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Free Radical Scavenger Activity of Cinnamyl Chitosan Schiff Base

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ABSTRACT

In this study, new cinnamyl chitosan schiff base was evaluated as antioxidant material. Antioxidant activity was measured by two different popular methods (uninhibited/inhibited hyaluronan degradation and decolorization of ABTS methods). the results show decrease the hydrogen donation behavior of chitosan after coupling with cinnamaldehyde, in the other hand, ABTS method show increase the electron donation activity of cinnamyl chitosan than the chitosan itself.

INTRODUCTION

Oxidation stress of free radicals attracted the attention of scientists over last years. Role of these free radicals as primary cause of several diseases driving them to condense their studies to identify their origin, mechanisms of their action and of course the best ways to scavenge them and decrease their harmful. Phenolic components have a good scavenger activity against free radicals and forming with it a more stable intermediated that limited its risk (Huang *et al.*, 2011; Muchuweti *et al.*, 2007, Shan *et al.*, 2005). Chitosan is biocompatible, biodegradable, nontoxic renewable biopolymer produced by alkali treatment of chitin (the second most abundant natural polysaccharide next to cellulose). Chitin found in the composition of crustacean shells. Chitin consists of ß (1→4)-2-acetamido-2-deoxy-d-glucopyranose (GlcNAc) as a repeating unit. Deacetylation of chitin yields chitosan, which is actually a copolymer of GlcNAc and ß-(1→4)-2-amino-2-deoxy-d-glucopyranose with deacetylation greater than 60%. Chitosan was found in several applications including cosmetics, artificial skin, photography, food and nutrition, ophthalmology and wastewater treatment, wound healing (Kumar *et al.*, 2007; Shahidi *et al.*, 1999; Dodane *et al.*, 1998; Jeon *et al.*, 2000; Mohy eldin *et al.*, 2008; 2015a).

Presence of different functional groups along chitosan backbone (i.e.; hydroxyl and amine groups) simplifies its chemical modifications. Several derivatives of chitosan were prepared recently including methylation (Curti *et al.*, 2003), carboxylation (Chen and Park., 2003), sulfonation (Vongchan *et al.*, 2002), schiff base formation (Kenawy *et al.*, 2015; Mohy Eldin *et al.*, 2015b; Soliman *et al.*, 2013).

This study was performed to evaluate antioxidant properties of cinnamyl chitosan schiff base and comparing its activity to citosan itself.

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MATERIALS AND METHODS

Materials

Shrimp shells was collect from wastes of seafood restaurants in Alexandria - Egypt, acetic acid (purity 99.8%) and sodium hydroxide pellets (purity 99–100%,) were obtained from Sigma Aldrich (Germany). Cinnamaldehyde (Purity 98%) was obtained from Scharlau, (Spain). The high-molar-mass hyaluronan sample Lifecore P9710-2A, kindly donated by Lifecore Biomedical Inc., Chaska, MN, USA (Mr 808.7 kDa; Mr/Mn¼1.63), was used on experiments. The analytica purity grade NaCl and $CuCl_2 \cdot 2 H_2O$ (Slavus Ltd., SK-Bratislava); L-ascorbic acid and $K_2S_2O_8$ (p.a. purity, max 0.001% nitrogen; Merck, Germany); 2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid] (ABTS; purum, >99%; Fluka, Germany) were used.

Methods

Extraction of chitin from shrimp Shells

According to Islama, *et al.*, (2011), the process mainly involved the following steps: Demineralizations of shells. In this step, the shells were dispersed in 5% HCl at room temperature in the ratio of 1:14 (w/v). After 24 hours, the shells were quite squashy and were rinsed with water to remove acid and calcium chloride., The de-mineralized shells were then treated with 5% NaOH at room temperature for 24 hours in ratio of 12:1 (v/w). The residue was then collected and washed to neutrality in running tap water and then distilled water. The obtained product is chitin.

Preparation of chitosan from chitin

According to Rigby method (1936) preparation of chitosan is simply deacetylation of chitin in alkaline medium (figure 1). Removal of acetyl groups from the chitin was achieved by using 50% NaOH solution with a solid to solution ratio of 1:50 (w/v) at 100-120 ^oC for 12 hours. The resulting chitosan was washed to neutrality with distilled water.

