Scientific paper

New Type of Chitosan/2-hydroxypropyl-β-cyclodextrin Composite Membrane for Gallic Acid Encapsulation and Controlled Release

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Abstract

A new type of chitosan/2-hydroxypropyl-β-cyclodextrin composite membrane have been developed for the encapsulation and controlled release of gallic acid. The morphology of the composite membrane was investigated by infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM), whereas swelling gallic acid and release properties were investigated by UV-visible spectroscopy. The release behavior with pH changes was also explored. The composite membrane based on chitosan/2-hydroxypropyl-β-cyclodextrin with gallic acid included showed improved antioxidant capacities compared to plain chitosan membrane.

The information obtained in this study will facilitate the design and preparation of composite membrane based on chitosan and could open a wide range of applications, particularly its use as an antioxidant in food, food packaging, biomedical (biodegradable soft porous scaffolds for enhance the surrounding tissue regeneration), pharmaceutical and cosmetics industries.

Keywords: Composite membrane, chitosan/2-hydroxypropyl-β-cyclodextrin/ gallic acid

1. Introduction

A large number of plant extracts and their constituents, already employed in the food industry, have been adapted to serve as major active ingredients in both cosmetic and health products. However, the effectiveness of these active compounds deepens on preservation of their stability, bioactivity and bioavailability. Therefore, new approaches have been developed in order to overcome these drawbacks. Gallic acid, a natural phenolic antioxidant extractable from medicinal plant and culinary herbs, has several biochemical properties such as: anti-inflammatory and antimicrobial agent,[1] as antioxidant,[2] and an anti-hyperglycemic agent;[3] it prevents also the oxidative stress.[4]

The synthesis of new polymeric membrane by grafting the antioxidant molecules on macromolecules with biodegradability, bio-compatibility and bio-activity has lately focused major interest in research.[5,6] These molecules have a hydrophilic exterior (due to the hydroxyl groups) and a hydrophobic inner cavity, which can encapsulate hydrophobic molecules or moieties.[7,8] It therefore becomes more bio-available (by increasing the active species solubility in the water phase) and proving also controlled release properties.[9] Cyclodextrins are inexpensive, friendly to humans, and also capable of improving the biological, chemical and physical properties of bioactive molecules.[10] Plants polyphenolic compounds have restricted application as pharmaceutical products since they have limited water solubility, poor bioavailability, and can be easily modified by environmental factors such as temperature, pH and light. Therefore, in order to preserve bioactive molecules structural integrity, they need to be protected by a finishing formulation with the capacity to deliver them to the physiological targets without losing any bioactivity. Recently, several papers have been published on the complexation of CDs with antioxidants, such as quercetin,[11–13] morin,[13,14] rutin,[15,16] chlorogenic acid,[17,18] resveratrol,[19]

isoquercitrin,[20] and kaempferol.[11,12] In practice, cyclodextrin derivatives such as hydroxypropyl-β-cyclodextrin are preferred to natural cyclodextrins for their increased water-solubility and for their better bio-compatibility profile.

In recent years many reports proposed that appropriate macromolecular systems, namely antioxidant-polymer conjugates, could combine the merits of polymer and natural antioxidants. This is due to their capability to retain the antioxidant molecule's biological activities and to reduce the degradation rate, as compared to the antioxidant molecules.[5,6,21] Chitosan, abundant natural polysaccharide, has already been well studied and sufficiently reported that chitosan is non-toxic, biodegradable and biocompatible.[22] This polymer has great potential for applications in the pharmaceutical industry, as a lipophilic encapsulation for drugs[23] and in the food industry, as an encapsulation for probiotics and prebiotics,[24] aromatic compounds,[25] enzymes[26] and antioxidants.[27,28]

To design some more advanced and controllable antioxidants carriers the present work focuses on development of the new composite membrane based on chitosan/2-hydroxypropyl-β-cyclodextrin (HP-β-CD) with gallic acid, as model compound, included. The complex of gallic acid with 2-hydroxypropyl-β-cyclodextrin/chitosan composite membrane was evaluated by Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and UV–visible spectroscopy (UV–Vis).

