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PREPARATION AND ANTIBACTERIAL EFFECTS OF SILVER LOADED CHITOSAN BEADS

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Abstract

In this study chitosan beads were prepared and cross-linked with glutaraldehyde then loaded with silver ions (Ag^+). Effects of cross-linking and (Ag^+) loading on the morphology and structure of beads were investigated by FTIR and SEM respectively. The water absorption capacity of beads were identified by swelling %. The release profile of (Ag^+) was demonstrated by inductively coupled plasma atomic absorbance spectrometer. *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were used to study the antibacterial effect of released (Ag^+). It was observed that prepared Ag loaded cross-linked chitosan beads have drop-like shape and rough surface with maximum swelling, about 27.07%. Obtained experimental results show early burst-like releasing profile of (Ag^+) from the chitosan beads. (Ag^+) released from prepared beads demonstrated strong antibacterial activity with complete inhibition activity against *S. aureus* and *E. coli* after 4 hrs from incubation with cumulative amount of (Ag^+) reach ~ 8.414 ppm.

Key words : Antibacterial activity, Chitosan, beads, Silver ions.

Introduction

Nowadays, a major global health concern is bacterial resistance towards traditional and conventional antibiotics (Arakha *et al.,* 2015). Recently, as a result of their antibacterial activities and infrequently bacterial resistance, metallic ions and their nanoparticles species were commonly used in several fields, such as in medicine, in wastewater treatment, and in textiles. The most important metallic ions with broad-spectrum antibacterial activities are silver ion (Ag^+) (Khalil *et al.,* 2017; Mokhtar *et al.,* 2018), which can be a therapy for human cells without any toxicity. The bactericidal action of (Ag^+) results from strong interaction between (Ag^+) and various electron donating groups in biological molecules with different mechanisms, for instance proteins inhibitions via binding to protein thiol groups and prevents DNA replication by its condensation (Pandian *et al.,* 2010). In addition, for sustaining release of Ag^+ and reduce silver toxicity, the direct inclusion of these silver agents in polymer or composite had recently been suggested (Pomogailo and Kestelman, 2005; Jin *et al.,* 2018).

Chitosan (CS), which is a deacetylated form of natural polymer chitin with randomly distribution of β-[1→4]-linked between D-glucosamine and N-acetyl-D-glucosamine units. CS has been described as biodegradable, biocompatible, hydrophilicity, and ease of chemical modification (Hamman, 2010), antimicrobial activity (Tao *et al.,* 2011; Benhabiles *et al.,* 2012). It has also been used in varied areas such as food industry, biotechnological, and biomedical, as well as in the pharmaceutical fields (Ghasemzadeh *et al.,* 2016). On the other hand, CS shows good solubility only in an acidic condition which restricts its usage as antibacterial polymer (Ma *et al.,* 2008; Zhou *et al.,* 2009; Kamari *et al.,* 2009). The facility of CS to undergo versatile modifications via chemical and non-chemical methods provides a significant opportunity for the industry and scientific community (Shanta & Paras, 2019). As a result of present both amino and hydroxyl groups CS has a significant capacity to absorb heavy metal ions either by chelation, ion exchange or electrostatic attraction based on it's crystallinity, water

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affinity, degree of deacetylation, the pH of environment and nature of metal ion (Zhou *et al.,* 2009; Muhammad *et al.,* 2014). Also CS can be adapted in multiform among others powder, films, gels, fibres, and beads (El-hefian *et al.,* 2011). Converting CS into gel beads may lead to improved absorbent ability, increased polymer chains, enhanced surface area and improved adsorption capacity (Tezcan *et al.,* 2017; Vakili *et al.,* 2019).

The aim of this study is to extend the antibacterial activity of CS by converting it into beads and then loading it with silver ions. The effect of cross-linking and silver loading on CS beads was investigated by FTIR, SEM and swelling ratio. Moreover, (Ag^+) release profiles and antibacterial activity of released (Ag^+) were also identified.

Materials and Methods

Chitosan (75-85% degree of deacetylation) was provided from Sigma Aldrich**.** Both sodium hydroxide and silver nitrate (AgNO3) were supplied from Merck. Glutaraldehyde (GDH) (25% solution) were purchased by Aldrich, Buchs, Switzerland**.**

Preparation of CS beads (CBs) and cross- linked CS beads (CGBs)

About 1.00 g of CS was overnight stirred with 30 mL of 2% acetic acid. Then CS solution was dropped into a precipitation bath of 500 ml from 0.5 M NAOH with moderately agitate, thus the homogeneous spherical beads were formed with continuous stirring. Finally CS beads were washed intensively with water to eliminate remaining NAOH. (CGBs) were synthesized according to Patrulea et al method when, wet CBs suspending in 0.5% GDH solution for 24 hrs at 25°C. Subsequently, CGBs were filtered and extensively washed to eliminate un-reacted GDH. In the end, a few drops of chloroform were added to CGBs and were reserved at 4°C (Patrulea *et al.,* 2013).