Preparation of cinnamyl chitosan from chitosan

Cinnamyl chitosan schiff base was prepared according to our previous work (Mohy Eldin *et al.*, 2015b). Briefly, Previously purified chitosan (1g) was dissolved in 50 ml of 2% acetic acid and stirred at room temperature for 6 h, the resulting viscous solution was filtered through cloth cheese to remove undissolved particles and 10 ml of ethanol containing definite amount of cinnamaldehyde was added to solution under stirring to have viscous solution. This mixture was stirred for 6 h at 50 ^oC. The formation of a deep yellow gel refers to formation of the chitosan schiff base (figure 2). The resulting product was added to excess of 5% sodium hydroxide solution. The precipitate was filtered and washed with water and ethanol several times to remove un-reacted cinnamaldehyde, the product was filtered and dried in a vacuum oven at 60 ^oC overnight.

Antioxidant evaluation

ABTS method

For the ABTS decolorization assay, the radical cations were pre-formed by the reaction of an aq. soln. of $K_2S_2O_8$ (3.30 mg) in H_2O (5 ml) with ABTS (17.2 mg). The resulting bluish green radical cation soln. was stored overnight in the dark below 0 ^oC. Before experiment, the soln. (1 ml) was diluted into a final volume (60 ml) acetic acid solution (0.5%). chitosan (or aminated chitosan) solution (0.5%) stock solutions were prepared in acetic acid (0.5%). A modified ABTS assay (Rapta *et al.*, 2009) was used to test the radical-scavenging efficiency applying a UV-1800 spectrophotometer (SHIMADZU, Japan). The UV/VIS spectra were recorded in defined times, in 1-cm quartz UV cuvette after mixing of antioxidant solution (50 µl) with an ABTS. soln. (2 ml).

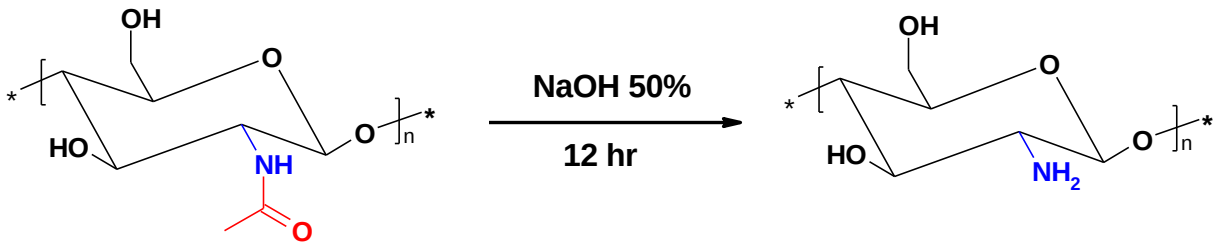

Fig. 1: Preparation of Chitosan.

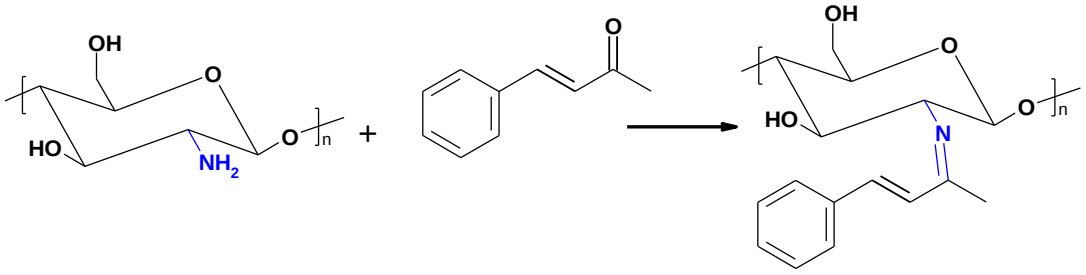

Fig. 2: Schematic preparation of Cinnamyl chitosan from chitosan.