2. Experimental

2. 1. Materials

Chitosan medium molecular weight (Aldrich), polyvinyl pyrrolidone K90 (PVP; Fluka), 2-hydroxypropil-β-cyclodextrin (HP-β-CD) (Aldrich), gallic acid (GA) (Fluka) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Aldrich) were used in this work. All other chemicals used were analytical grade.

2. 2. Composite Membrane Preparation

Chitosan/2-hydroxypropil-β-cyclodextrin-gallic acid membranes (CH/HP-β-CD-GA) were manufactured using a solvent casting technique.[29] First, the complex mixture (2-hydroxypropil-β-cyclodextrin-gallic acid) was prepared at a 1:1 molar ratio, according to procedure reported by Chikuno and Terao, with minor modifications.[30] Briefly, HP-β-CD (0.05 M) and GA (0.05 M) were dissolved in 5 mL of water and the mixture was stirred at 50 °C for 8 hrs. Then the solution was filtered to remove the gallic acid which had not reacted. After drying, a white powdered product of the complex was obtained, 1.5% (w/v) chitosan solution was prepared by dissolving certain chitosan powder in 2% acetic acid, 1% PVP and 0.8% complex HP-β-CD-GA. The chitosan membranes were obtained by adding 1% of glycerol in chitosan solution. The solution was stirred at the ambient temperature for 12 hrs and then filtered. The homogenized mixture was cast onto a Petri dish, which was placed for 24 hrs in the oven, at 60 °C. Finally, the membrane was successively washed with 1.0 M aqueous NaOH solution and deionized water. The prepared CH/HP-β-CD-GA composite membrane was dried at 50 °C overnight.

Previous research suggested the molecular encapsulation of phenolic acid in cyclodextrin cavity by replacing of crystallisation water molecules with the more hydrophobic guest compounds[31].

2. 3. Characterization of CH/HP-β-CD-GA Composite Membrane

FT-IR spectra were obtained with a Tensor 27–Bruker spectrometer equipped with a source for mid infrared (4000–400 cm^{-1}), KBr beam splitter and RT–DLaTGS detector. FT-IR analyses were performed in transmittance mode with following operational parameters: resolution of 4 cm^{-1}; co-added scans 96; 4 mm aperture and 20 kHz scanner velocity. The opus 6 software was used to record and process the spectra.

The morphological characterization of the composite membranes was obtained by scanning electron microscopy (JEOL JSM-7500F, Japan). The dried membrane specimen was covered with sputtered gold before scanning.

2. 4. Antioxidant Activity: DPPH (2,2'-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The free radical scavenging activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to the modified method of previous previous studies.[32,33] To this end, dry films (25 mg) were previously placed in 30 mL of 0.1 M sodium phosphate buffer and maintained under magnetic stirring for 12 h at 25 °C, and then a 100 μL of film extract solution were mixed of this solution were mixed with 1mL of DPPH solution (0.25 mM) and 1.9 mL methanol. The decreasing of the DPPH radical absorption at 516 nm by the action of antioxidants could be used for measuring the antioxidative activity. The antioxidant activity (radical scavenging activity) was calculated using the expression:

$$\% \text{ inhibition} = [(A_0 - A_s)/A_0] \cdot 100 \quad (1)$$

where: A_0 = blank absorbance; A_s = sample absorbance.

2. 5. Swelling Studies

The dried composite membrane was immersed in phosphate buffer solution of pH 3, pH 7.5, pH 8.5 and pH 9 at ambient temperature. After predetermined time, the samples were removed and the weights (w_s) were measured. The degree of swelling (S, %) was then calculated on

the basis of the weight of swollen and dry membrane using the following equation.

$$s = \frac{w_s - w_d}{w_d} \times 100 \quad (2)$$

where w_d is the weight of the dry sample and w_s is the weight of sample at time t (after immersion in the aqueous medium).

2. 6. In Vitro–release Studies

Gallic acid released from CH/HP-β-CD-GA composite membrane was quantified over time by an in vitro release assay. 25 mg of each composite membrane type were placed in containers containing 30 mL of 0.1 M sodium phosphate buffer (PBS), pH 3÷9, at 22 °C. At designated time points (1, 2, 3, 4, and 7 days), 3 mL aliquots of the release medium were sampled and the same amount of fresh distilled water was added into the containers. In the collected fractions, the cumulative amount of gallic acid released as a function of time was determined by UV spectrophotometer (Thermo Scientific Evolution 260 Bio UV–Vis) at 260 nm.

