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RESEARCH ARTICLE

Triclosan-Loaded Alginate Films for Topical Medical Applications

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Manuscript Info *Abstract*

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This paper focuses on enhancing the physico-mechanical and antibacterial properties of alginate films via incorporation of triclosan into the ionicallycrosslinked alginate for topical medical applications. Results indicated that the prepared bioactive films exhibited a range of physicomechanical and functional properties depending on type of crosslinker, Ca2+, Ba2+ or Zn2+, kind of matrix, alginate or alginate/CMC blend (50/50), degree of crosslinking and swelling properties as well as extent of embedding and releasing of the loaded triclosan. The obtained bioactive alginate films using CaCl2 (2% w/w), as ionic crosslinker salt, triclosan (20% w/w) as antibacterial agent, along with glycerol (0.25% w/w) as a plasticizing agent, showed proper physico- (water retention, weight loss and water vapor permeability), mechanical properties (tensile strength) along with a remarkable antibacterial activity against the tested G+ve (S. aureus) and G-ve (E. coli) bacteria. Both surface morphology and surface composition of alginate films using different ionically crosslinking divalent metal cationswere investigated using SEM and EDX analysis.

INTRODUCTION

Environmental and ecological concerns have increased the interest in utilization of natural biopolymers such as cyclodextrin, chitosan, alginate and sericin protein, as proper and renewable materials, in the development of bioactive materials(UL-Islam, Shahid, & Mohammad, 2013a; UL-Islam, Shahid, & Mohammad, 2013b).

Alginate is a naturally occurring anionic and hydrophilic polysaccharide harvested from brown algae. Alginate comprises the linear chain of (1,4)-β-D-mannuronic acid (M) and (1,3)-α-L-guluronic acid (G) monomers, which vary in composition and sequence depending on biological source, growth and seasonal conditions(Sun & Tan, 2013; Lee & Mooney, 2012). Due to its unique properties such as biocompatibility, biodegradability, ion exchange/gel-forming and film-forming abilities as well as inexpensiveness, alginate has been widely used in a variety of potential biomedical applications, e.g. tissues engineering, wound healing, drug delivery (Sun & Tan, 2013; Lee & Mooney, 2012; Pawar& Edgar, 2012).

Owing to their low antibacterial activity, alginate biopolymer can be incorporated and/or loaded with several antibacterial agents along with proper cross-linking to produce highly absorbent antibacterial cross-linked alginate films(Qin, 2005; Brachkova, Duarte, & Pinto, 2012; Zactiti&Kieckbusch, 2006).

Consequently, the aim of the present research article was to develop a new facile procedure to impart antibacterial functionality to cross-linked alginate-based films via incorporation of triclosan, as antibacterial agent, into the film-forming formulations using alginate, as a film-forming material, glycerol, as a plasticizing agent, and Ca-, Ba- or Zn Chloride, as a cross-linking agent. Moreover, partial replacement of Na-alginate with carboxymethyl cellulose (CMC) and its impacts on the physico-mechanical and antibacterial properties of the obtained films were investigated, SEM and EDX images of some bio-films were also demonstrated.

2. Experimental

2.1. Materials

Sodium alginate with an average molecular weight of 150,000 Dalton and M/G ratio of 1.56 was provided by Sigma-Aldrich (USA). Tinosan® CEL (antimicrobial agent based on triclosan, Ciba) and carboxymethyl cellulose (CMC, MW = 4000 Dalton) were used as received. Glycerol, Zinc, calcium and barium chlorides ($ZnCl_2$, $CaCl_2$ and $BaCl_2$) were of laboratory grade chemicals.

2.2. Methods

2.2.1. Preparation of bio-films

Solutions of Na-alginate (2%) alone and in admixtures with CMC, and triclosan, (0-30% w/w, based on weight of the film-forming material), were initially prepared through the dissolution in distilled water at 80 ^{O}C for 45 minutes with continuous stirring until the mixture dissolved completely, followed by the addition of glycerol (0.25%w/w) as a plasticizing agent to improve the flexibility of the obtained bio-films. Afterwards, the homogenous solution was casted into a level Teflon coated glass plates and left to dry at ambient conditions, followed by peeling off after drying. Thereafter, the dried films were immersed for 10 min into a $CaCl_2$aqueous solution either 2% or 4% for cross-linking to obtain the cross-linked bio-films. Finally the obtained films were washed in distilled water to remove unfixed and/or extra $CaCl_2$ for 10 second, dried at room temperature and stored in desiccators at relatively humidity of 52% for 24 hr before characterization and testing.