Rotational viscometry method

For the rotational viscometry measurements, a hyaluronan soln. (2.5 mg/ml) was prepared in the dark, standing at room temperature in an aqueous. NaCl soln. (0.15 M) in two steps: first, the solvent (4.0 ml) was added to a hyaluronan powder (20 mg), and, within 6 h, the same solvent (3.85 ml) was added. The stock solutions (16 µM) of ascorbic acid, polymer solution (0.5%) stock solutions were prepared in acetic acid (0.5%). $CuCl_2$ (160 µM) were also prepared. Before starting to monitor the kinetics of the hyaluronan degradation via rotational viscometry, the mixture (8.0 ml), consisting of the biopolymer soln. of the concentration as stated above, ascorbic acid (100 µM), Cu II ions (1.0 µM), and certain amount of chitosan (or aminated chitosan) solution were transferred into the Teflon cup reservoir of the Brookfield LVDV-IIPRO digital rotational viscometer (Brookfield Engineering Labs., Inc., Middleboro, MA, USA). The experimental set was carried out by adding the chitosan (or aminated chitosan) solution at the beginning of degradation and also after 1 h of degradation start. The data acquisition of the viscometer output parameters was performed by recording within 2 min after the onset of the experiment. Time-dependent changes of the dynamic viscosity values of the system were measured at 25.0 ± 0.1 ^{o}C within 3 min intervals for up to 5 h. The viscometer teflon spindle rotation rate was 180 rpm, i.e., at the shear rate equaling 237.6 s^{-1} (Soltes *et al.*, 2007; Soltes *et al.*, 2005).

RESULTS AND DISCUSSION

Antioxidant evaluation

In this study, antioxidant activity of new chitosan derivate was done using our two established methods. Hydroxyl and alkyl free radical scavenger activity was measured by rotational viscometry method beside measure the free radical scavenger activity via ABTS method.

Rotational viscometry method

In this method, uninhibited and inhibited hyaluronan degradation under free radical stress was used to evaluate chitosan antioxidant activity. This standardized method is, in general, used to mimic the pathophysiological situation, which may occur at the early stage of acute joint inflammation (Hrabarova *et al.*, 2010) according to method, Hyaluronan was induced to degradation using Weissberger's oxidative system (ascorbate plus Cu II ions) that generate hydroxyl radical attract hyaluronan to start a chain radical degradation reaction let to split the hyaluronan chains. See reaction scheme (figure 3,4)

On application of Weissberger's system, the hyaluronan degradation was evidenced by the gradual decline of its dynamic viscosity. Figure (5) show the time dependant of hyaluronan degradation in neutral and inflammation conditions in absent and presence of chitosan and cinnamyl chitosan. by applying chitosan to inflammatory induce degradation (i.e. acidic condition) in the beginning of degradation (where OH radical is the dominant free radical species) chitosan show moderated scavenger of hydroxyl radicals results its functional groups (hydroxyl and amine groups) (Xue *et al.*, 1998; Muzzarelli *et al.*, 1997; Park *et al.*, 2004; Sousa *et al.*, 2009;Xie *et al.*, 2001), this effect was depressed in cinnamyl derivatives that may be attributed to consumption of chitosan amine groups in new schiff base bond.

The same results were obtained when applying chitosan and cinnamyl chitosan after one hour of reaction onset where the alkyl and hyaluronan macroradicals were dominated. (Figure 6).