Loading of Ag ion into cross- linked CS beads (Ag-CG Bs)

As the method done by Sowmya & Meenaksh (2014) with minor modifications when dry CG Bs were immersed in 10 mL of 5% $AgNO_3$ and serried in dark hood for 24 hrs. Then beads were washed with deionized water and were dried at room temperature for further studies.

Characterisation of CS beads

SEM Assay

The surface characteristics of CBs, CGBs and Ag-CG Bs was evaluated with Scanning Electron Microscope (Stereoscan 360, Cambridge) at 10 kV.

FTIR Assay

The structure of KBR sample of CBs, CGBs and Ag-CG Bs was analysed using (Mattson Satellite 5000 FTIR spectrophotometer).

Swelling behaviour

Swelling ratio of CBs, CGBs and Ag-CGBs was evaluated along with the previously methods (Barkhordari *et al.,* 2014). In 50 ml of buffer solutions (pH =7.4) 0.3 g of dry CGBs and Ag-CGBs were immersed for 40 hrs at room temperature to achieve swelling equilibrium. The swelling ratio of CGBs was decided via Equation 1.

Swelling ratio = $((W_2 - W_1) / W_1) \times 100\%$, where W1 and W2 is initial weight and swollen weight of CGBs respectively.

Release study of Ag ions from Ag-CGBs

The release profiles of (Ag^+) from the Ag-CG Bs were done according to Radhesh Kumar method when, 0.3 g from Ag-CG Bs was stored with 10 mL of medium consist of (9.5 mL water acidify with 0.5mL of 0.1N HNO_3) in a rotary shaker with 60 rpm at 37°C. The ICP-AAS (SPS-1500 VR, Seiko Instruments) was used to determine the releasing of silver ions concentrations from Ag-CGBs (Kumar and Unstedt, 2005).

In vitro antibacterial activity of Ag-CGBs

The antibacterial activity of Ag-CGBs was tested against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. Test sample and negative control were prepared by adding an amount of 0.3 gm from Ag-CGBs and CGBs into nutrient broth tubes that have been inoculated with 105 CFU of bacteria and incubation in an incubator shaker at 37°C, respectively. While a positive control sample was prepared by incubating nutrient broth under the same conditions of study, but without adding any bead samples. After 0, 2 and 4 hrs from incubation, 0.1 mL of the broth was withdrawn and spread on a nutrient agar plate and incubated for 24 h at 37°C.

Statistical analysis

All study data are showed as mean values ±SD and analyses statistically with the Graph pad Prism version 8.4.2(679).

Results and Discussion

SEM Assay

SEM for CBs, CGBs and Ag-CG Bs are represented in Fig.1 a-c and Fig.2 a-d. In Fig.1 a-f Low SEM magnification of prepared dried beads, before and after cross-linking with GDH and loading Ag are compared. Actually, all beads have drop-like shape with the exception

of the CBs ones that has a hollow centre before cross-linking, while after being cross-linked with GHA CGBs beads conserved their shape and looked more tough and strong. Moreover, the surface beads changed from smooth and few wrinkles in CBs to roughness in CGBs and Ag-CGBs, thus due to GDH chains interaction on the surface of beads (Benucci *et al.,* 2016). In Fig.2 a–c high magnification SEM of CGBs and Ag-CG Bs are compared. It was found that dots on the surface of Ag-CGBs demonstrate that immobilization Ag ions on the surface of the CGBs. (Nguyen & Juang, 2015).