Statistical Analysis: The measurements were performed in triplicate and for statistical processing Excel 2007 was used, standard deviation (STDV) was < 10%.

3. Results and Discussion

3. 1. Characterization of CH/HP-β-CD-GA Composite Membrane

FT-IR spectroscopy was used to confirm changes in chemical structure of the membrane prepared using the developed methodology, since changes in covalent bonds and chemical interactions can be tracked by modifications in characteristic spectroscopic signals. Figure 1 shows the FT-IR spectra of CH membrane (a), CH/HP-β-CD (b) and CH/HP-β-CD-GA (c) composite membrane.

Fig. 1a displays the FT-IR pattern characteristic of the CH membrane. The broad band due to the stretching vibration of $–NH_2$ and –OH groups can be observed at 3400–3500 cm^{-1}. The bands assigned to stretching vibration of C=O and deformation vibrations of N-H from carboxamide (OC–NHR) groups appeared at 1660 cm^{-1} and 1493 cm^{-1} respectively in pure chitosan spectrum and were preserved after membrane modification. Bands at 2881 cm^{-1}, 1424 cm^{-1}, 1320 cm^{-1} were assigned to CH_2 vibrations of carbohydrate ring and bands at 1291 cm^{-1} were assigned to tertiary amine C-N stretching from PVP. The band located near = 1152 cm^{-1} is related to asymmetric vibrations of CO in the oxygen bridge resulting from deacetylation of chitosan. Absorption bands at 1152 cm^{-1}, 1082 cm^{-1} and 1031 cm^{-1} (skeletal vibrations involving the C–O stretching vibrations) are characteristic of chitosan saccharide structure.[34] The band assigned to C–N vibration appears in fingerprint region at 896 cm^{-1}. The characteristic bands of CH membrane (Fig.1a) have been appeared in the spectra of CH/HP-β-CD (Fig.1b) and CH/HP-β-CD-GA (Fig.1c) composite membrane. The FT-IR spectra of the composite membrane and of the inclusion complex are similar to that of CH membrane, due to the low quantity of HP-β-CD and GA included in the system. The larger differences can clearly be observed between composite membrane with gallic acid (CH/HP-β-CD-GA) (Fig. 1c) and those without gallic acid (Fig. 1a and 1b), with the main spectral differences at 1260–1500 cm^{-1} and 800–850 cm^{-1}. These bands can be assigned to the presence of the gallic acid aromatic ring, since -OH in plane bending of phenolic ring can be attributed to 1365 cm^{-1}, the aromatic ring CC stretching is

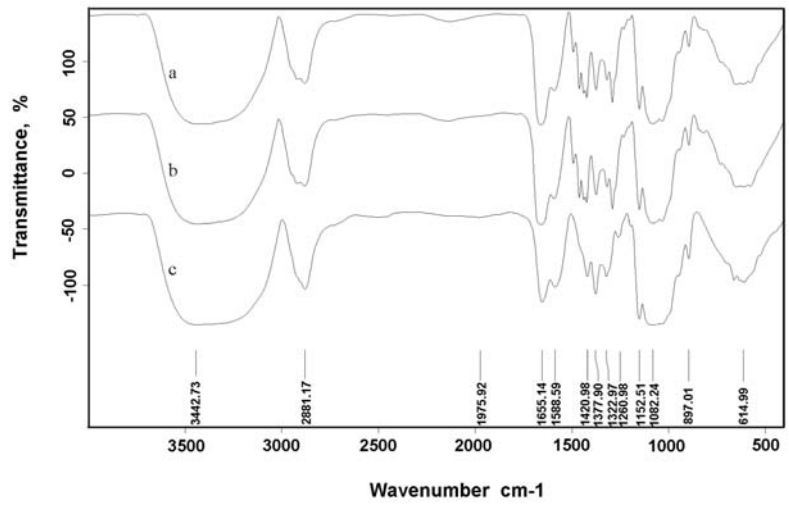

Fig. 1. FT-IR spectra of (a) CH membrane, (b) CH/HP-β-CD and (c) CH/HP-β-CD-GA composite membranes