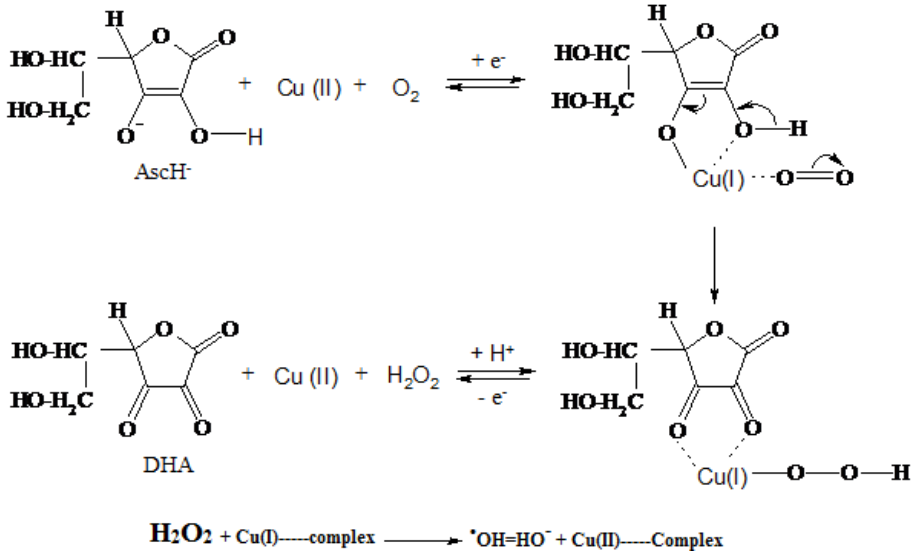

Fig. 3: Weissberger's biogenic oxidation system (WBOS) (Fisher and Naughton., 2005).

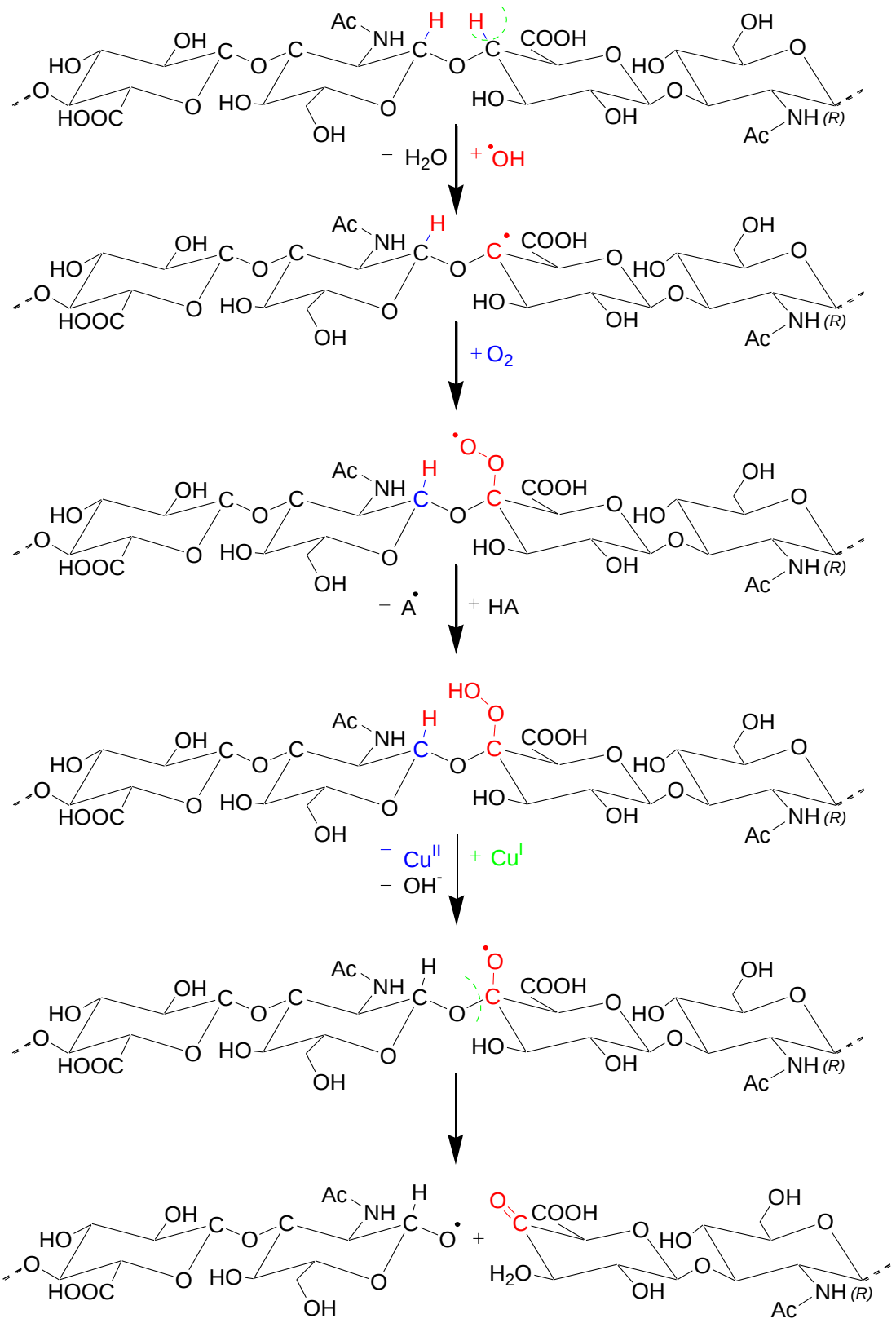

Fig. 4: Schematic degradation of HA under free radical stress (Hrabarova *et al*., 2012).