FTIR Assay

The FTIR spectra of CBs, CGBs and Ag-CG Bs are presented in Fig 3. In the case of CBs Fig. 3, a the main peaks for CBs appear at 3431 cm^{-1} related to stretching vibration of O–H and N–H,while 2924 cm^{-1} corresponded to symmetric stretch of CH_3. Whereas, spectra at 1658 cm^{-1} due to stretching vibration of C=O and band at 1381 cm^{-1} related to CH_3 bending vibration. Bending vibration of C–O–C was found at 1150 cm^{-1}, while stretching vibration of C–OH was found at 1073 cm^{-1}. As shown in Fig. 3, b the cross linking interaction in CGBs was identified by shifting the stretching vibration of N–H and O–H to 3417 cm^{-1}, the C=O stretching vibration shifts to 1641 cm^{-1} and the C–OH shifts to 1030 cm^{-1}. In addition, both CH_3 and C–O–C bending vibration were not observed in spectra of CGBs. The decrease in peaks' intensity of CGBs than CBs can be related to the change in the number of hydrogen bonding that formed after cross-linking with GDH (Dlugunovich *et al.,* 2006; Vakili *et al.,* 2019). In Fig. 3,c in the spectra of Ag-CG Bs the interaction between cross-linked CS and Ag ions were identified by shifting both the stretching vibration of N–H and O–H to 3424 cm^{-1} and C=O to1634 cm^{-1} while, spectra of C–OH shifts to 1086 cm^{-1}. The appearance of such red frequency shift due to chelating effect of (Ag^{+}) with some hydroxyl and amino groups of CS (Bardajee *et al.,* 2012; Sherif *et al.,* 2017).

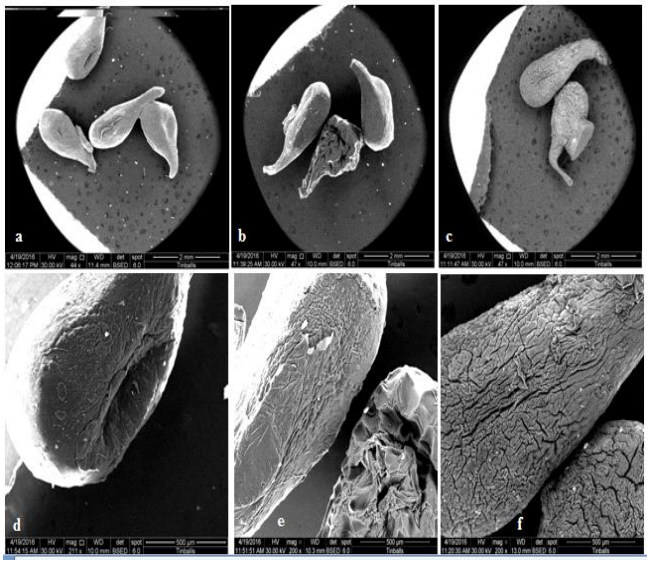

Fig. 1: SEM micrographs at low magnification of (a) CBs (b) CGBs (c) Ag-CG Bs at (47 x) and (d) CBs (e) CGBs (f) Ag-CG Bs at (200) x.

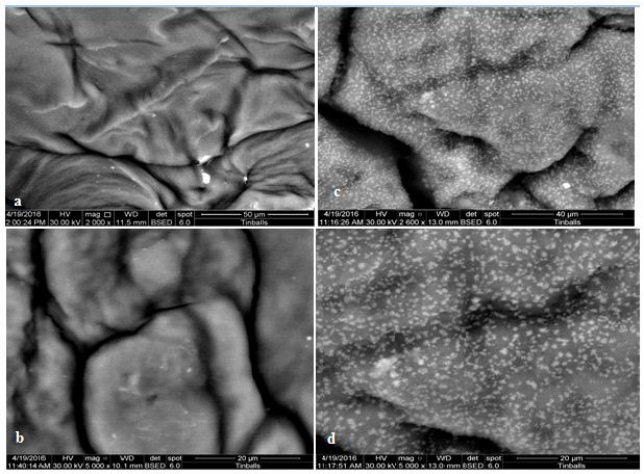

Fig. 2: SEM micrographs at high magnification of the dried CS beads surface (a) (b) after GDH cross-linking (c) (d) after immobilization of Ag on cross linked CS beads at 2000 x and 5000 x respectively.

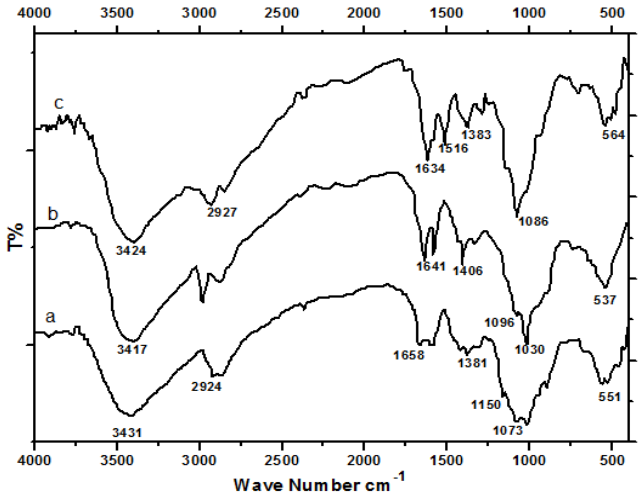

Fig. 3: The FTIR spectra of CBs (a), CGBs (b) and Ag-CG Bs (c).