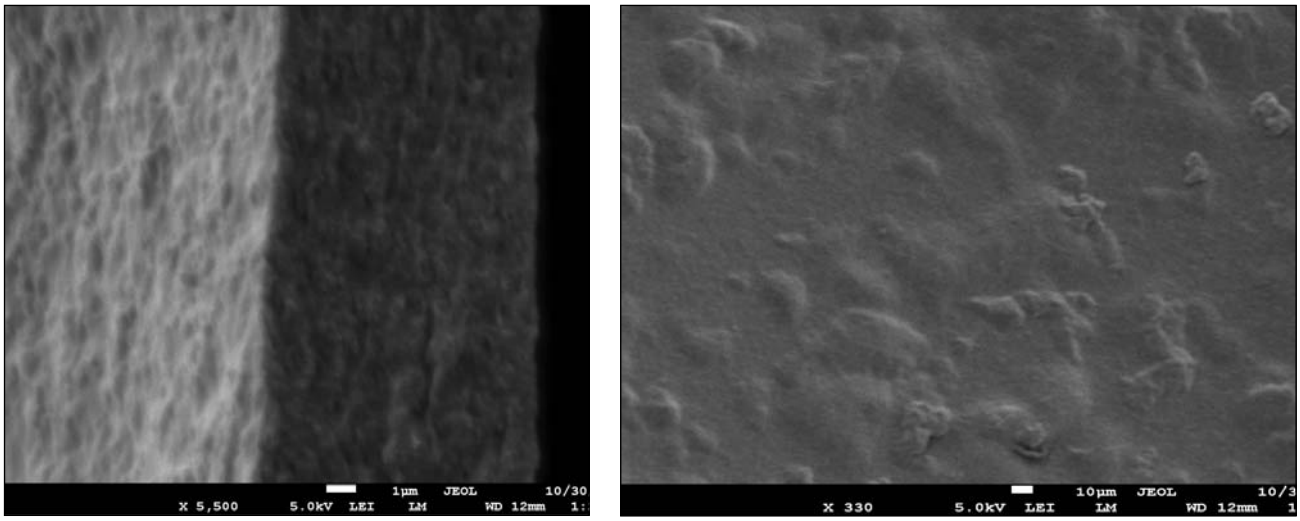

Fig. 2. Morphology of CH/HP-β-CD composite membrane composite membranes from SEM

within the 1450–1600 cm^{-1}, CO/CC stretching vibrations are within the 1200–1300 cm^{-1}, and the CH bending vibration is between 800 and 900 cm^{-1}. The band assigned to the ν_{NCO} stretching vibrations was shifted to higher wave numbers (from 2134 in CH/HP-β-CD to 2199 cm^{-1} in CH/HP-β-CD-GA).[35]

Fig. 2 shows the SEM images of CH/HP-β-CD composite membrane. SEM analysis of the cross-section and top surface of these composite membranes provides information about the membrane thickness, density and homogeneous structure. The HP-β-CD-GA loading in chitosan solution is somewhat not well dispersed and shows the agglomeration in the composite membrane.

3. 2. Antioxidant Activity

The DPPH radical scavenging percentage of samples was determined by the decrease in absorbance at 516 nm. Trolox and ascorbic acid were used as positive control. Compared to chitosan, composite membrane with gallic acid showed increased radical scavenging of up to 38.6% (Figure 3), which is probably related to the intermolecular hydrogen bonds between GA and HP-β- CD.[36] However, the increased free radical scavenging capacity of the gallic acid-loaded CH/HP-β-CD is in direct correlated to the high antioxidant activity of incorporated gallic acid. Similar results are also reported by other researchers.[5,6,37]

