

Microbial activity and swelling behavior of chitosan/polyvinyl alcohol/sodium alginate semi-natural terpolymer interface containing amoxicillin for wound dressing applications

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ABSTRACT

Samples of equimass fraction chitosan/polyvinyl alcohol/sodium alginate semi-natural terpolymer with and without gradient concentration of amoxicillin, a topical antibiotic drug were prepared via traditional casting techniques. Changing in antibacterial activity and swelling degree have been assessed using the zone of inhibition method and pH sensitivity study. Upon increasing the amoxicillin concentration, the enhancement of antibacterial activity was performed against (2g) positive bacteria (*S. aureus*, *B. subtilis*), (2g) negative bacteria (*E. coli*, *P. aeruginosa*) and fungus (*C. albicans*). Increasing the concentration of amoxicillin caused a decreasing in the swelling ratio (SR) at neutral (pH= 7) and basic medium (pH= 8, 9) due to increase cross-linked and rigidity of the network. Hence the results strongly support the possibility of using this novel Cs/PVA/SA with different amounts of amoxicillin to compatible used in tissue regeneration and antimicrobial applications.

Keywords: Chitosan; Polyvinyl alcohol; Sodium alginate; Amoxicillin; Antibacterial Activity; Swelling ratio; Tissue regeneration

1. INTRODUCTION

Polymers in nature are popularly utilized for pharmaceutical applications due to its plentiful supply, biocompatibility, renewability, high porosity and chemical stability while the artificial polymers which synthesized in the laboratory from low molecular weight compounds exhibit lack of biocompatibility so it is mostly blended with another component to improve such properties [1-3]. Recently, many different types of polymers found extensive applications as dressing to preserve wound and improve healing [4].

Chitosan (Cs) is a plentiful natural biopolymer, extracted from the chitin the second most abundant polymer after cellulose. Chitosan has been used in the biomedical field since it shows several important properties, such as antimicrobial, biocompatibility, biodegradability, homeostasis, wound healing efficiency, bio-adhesion and its capability to absorb exudates [5]. These properties also permitted (Cs) to be an essential applicant in biomedical uses.

Polyvinyl alcohol (PVA) uses in pharmaceutical purposes such as blood vessels, wound healing and drug delivery systems because of its biocompatibility with human organs, inveterate excellent mechanical strength eligible physical properties, non-carcinogenicity, nontoxicity and swelling behavior [6]. However, insufficient rigid and confined hydrophilicity characteristics make it difficult to use alone as a wound dressing [7], so the blending of synthetic polymers with natural polymers perform attractive physical characteristics as well as biocompatibility [8].

Sodium alginate (SA) is a nontoxic polysaccharide manufactured from brown seaweeds. It has many desirable biological and physical characteristics such as physiological moist microenvironment, high availability, natural low cost, solubility in water and superior biocompatibility, these properties aid (SA)

used in some biomedical purposes such as tissue engineering, wound dressings and drug delivery [9].

Amoxicillin (Amox) belongs to the penicillin class of antibiotic which has excellent properties such as broad utilize to treat bacterial infections caused by susceptible microorganisms and spectrum antibiotics. Also, the crystal structure of amoxicillin can improve the antimicrobial effect and the stability of the drug, prolong the action duration of the drug and slow down the dissolution of the drug [10].

Sarhan *et al.* [11], investigated the effect of increasing honey concentration to polyvinyl alcohol/chitosan nanofibers combined with different concentration of honey for determining the effect of different concentration of honey on the properties of besides, antibacterial study and swelling degree verified the successful combination of honey in nanofibers. Due to antibacterial properties and high cross-linked network, they concluded the process parameters have improved some properties of nanofibers to compatible their applications in wound healing/dressing. The miscibility highly found in ternary blend due to the composing of three polymers varying in properties may suggest a special opportunity to promote a new polymer material with flexible combination from three constituents. Antibacterial factors with wound healing effects are becoming essential in time. Yavaraja *et al.* [5] examined the factors used in a diversity of the healthcare applications, industries, laboratories and environments. Skin wounds are oversensitive to infections by both gram-positive, gram-negative bacteria and fungi. The Contagion of wound surfaces often causes retarded wound healing or increases the hazard of septicemia, which fatality is the main cause of wound-associated death.