2.3. Measurements

- The thickness of the cross-linked bio-films was determined by a manual micrometer (Dial thickness gauge 7301, Mitutoyo Co. Japan, at 0.01mm accuracy).
- The tensile strength (TS) of the cross-linked films, in the warp direction, was measured according to the ASTM standard method D882 (ASTM, 1995a).
- Water vapor permeability (WVP) was determined gravimetrically using a modified ASTM method E96-95 (ASTM, 1995b).
- The swelling behavior of the obtained cross-linked bio-films was assessed gravimetrically by the following equation:

$$DS\% = \frac{Wh - Wd}{Wd} X\ 100$$

where DS, Wh and Wd are the degree of swelling, the hydrated weight and the sample dry weight respectively.

- Antibacterial activity of the cross-linked bio-films against G+ve bacteria (*S. aureus*) and G-ve bacteria (*E.coli*) was determined qualitatively according to the disc diffusion method AATCC Test Method (147-1988), and expressed as zone of growth inhibition (ZI, mm).
- Scanning electron microscope (SEM) images of the selected bio-films were obtained with a JEOL, JXL 840A electron probe micro analyzer equipped with energy disperse X-ray (EDX) spectroscopy for the composition analysis.

3. Results and Discussion

This study focused on the technical feasibility of developing Ca-Alginate films with remarkable antibacterial via incorporation of triclosan, as antibacterial agent/gust molecule, into cross-linked alginate, as a biopolymer matrix material; under a wide of experimental parameters to produce antibacterial polysaccharide films have potential for topical biomedical applications. Discussion of the experimental results follows.

3.1. Triclosan concentration

The results for antibacterial activity, expressed as ZI, of triclosan-loaded ionically cross-linked alginate films against G+ve (*S.aureus*) and G-ve (*E.coli*) bacteria are illustrated in Figure 1. For a given set of film-forming conditions, it can be seen that: i) increasing the concentration of triclosan up to 20% (based on weight of Na-alginate) results in a remarkable improvement in the antibacterial activity of the obtained bio-active films, regardless of the ionic cross-linker, $CaCl_2$, concentration, ii) the higher the $CaCl_2$ concentration, the higher the extent of ionic cross-linking, but lower the extent of releasing the embedded triclosan, thereby minimizing the antibacterial properties, iii) further increase in triclosan concentration beyond 20% has practically no significant increase in the conferred biocidal activity, iv) the imparted antibacterial efficacy against the tested bacteria follows the decreasing order: G+ve> G-ve, reflecting their differences in their cell wall structure and amenability to disruption (Ibrahim,

Amr, Eid, Mohamed, &Fahmy, 2012; Ibrahim et al., 2013;Ibrahim, Khalil, El-Zairy, &Abdalla, 2013; Orhan, Kut, &Gunesoglu, 2009), and v) the remarkable improvement in the antibacterial activity of the obtained bio-films reflects the negative impacts of the released triclosan on inhibiting biosynthesis of fatty acid via blocking lipid biosynthesis, which in turn hinders both the building of cell membranes and reproduction(Orhan, Kut, &Gunesoglu, 2009;Lam, Kan, & Yuen, 2012; Orhan, Kut, &Gunesoglu, 2007).

3.2. Type of divalent metal cation

Table 1 shows the effect of using different ionic cross-linking agents namely $CaCl_2$, $BaCl_2$ and $ZnCl_2$ on the performance and anti-bacterial functional properties of the obtained bioactive alginate films. Table 1 clearly demonstrates the following orders:

- the water retention percentage decreased in the order:
  $Ca^{2+} > Zn^{2+} > Ba^{2+}$
- the weight loss decreased in the order:
  $Zn^{2+} > Ba^{2+} > Ca^{2+}$
- the water vapor permeability increased in the order:
  $Zn^{2+} < Ba^{2+} < Ca^{2+}$
- the tensile strength, warp, increased in the order:
  $Ca^{2+} < Zn^{2+} < Ba^{2+}$, and
- The antibacterial activity of the triclosan loaded films enhanced in the order:
  $Ba^{2+} < Zn^{2+} < Ca^{2+}$, keeping other parameters constant.