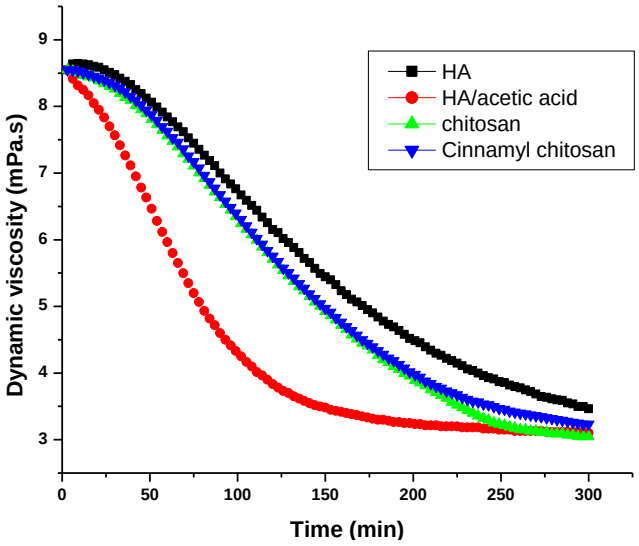

Fig. 5: Effect of chitosan and Cinnamyl chitosan dissolved in acetic acid (0.5%) on the hyaluronan degradation induced by WBOS (black) when added to the reaction system before initiating the degradation of HA

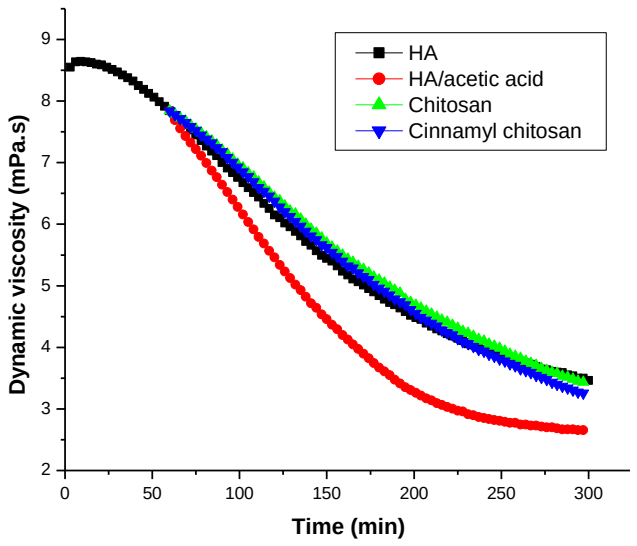

Fig. 6: Effect of chitosan and Cinnamyl chitosan dissolved in acetic acid (0.5%) on the hyaluronan degradation induced by WBOS (black) when added to the reaction system after 1 hour of initiating the degradation of HA

