1} (cos s) with similar transmittance which with the peaks at 1012-1016 cm^{-1}, 1052-1060 cm^{-1}, and 1152 cm^{-1} are related with the habitual behave of saccharide structure [26, 27]. The peaks at 1258-1262 cm^{-1} and 1374-1377 cm^{-1} can be related with the presence of amide III associated with GlcNAc units [1, 26-29], the peaks at 1403-1420 cm^{-1} are related to bend vibration of O-H and C-H bonds [1, 26, 27, 30], the presence of peaks around 1531-1539 cm^{-1} are related to vibrations of amide II and C-OH bonds [1, 26, 27], the peaks in 1632-1634 shows the presence of amide I and the peaks around 1700-1702 cm^{-1} are related with carboxyl group vibrations and the peaks at 2866-3069 are related to O-H and C-H bond vibrations [1, 26, 27].

Table 1. Dynamic viscosity related to COS obtention from chitosan.

Sample	Viscosity (Pa)
Chitosan	41.6
COS H_2O_2	3.2
COS H_2O_{2-m}	0.3239

COS H_2O_2 are for COS obtained by oxidative hydrolysis with hydrogen peroxide and COS H_2O_{2-m} for COS obtained by oxidative and mechanical hydrolysis.

The nanoparticles systems synthetized with silica nanoparticles do not show significant differences with the previously discussed spectrums which can be due to a signal overlap between similar bond energy. The siloxane bonds (Si-O-Si) could be overlapped by the glycosidic bond in the saccharide (C-O-C) at 467-586 cm^{-1} respectively and in 1000-1062 cm^{-1} peaks, the peak at 892 cm^{-1} also can be attributed to the bonds Si-C and NH$_2$, the peaks at 2000-2332 cm^{-1} can be related to S-H or C=O bonds [7, 25, 31].

Dynamic Light Scattering Analysis

Dynamic Light Scattering Analysis shows an increment in size of nanoparticles silica (Figure 2. A) well defined and monodispersed histogram presents a particle size of 6.74 nm with zeta potential (ζ) of -32.80 mV related to deprotonated hydroxyl groups rich in the surface of SiNPs. The first A-Si-COSNPs (Figure 2. B) shows an increment in particle size to 251.8 nm with $\zeta=30.40$ mV which reflects the formation of coat made of COS at the surface of SiNPs. Similar results are shown by C-Si-COSNPs (Figure 2. C) with particle size increment to 139.35 nm and $\zeta=34.67$ mV. By DLS results the nanosystem synthetized with 0.5% of COS (C-Si-COSNPs) can be correlated to more effective and stable nanosystem thus related to the increment of potential zeta in 4.27 mV which reflects more repulsive forces between particles and the smaller particle size (139.35 nm) improve the efficiency of the nanosystem. Additionally, the nanosystem C-Si-COSNPs shows a distribution of size particles more related to monomodal nanoparticles system. The DLS results in Chi-SiNPs systems were discarded due to the trend of the system to precipitate due to molecular weight and incompatible pH regions of chitosan an SiNPs [7].
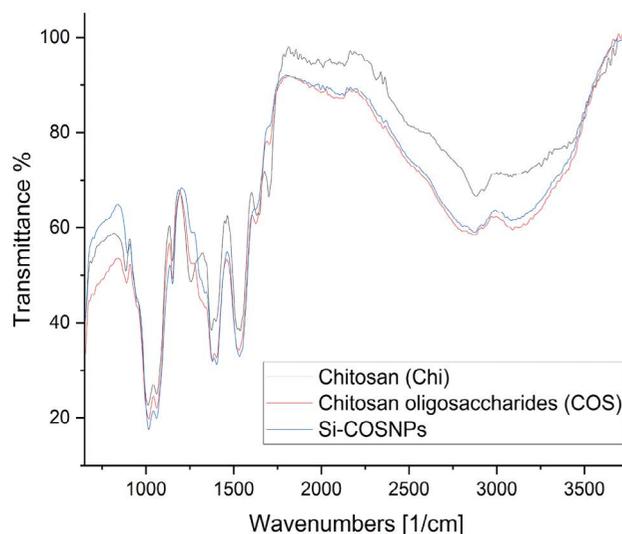