The variation in the above mentioned properties reflects the differences among the nominated divalent cations in: i) ionic radius (Nakamura, Nishimura, Hatakeyama, &Hatakeyama, 1995), ii) their ability to build cross-linked network via complexion with the carboxylate groups of guluronate component of alginate, iii) cross-linking density and consequent decrease of porosity, water swell ability and water absorption, iv) extent of embedding and releasing of the bioactive agent, v) competition between triclosan with the alginate for bivalent cations, vi) extent of inclusion of glycerol, as plasticizing agent, between the cross-linked polymer chains, as well as, vii) location and extent of distribution and fixation of both the divalent cations and the triclosan onto and/or within the ionically cross-linked alginate film (Nakamura, Nishimura, Hatakeyama, &Hatakeyama, 1995; Seixas, Turbiani, Salomão, Souza, &Gimenes, 2013; Bayer, Herrero, &Peppas, 2011; Sikareepaisan, Ruktanonchai, &Supaphol, 2011).

3.3. SEM image and EDX spectrums

The variation in SEM images of control film (Fig. 2a) in contrast to ionically cross-linked films (Fig. 2 b, d, f) is governed by film smoothness, homogeneity extent of dispersion and distribution of the ionic cross-linker throughout the prepared films as well as degree of cross-linking. On the other hand, EDX-elemental analysis (Figure 2c,e,g) results confirmed the presence of Ca, Ba or Zn element in different contents close to or at the surface of the obtained films (Figure 2c,e,g).

3.3. Type of matrix

As far as the changes in the physico-mechanical properties of the triclosan-loaded ionically cross-linked films as a function of using Na-alginate alone or in combination with CMC (50/50) as a matrix, Figure 3 a-d demonstrates that: i) including CMC with Na-alginate results in a significant increase in WR% (Figure 3a), WL% (Figure 3b) values along with a slight decrease in WVP (Figure 3c) as well as a noticeable reduction in TS (Figure 3d) values compared to the blank alginate films, ii) the presence of CMC component enhances both the degree of swelling by increasing the number and accessibility of hydrophilic active sites, carboxylate groups, as well as increases the WL (%) as a direct consequences of partial disintegration and solubility of the formed films in the aqueous medium, iii) incorporation of CMC to alginate matrix is accompanied by a slight decrease in WVP values most probably due to an increase in the pathways to water molecules to pass through the blended films (Sirviö, Kolehmainen, Liimatainen, Niinimäki, &Hormi, 2014), along with a significant reduction in TS properties as a direct consequence of decreasing the extent of cross-linking of alginate and Ca^{2+} and increasing the water content compared to the alginate films without CMC (Rhim, 2004), and iv) the change in the above mentioned physico-mechanical properties is governed by the ionic interactions among the alkaline divalent metal, Ca^{2+}, alginate and/or CMC carboxylate groups, in the presence of the bioactive agent triclosan.

On the other hand, Figure 4 clearly shows that: i) incorporation of triclosan into ionically cross-linked alginate or alginate/CMC (50/50) blended films brings about a remarkable improvement in their antibacterial activity against both the tested G+ve and G-ve bacteria, ii) the imparted antibacterial activity is determined by the nature of matrix i.e. alginate /CMC (50/50) > alginate, as well as the type of bacteria, i.e. G+ve (*S.aureus*) > G-ve (*E.coli*), iii) the higher the degree of cross-linking, as in case of using alginate as a matrix, the lower the extent of releasing the bioactive agent, triclosan, i.e. less antibacterial activity compared with alginate/CMC films, and iv) the

variation in antibacterial activity against the nominated bacteria reflects the differences in their cell wall structure as well as ability to offer enough protection against the released triclosan moieties from the loaded triclosan films.