The swelling degree of samples has an important role in the antibacterial activity and wound restoration due to solvent-

holding capacity which can absorb predict exudates by swelling and help a quick restoration of wounds [11].

The recent analysis, study the combination of chitosan, polyvinyl-alcohol and sodium alginate for determining the effect of swelling

behavior on these physical characteristics and developing the antibacterial activity to use in several biomedical applications like wound healing.

2. MATERIALS AND METHODS

2.1. Materials used.

Chitosan (Cs) with a deacetylation degree of 86% extra pure was obtained from exporter lab chemicals Co. (Alpha Chemika, India). Polyvinyl alcohol (PVA) (MW 14,000) was obtained from (Rasayan Laboratories). Sodium alginate (SA) was purchased from (Carlroth Co.). Amoxicillin (Amox) powder was purchased from Pharmaceutical Chemicals, Egypt.

2.2. Preparation method of films.

2.2.1. Preparation of ternary films. A certain amount of the Cs solution (100 mL) was mixed with PVA solution (100 mL) and SA solution (100 mL) at 60 °C and stirred for 60 min by utilizing the casting/solvent evaporation technique. Then mixed solution poured onto clean Petri dishes and dried at 50 °C.

2.2.2. Preparation of Cs/PVA/SA/Amox films. The certain amounts of Cs/PVA/SA (300 mL) solutions were prepared with various loading concentrations of amoxicillin (antibiotic drug). The solution of Cs/PVA/SA divided into 6 beakers that means each beaker contained (50 mL) of the solution then Amox was dropped through a syringe needle with different amounts 0.08, 0.16, 0.32, 0.48 and 0.64 g (S_0 , S_1 , S_2 , S_3 , S_4 , S_5). All samples were fabricated by the same technique and poured onto clean Petri dishes and dried at 50 °C. **Table 1** summarizes the different concentrations of the prepared films.

Table 1. Samples with different amount of amoxicillin (Amox).

Sample	(Cs/PVA/SA)/(Amox) (mL)	(Cs/PVA/SA)/(Amox) (g/g)
S_0	0	0
S_1	40	0.08
S_2	80	0.16
S_3	160	0.32
S_4	240	0.48
S_5	320	0.64

2.3. Experimental techniques (Characterization).

2.3.1. Antibacterial properties. The antibacterial activity of the amoxicillin-assimilated ternary blend (Cs/PVA/SA) films were studied on agar plates injected with (2g) positive bacteria (*S. aureus*, *B. subtilis*), (2g) negative bacteria (*E. coli*, *P. aeruginosa*) and fungus (*C. albicans*) utilizing the test of inhibition zone (IZ). Amoxicillin was progressive from films into

injected medium to stop the growth of bacterial strains of positive, negative and fungus. After 24 hours of incubation at 37°C, the diameter of the inhibitory circle around the amoxicillin-assimilated film was compared and estimated with the virgin film without amoxicillin content. Antibiotic (Ampicillin) and antifungal (Colitrimazole) used as a positive reference to determine the sensitivity of bacterial species tested [12].