Swelling behaviour

Swelling behaviour of CBs, CGBs and Ag-CG Bs are given in Fig. 4. The results demonstrated that CBs showed a higher swelling % about 43.23% in comparison to CGBs with swelling % about 28.42%. This could be related to the restricting role of cross linker on the movement of the polymer chains in CGBs network, while uncross-linked polymer chains of CBs could freely move in free spaces within the CBs network and adsorb more

water and can prospect that swelling % increase. [30].

However, Ag-CGBs revealed less swelling % about 27.07% in comparison to that of the CGBs. This could be related to knot tying functions of silver ions that restricts the swelling of polymer chains. Thus, due to chelating effect of between silver ion and number of amine and hydroxyl groups within CS (Bardajee *et al.,* 2012; Sherif *et al.,* 2017).

Release study of silver ions from Ag-CGBs

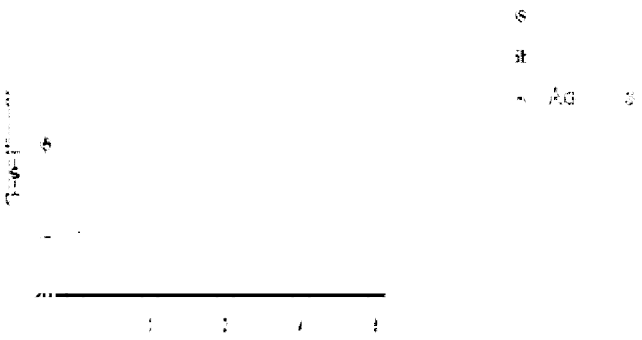

Fig. 4: Swelling behaviours of CBs, CGBs and Ag-CG Bs.

In this study the quantitative amount of the Ag ion released from the Ag-CGBs was shown in Fig. 5. The duration for whole releasing study of Ag ions was 72 hrs. As revealed in Fig. 5, the releasing profile of (Ag^+) from the CS beads is very rapid at the first 4 hrs with mean concentration of 8.414 ppm and gradually became slower with mean concentration of 9.33 ppm after 72 hrs. Thus, it shows that the release of Ag ions is related to the inter diffusion of the (Ag^+) inside the CS beads (Kawashita *et al.,* 2003; Mirzaei *et al.,* 2013).

The early burst-like release behaviour may occur as a result of drying process that effect on the migration and diffusion of silver ions, when water moved to the surface of the beads and evaporated. Silver ions may migrate with the water, leaving a heterogeneous distribution of silver ions across the Ag-CGBs, with elevated concentrations at the beads surface (Liu *et al.,* 2000).

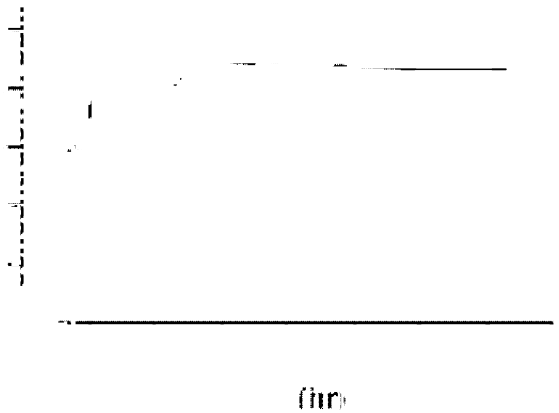

Fig. 5: The releasing profile of Ag ions from Ag-CGBs.

***In vitro* antibacterial activity of Ag-CGBs**

The antibacterial effect of silver is based on (Ag^+), when strongly attaches to sulphur, oxygen, or nitrogen of electron donating groups in different biological molecules (Feng *et al.,* 2000). In Fig 6.a-b the antibacterial activities of Ag-CGBs and CGBs and control sample were shown comparatively. In general the Ag-CGBs demonstrated strong bactericidal effect against *S. aureus* and *E.coli* after 2 and 4 hrs from incubation, while CGBs did not exhibit any inhibition effects against both bacterial strains.