4. Conclusion

Triclosan-loaded alginate films were successfully prepared as wound dressing using Ca^{2+} as ionic cross-linker. The physico-mechanical and antibacterial functional properties of the obtained films are highly dependent on type and concentration of ionic cross-linker, the content of the antibacterial agent as well as type of matrix, i.e. alginate or alginate/CMC blend (50/50). The differences in the imparted properties are attributed to the extent of cross-linking, degree of swelling, as well as the degree of leaching and releasing of the loaded bioactive agent. This work demonstrates that triclosan - loaded ionically cross-linked alginate films have proper physico-mechanical properties along with remarkable antibacterial activity to be used as effectively wound dressings.

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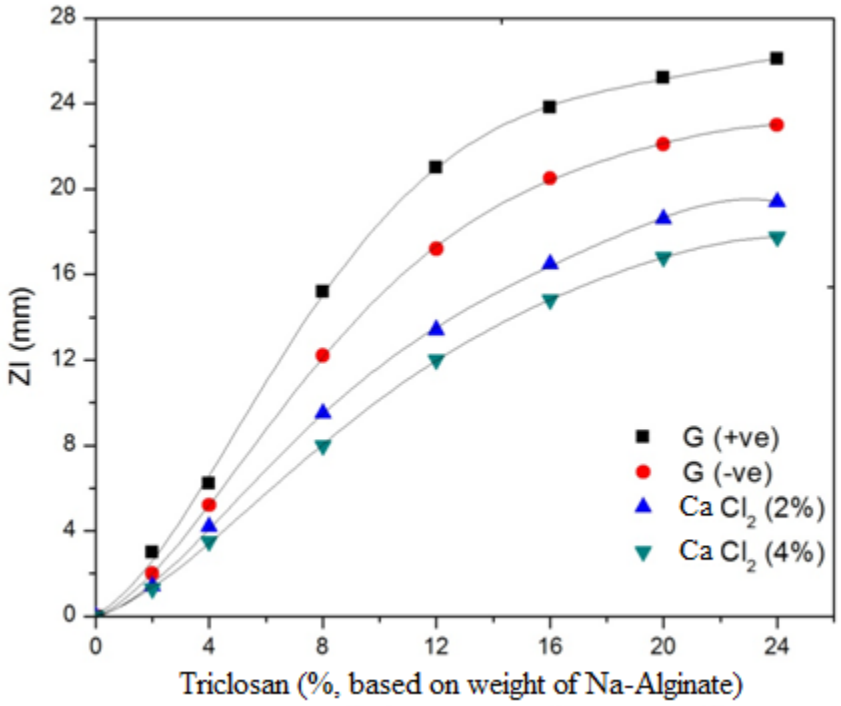

Fig. 1. Effect of antibacterial agent and crosslinker concentration on the antibacterial activity of triclosan – loaded Na-Alginate films. Na-Alginate, 20g/L; triclosan, 20% (OW Na-Alg.); glycerol, 4 g/L; crosslinking salt solution, 2% (OW Na-Alg.); immersion time, 5 min; film thickness (0.144±0.005 mm).