The % activity index for the samples was estimated by this equation [1]:

$$\text{Activity Index (\%)} = \frac{\text{Diameter of test compound (Zone of inhibition)}}{\text{Diameter of standard compound (Zone of inhibition)}} \times 100 \quad (1)$$

2.3.2. Swelling rate (SR) and pH-sensitivity study. The pieces of polymer samples (2cm×2cm) were dried at 50 °C under vacuum for 12 hours (W_d) (gravimetric method). Then pieces of film were placed or soaked (submerged) in buffer solutions with several pHs between (4-9) at room temperature (27 °C) then incubated at 37 °C for 24 hours until the samples reached the equilibrium state of swelling. In detail, Disodium citrate ($C_6H_6Na_2O_7$)/ Hydrochloric acid (HCl), Phosphate buffer solutions (PBS) and Distilled deionized water (DDIW) were utilized for pH 4 and pH from 5 to 8, respectively but Diluted sodium hydroxide (NaOH) solutions were utilized for higher pH values like (9) or more. After removal swollen films from the swelling medium, the polymer films were omitted or blotted carefully and quickly with filter paper to remove liquid on the surface of the sample and weighed. The water absorption of the polymer samples was measured every 2 hours by this equation by using an analytical balance.

$$\text{Swelling ratio (SR)} = \frac{W_s}{W_d} \times 100 \quad (2)$$

Weights after immediate soaking (W_s) were also possessed to reduce the error caused by the water surface. So the swelling ratio was then achieved according to the following formula.

$$\text{Swelling ratio (\%)} = \frac{W_s - W_d}{W_d} \times 100 \quad (3)$$

Where W_s and W_d the weight of swollen polymer soaked in different solvents and dry polymer at temperature (37°C) and (60°C) [13].

3. RESULTS

3.1. Antibacterial studies.

The antibacterial ability of ternary blend with different content of the antibiotic drug (amoxicillin) examines using the disk diffusion test. In **Figure 1. a- e**, no growth zones see around the circles of amoxicillin- assimilated films in agar plates injected with either (2g) positive bacteria (*S. aureus*, *B. subtilis*), (2g) negative bacteria (*E. coli*, *P. aeruginosa*) and fungus (*C. albicans*) [14, 15]. The antimicrobial activity of Cs/PVA/SA/nAmox compares against a set of standard antibiotics such as antibiotic Ampicillin and antifungal Colitrimazole. Similar inhibition zones do not see the good results with discs of drug-free

Cs/PVA/SA film, thus indicate that the amoxicillin (antibacterial agent) can immobilize in the Cs/PVA/SA blend and then release. **Table 2** the antibacterial activity of Cs/PVA/SA increases with increasing content of the drug.

From this table, the antimicrobial properties of the drug-including ternary blend are more efficient against the Fungus (*Candida albicans*) due to the growth of fungus more densely on the middle surface of agar tend to indicate the bigger inhibition zone as they were more exposed to amoxicillin. Among all studied samples, the S_5 sample Cs/PVA/SA/0.64g/g Amox performs the

best with a circular zone of inhibition around all strains and exhibits increasing in the antibacterial activities.

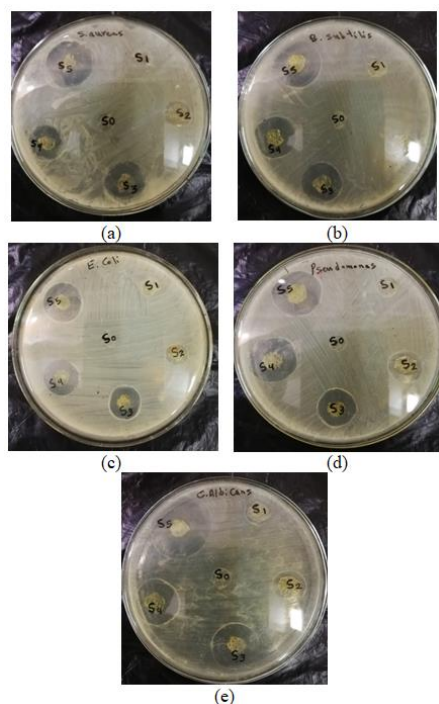
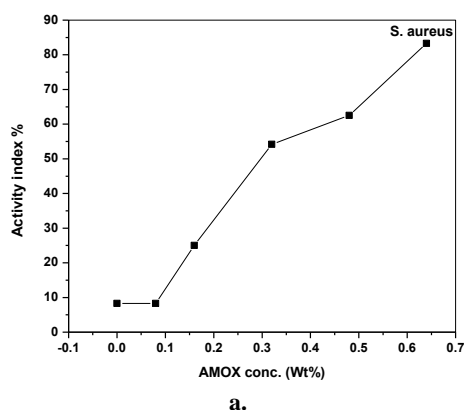