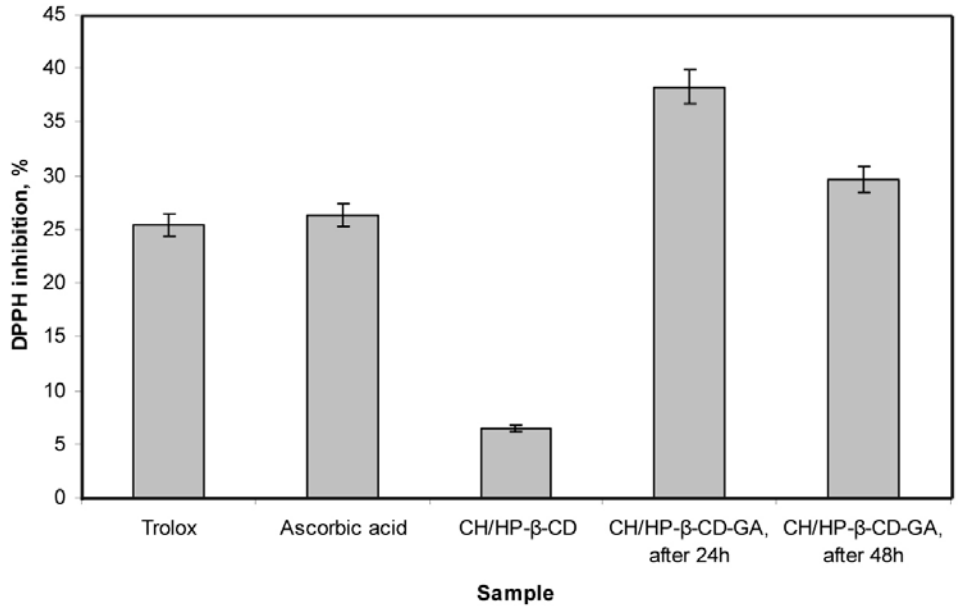

Fig. 3. DPPH radical scavenging capacity of CH/ HP-β-CD composite membrane and the complex of CH/HP-β-CD - GA after 24 h and 48 h

3. 3. Swelling Studies

Swelling studies are important to understand antioxidant release characteristics through CH/ HP-β-CD composite membrane. The pH of swelling medium has a significant effect on water uptake of these membranes. As can be noted from figure 4, the swelling values attained at equilibrium by the composite membrane were found to be higher at pH 3 than at pH > 7.5.

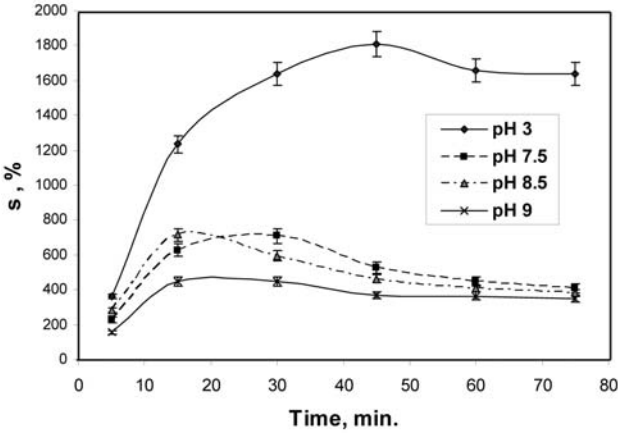

Fig. 4. *In vitro* swelling studies of CH/ HP-β-CD composite membrane in different buffer pH.

This can be explained by protonation of the unreacted NH_2 groups of chitosan at acidic pH, leading to dissociation of the hydrogen bonding involving the amino groups, and consequent facilitation of the entrance of solvent into the material.

The swelling values at pH > 7.5 will be lower than the values at pH 3 due to the increased hydrophobicity of the chitosan/2-hydroxypropyl-β-cyclodextrin membranes at higher pH values, thus preventing faster swelling in neutral and alkaline media.[38]

3. 4. Spectroscopic Studies of Complexes of CH/HP-β-CD-GA. In Vitro Release Studies

The absorbance of HP-β-CD-GA complex at 210 nm and 260 nm increased with the HP-β-CD concentration (Fig. 5).

Based on our previous effected experiments, only the 5.3×10^{-5}M HP-β-CD with entrapped GA was investigated for release. In order to determine the ability of composite membrane to release GA under in vitro conditions, GA released from the composite membrane was measured as a function of time and at different pH values (Fig. 6).