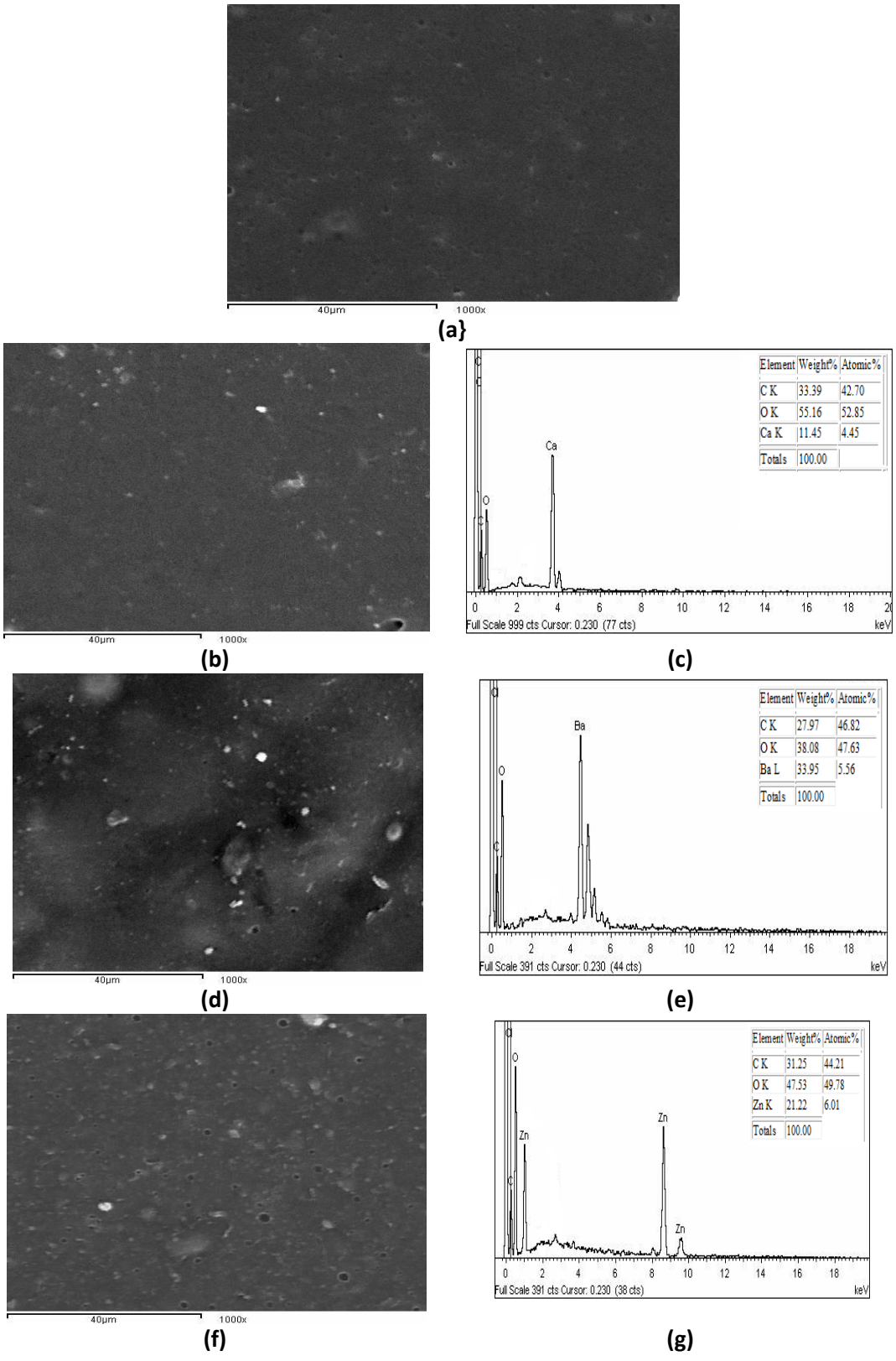

Fig. 2. SEM images and EDX spectra of sodium alginate crosslinked films. Sodium alginate, 20g/l; film thickness, 0.1444 ± 0.0110