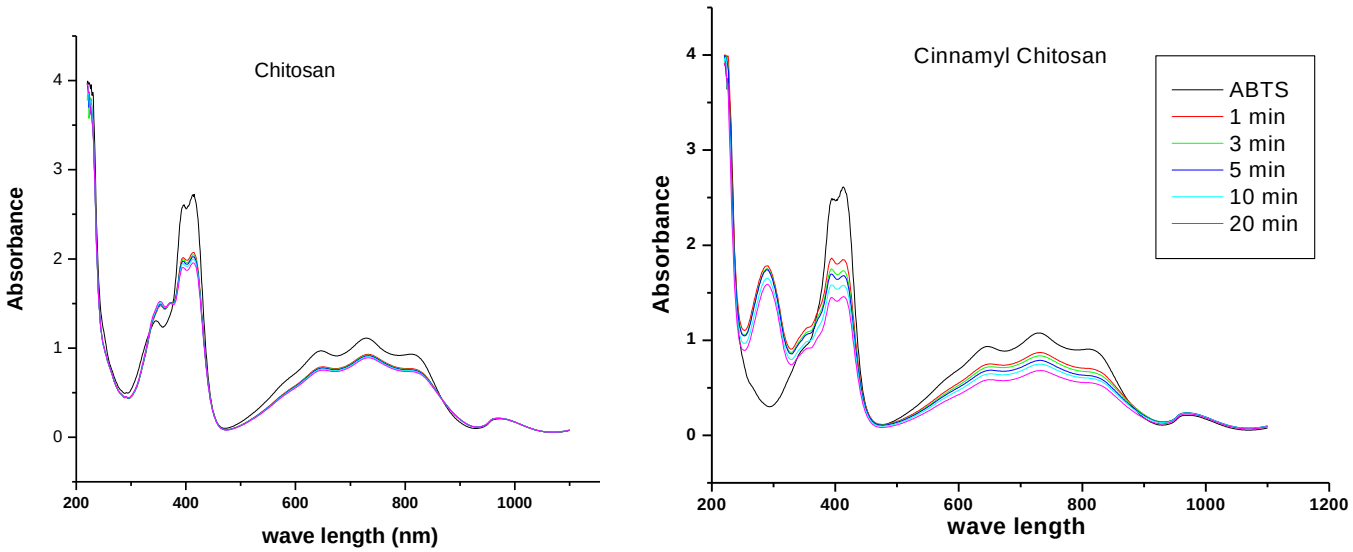

Fig. 7: Effect of Chitosan and cinnamyl chitosan (conc. 0.125 mg/ml) on reducing $ABTS^{\bullet+}$cation radical.

ABTS Method

Decolorization of $ABTS^{\bullet+}$ bluish green color was taken as monitor for measuring ability of antioxidant to donating electron. $ABTS^{\bullet+}$ exhibits a bluish-green color with maximum absorbance values at 645, 734, and 815 nm, this color rapidly decrease by acceptant electron from antioxidant substance (Re *et al.*, 1999; Hrabarova *et al.*, 2010). Figure (7,8) show effect of chitosan and cinnamyl chitosan on decolorization of $ABTS^{\cdot+}$ cinnamyl chitosan show increase of decolorization effect than chitosan itself that may be result of attaching phenolic group on backbone. Phenolic groups have a reported potency as an electron donor than amine group of chitosan. Figure (9, 10) show the effect of dose concentration on decolorization of $ABTS^{\cdot+}$. Again the figure show increases the ability of cinnamyl chitosan schiff base of chitosan itself on donating electron.

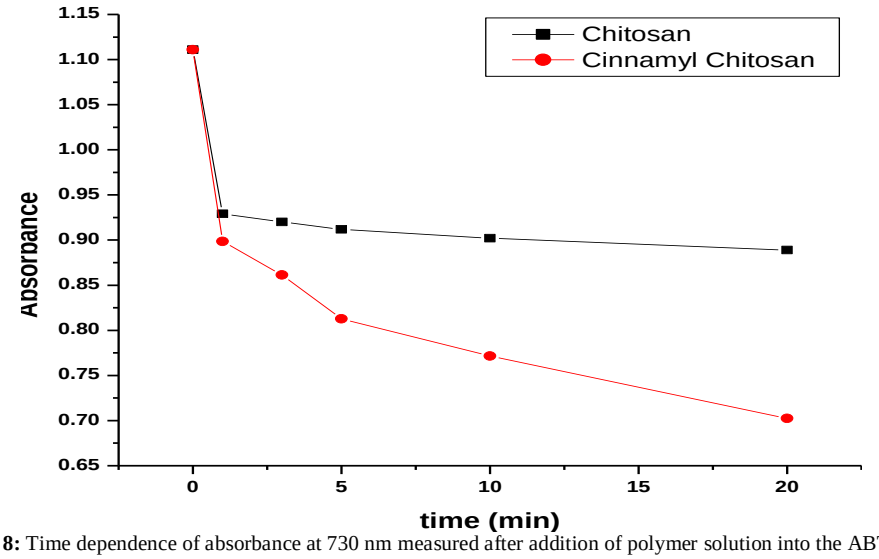

Fig. 8: Time dependence of absorbance at 730 nm measured after addition of polymer solution into the $ABTS^{\bullet+}$