Figure 1. Inhibition zones of Cs/PVA/SA with different amount of Amox against two gram positive (a) *Staphylococcus aureus*, (b) *Bacillus subtilis*, two gram negative bacteria (c) *Escherichia coli*, (d) *Pseudomonas aeruginosa* and fungus (e) *Candida albicans*.

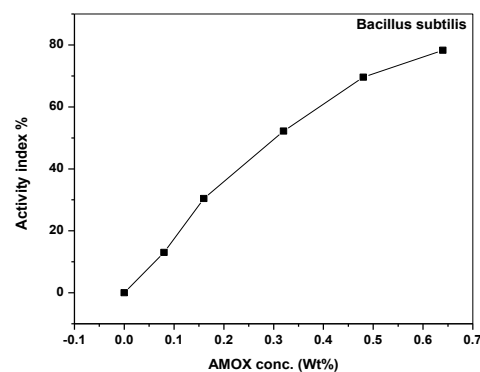
Also, that proves the best inhibition zone and activity index appear with *C. albicans* culture compared to *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. All tested samples show best inhabitation zones and activity index against bacterial and fungal with increase amount of model drug in the polymer blend.

The antibacterial mechanism of amoxicillin destruct of proteins and lipids of bacterial cell wall membranes, and ultimately, the exudation of cell contents and bacterial cell death. Ultimately, it can understand that we can use amoxicillin particles in a polymer matrix to strengthen the antimicrobial activity of the polymer matrix in wound dressing.

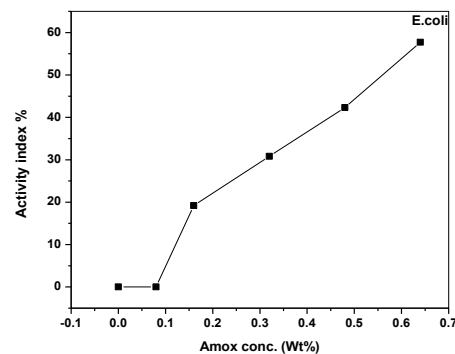
Figure 2. a- e, shows the relation between activity index (%) and amounts of amoxicillin drug (Wt. %) against different bacterial and fungi. The activity index great increased by increasing the amount of model drug (Amox) until reach (0.48 and 0.64 g/g) found plateau and no difference between the last two figures. So, constant at this concentration (0.64 g/g) shown in **Figure 2. e**.



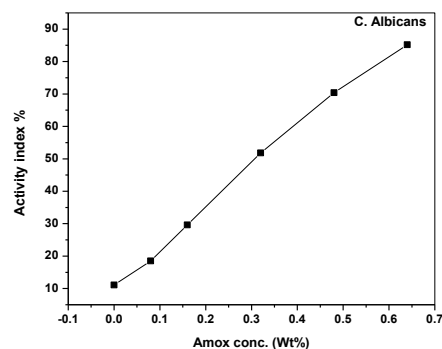
a.



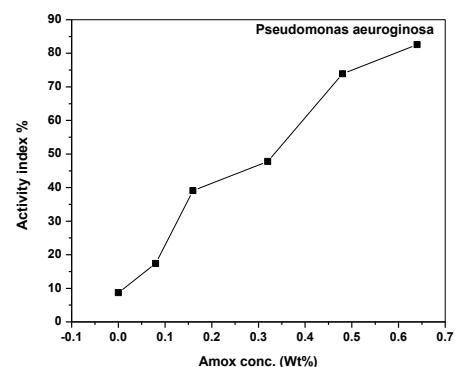
b.



c.



d.



e.