Figure 1. ATR-IR spectrums for Chitosan (Chi), Chitosan oligosaccharides (COS) and Silica-Chitosan Oligosaccharides Nanoparticles (Si-COSNPs).
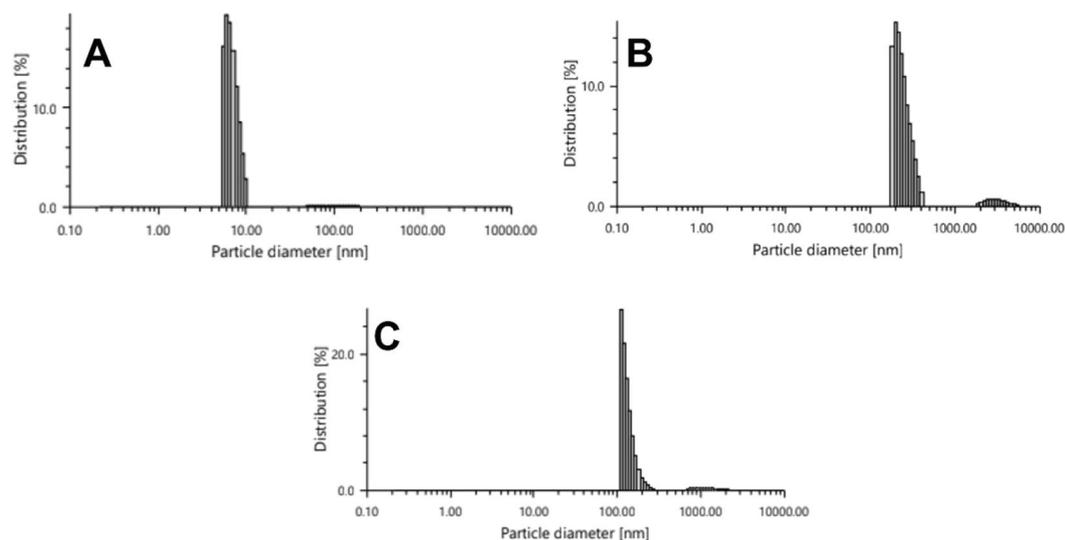
Figure 2. Particle size histogram for SiNPs (A), A-Si-COSNPs (B) and C-Si-COSNPs (C).

Inhibitory efficacy - antibiograms

The obtained chitosan oligosaccharides show inhibitory diameter significant bigger at the 24 and 48 h after the inoculation with *E. coli* and *S. aureus* then the chitosan solution and then silica nanoparticles (Table 2), the silica chitosan oligosaccharide nanosystems (A and C) also show significant inhibitory efficacy in contrast of the silica chitosan nanosystem. The improvement of antimicrobial activity of COS and COS-SiNPS can be related to the increment of amine and amide groups available after hydrolysis of glycosidic bonds, especially due to the increase of primary amino groups [32]. Also, pristine silica nanoparticles show zero-antimicrobial activity as was expected due SiNPs surface negative charge, in contrast, due the positive surface charge shows by COS and COS-SiNPs can be attracted and bonded to bacterial cell wall with negative charge due the carboxylic acid groups and this interaction can makes an impermeable coat of COS or COS-SiNPs blocking the nutrient uptakes and leading to the cell death [32, 33].

Table 2. Antibiogram results of tried nanosystems and precursors against *Escherichia coli* and *Staphylococcus aureus*.

Sample	*Escherichia coli*		*Staphylococcus aureus*	
	24 h (mm)	**48 h (mm)**	**24 h (mm)**	**48 h (mm)**
SiNPs	0	0	0	0
Chitosan	1.9	1.4	1.9	1.4
COS	2.8	2.6	2.8	2.6
SiNPs-COS A	1.9	1.8	1.9	1.8
SiNPs-COS C	1.7	1.6	1.7	1.6
SiNPs-Chi	0	0	0	0

CONCLUSIONS

A stable silica chitosan oligosaccharide nanosystem was successfully obtained with antimicrobial activity against *E. coli* and *S. aureus* by the synthesis of COS from low molecular weight chitosan by chemical and mechanical hydrolysis with hydrogen peroxide and 700W microwave. The obtained nanosystems maintain their main chemical composition characteristic of chitosan with more availability of functional groups due the breaking of glycosidic bond during the COS synthesis. The obtained COS-SiNPs shown a particle size 139.35 nm and $\zeta = 34.67$ mV with growth inhibitory diameter of 1.9 mm and 1.8 mm against *E. coli* 24 h and 48 h the inoculation, and 1.9 mm and 1.8 mm against *S. aureus* 24 h and 48 h the inoculation.

ACKNOWLEDGEMENTS

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