The GA release experiments have been performed in simulated gastric fluid of pH 3, intestinal fluid of pH 7.5,

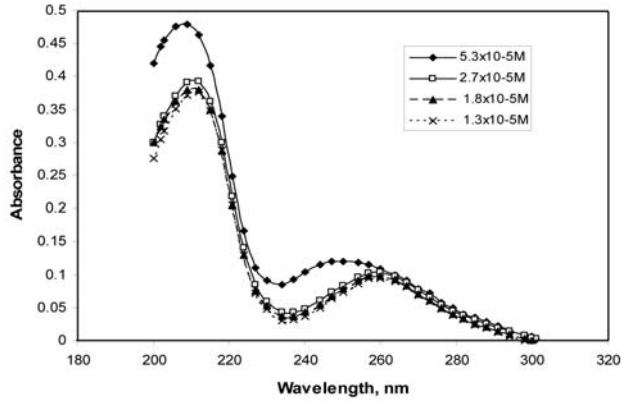

Fig. 5. Absorption spectra of GA in different concentrations of HP-β-CD. The concentration of HP-β-CD: 1.5–5.3 × 10^{-5} M (c_{GA} = 5 mg/L).

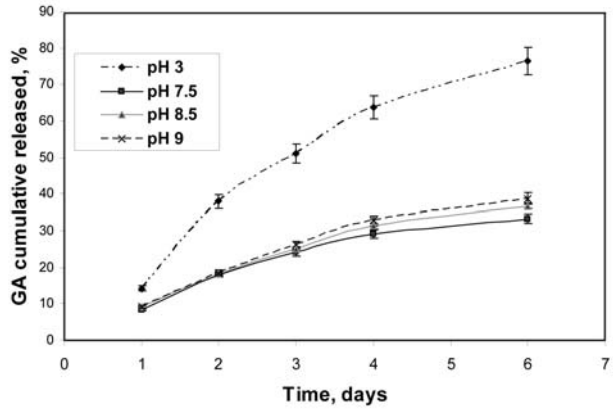

Fig. 6. In vitro cumulative amount of the gallic acid released from the CH/HP-β-CD – GA composite membrane, at pH = 3, 7.5, 8.5 and 9.

8.5 and 9. The percentage of cumulative amount of released GA was determined from standard calibration curves. Controlled release of GA was dependent on pH value of the media conditions, with slower release kinetics at higher pH values (7.5, 8.5 and 9). After 6 days, 35% of GA is released in pH 7.5, 8.5 and 9 buffers, while during the same period at pH 3 the released GA is about 76%. The higher rate of the released GA was attributed to amino groups of chitosan protonation, charged polysaccharide giving faster and more swelling in acidic medium. Tapia et al. showed that, chitosan-alginate complex erodes slowly in phosphate buffer at pH higher than 6.5 and this behavior leads to suppression of the drug release.[39]

4. Conclusion

Composite membranes based on chitosan/2-hydroxypropyl-β-cyclodextrin (HP-β-CD) with gallic acid included were prepared for the first time. The mem-

branes were characterized by SEM and FT-IR. The composite membranes can deliver gallic acid with a sustained slow release rate at basic pH values (greater than 7). The DPPH radical scavenging ability of GA incorporated in CH/HP-β-CD composite membrane increased, as compared to CH/HP-β-CD composite membrane and exhibited long-acting release properties.

The improved antioxidant property of this composite membrane with gallic acid could open a wide range of applications, particularly its use as an antioxidant in biomedical, pharmaceutical and cosmetics industries.

5. References

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Povzetek

Nova vrsta hitosan/2-hidroksipropil-β-ciklodekstrin kompozitne membrane je bila razvita za »zajetje« in kontrolirano sproščanje galne kisline. Morfologijo membrane smo preučevali z infrardečo spektroskopijo (FT-IR) in vrstičnim elektronskim mikroskopom (SEM). Preučevali smo tudi »nabrekanje« in mehanizem sproščanja galne kisline z UV-VIS spektroskopijo. Mehanizem sproščanja galne kisline pri različnih vrednostih pH je bil prav tako predmet raziskave. Antioksidativne lastnosti kompozitne membrane hitosan/2-hidroksipropil-β-ciklodekstrin z dodano galno kislino so izboljšane v primerjavi s samo hitosan membrano. Informacije, pridobljene v tej študiji, bodo olajšale načrtovanje in pripravo sestavljenih membran, ki temeljijo na hitosanu. S tem pa bi se lahko odprla cela paleta področij uporabe: antioksidanti v živilih, pakiranje hrane, biomedicina (npr. priprava biološko razgradljivih mehkih poroznih oblog za hitrejšo regeneracijo tkiv), farmacevtska in kozmetična industrija.