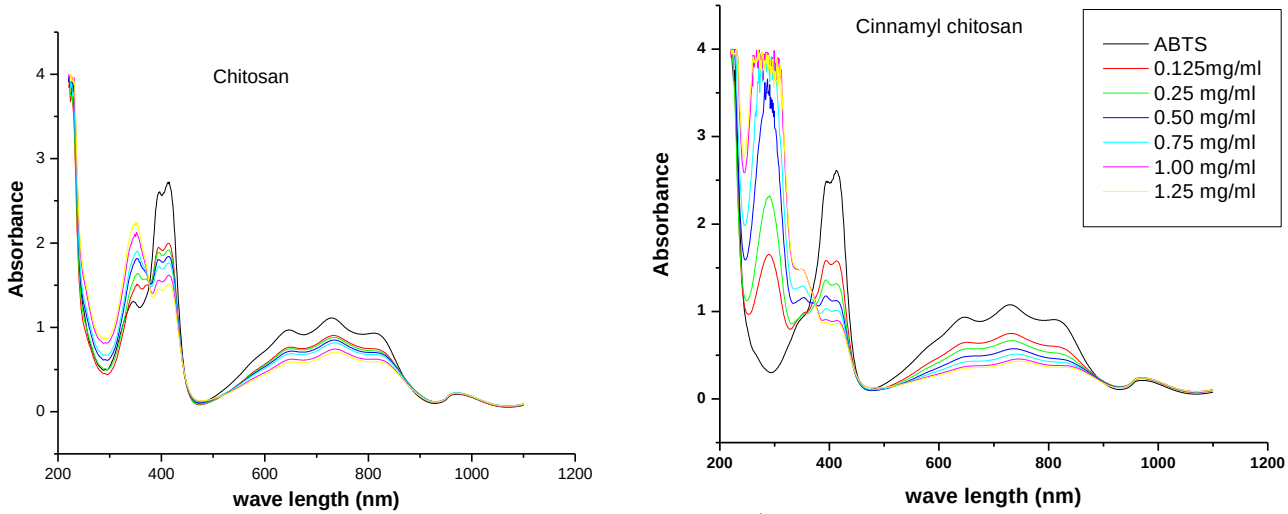

Fig. 9: Effect of Chitosan and cinnamyl chitosan concentration on reducing $ABTS^{\bullet+}$ cation radical measured 10 min after the reaction onset.

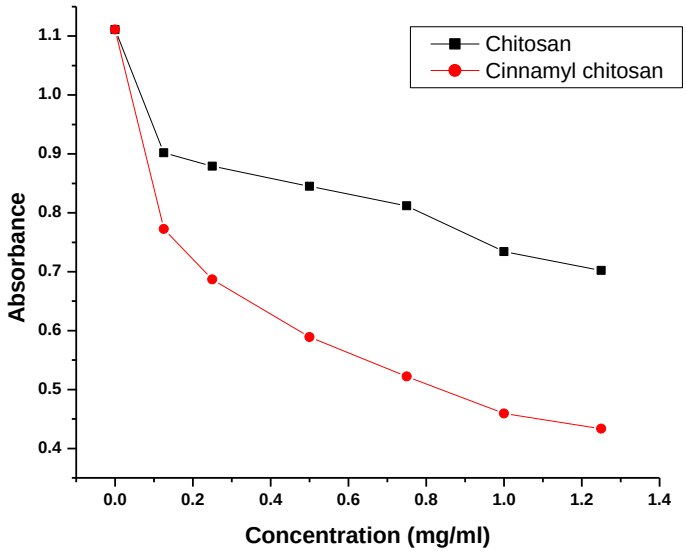

Fig. 10: dose dependency of $ABTS^{\bullet+}$ cation scavenging activity of Chitosan and cinnamyl chitosan measured 10 min after the reaction onset.

CONCLUSION

Obtained results show no improves in chitosan hydroxyl free radical scavenger activity by modification. In the other hand it illustrates a clear increase in polymer electron donor tendency. This behavior may be attributed to immobilizing phenolic nucleus to chitosan backbone.

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