Our results were revealed good bactericidal efficacy of released (Ag^+) against *S. aureus* than *E. coli* after 2 hrs from incubation. These results are in disagreement with previous research works that identified silver ions have showed higher bactericidal effect against gram–negative *E. coli* than gram-positive *S. aureus* (Pal *et al.,* 2007). Thus, complete inhibition activity of the release Ag^+ was showed against *S. aureus* and *E. coli* after 4 hrs from incubating with a cumulative amount of silver ions reach ~ 8.00 ppm as shown in Fig. 5. Our finding which is consistent with previous study done by Liau et al and Matsumura *et al.* that identified MIC of $AgNO_3$ was 5 m*M* (Liau *et al.,* 1997; Matsumura *et al.,* 2003). Thus, the different antibacterial activity of $AgNO_3$ can be imparted depending on its concentration. It was established that silver nitrate at high concentrations can completely inhibit bacterial growth, while the bacteria stayed alive at low concentrations such as 1 m*M* and restarted their growth once the silver is eliminated from their growth medium (Kalimuthu *et al.,* 2008).

Conclusion

In summary**,** structural details of beads were studied by FTIR and SEM. Additionally, the effect of glutaraldehyde and (Ag^+) on the swelling, releasing profile and bactericidal properties of the prepared beads was investigated. The (Ag^+) was productively loaded on the CS beads and had noticeable influence on the morphology of surface as confirmed by SEM and FTIR analysis. Prepared (Ag^+) loaded cross-linked chitosan beads demonstrated prolonged (Ag^+) release profile with complete inhibition activity against *S. aureus* and *E. coli* after 4 hrs from incubation. Based on these findings, the simple way for preparation of silver ion loaded chitosan beads could be hopeful methods to extend the antibacterial

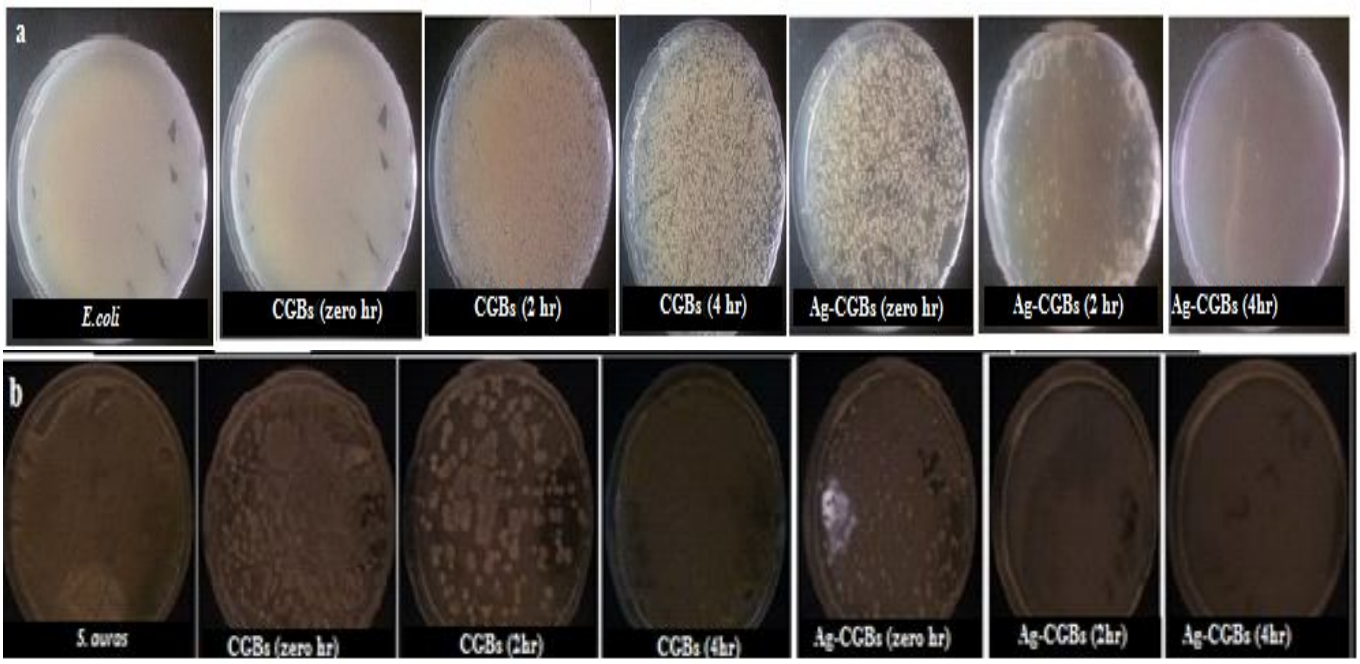

Fig. 6: The antibacterial activities of Ag-CGBs and CGBs at zero, 2 and 4 hrs from incubation against *E. coli (a) S. aureus* (b).

activity of chitosan.

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