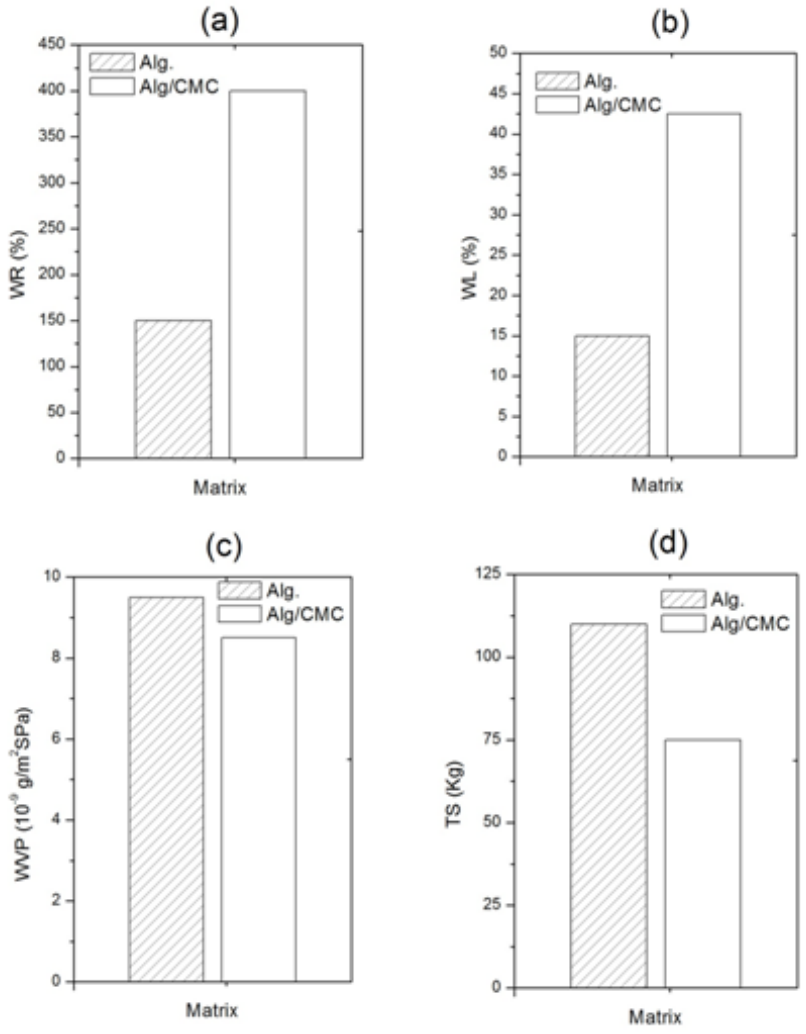

Fig. 3. Effect of matrix type on the physico-mechanical properties of the triclosan – loaded films.

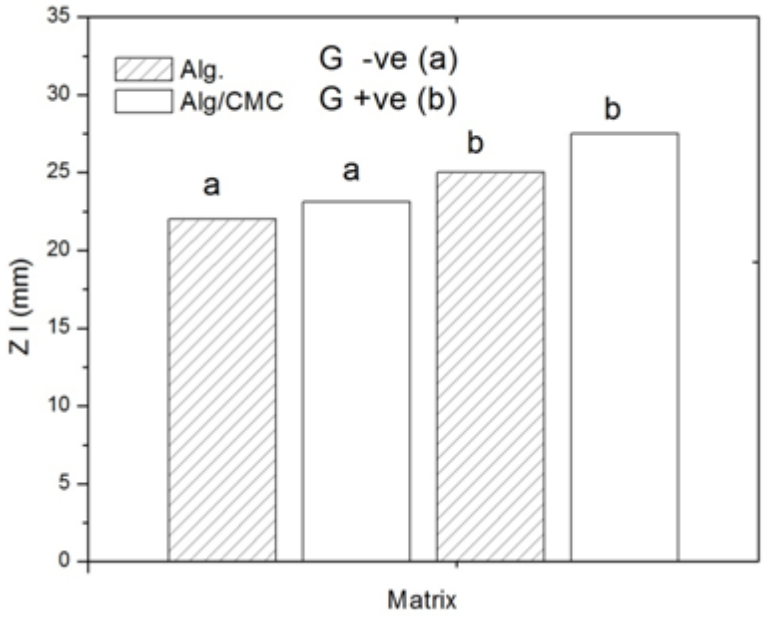

Fig. 4. Effect of matrix type on the antibacterial activity of the triclosan – loaded films.

Table 1: Effect of using of different crosslinkers

Performance property		Crosslinking salt (2% ow Na-Alg.)		
		$CaCl_2$	$BaCl_2$	$ZnCl_2$
Water retention (WR, %)		158	102	152
Weight Loss (WL, %)		15.0	17.3	21.4
Water vapor permeability (WVP, 10^{-9} g/m^{2}sPa)		9.5	8.8	8
Tensile strength (TS, Kg)		110	135	120
Antibacterialactivity (Z I, mm)	**G +ve**	25	17	22
	G -ve	22	13	20

Na-Alg. (20g/L); triclosan (20% OW Na-Alg.), glycerol (4 g/L), crosslinking salt solution (2% OW Na-Alg.), immersion time (5 min.); film thickness (0.144±0.005 mm)