Figure 2. a- e. Activity index of (a) *S. aureus*, (b) *B. subtilis*, (c) *E. coli*, (d) *C. Albicans* and (e) *P. aeruginosa* versus concentration of amoxicillin.

Figure 3. a- e, represents the optical gap (eV) related to the activity index (%) against different types of bacteria and fungi. The conclusion from this figure is when the optical energy gap decreases the activity index increase.

3.2. Swelling Studies.

Biologically, the skin topmost layer is described stratum layer and features of its structure including homeostasis, permeability barrier and cohesion, etc. These features are controlled by the pH of the skin. The term (acid mantle) is called the stratum layer because of the pH of the healthy (normal) skin in

the domain of (5 – 6). The pH of this layer is affected by numerous agents like (eccrine, sebaceous and apocrine) glands, epidermal cells, gender and age.

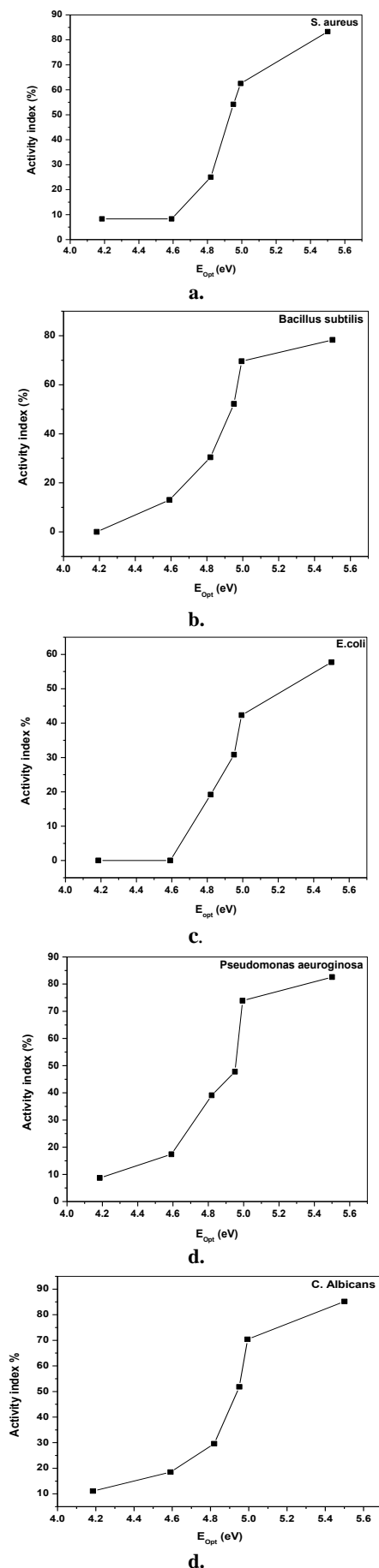


Figure 3. a- e, optical gap (eV) in correlation with the activity index (%) against different types of bacteria (a) *S. aureus*, (b) *B. subtilis*, (c) *E. coli*, (d) *P. aeruginosa* and fungi (e) *C. albicans*.

The unbalanced pH of the skin causes numerous disorders or illnesses such as skin irritation (inflammation) as well as reduced cell cohesion and permeability barrier in the stratum corneum. The micellization resulted when the pH of the skin is more than 6 but the structural disorders resulted when the pH of the skin below 4.5. The swelling behavior is admitted essential for polymer films which used for wound healing because tissue needs fluids to facilitate regeneration. The new blood vessel formation (skin tissue regeneration angiogenesis) from existing vessels is important for normal healing depends on the pH-sensitive films. So, the very effective factors of pro-angiogenic can found by using ideal candidates of pH-sensitive films to enhance angiogenesis and control release at the wound site [16].

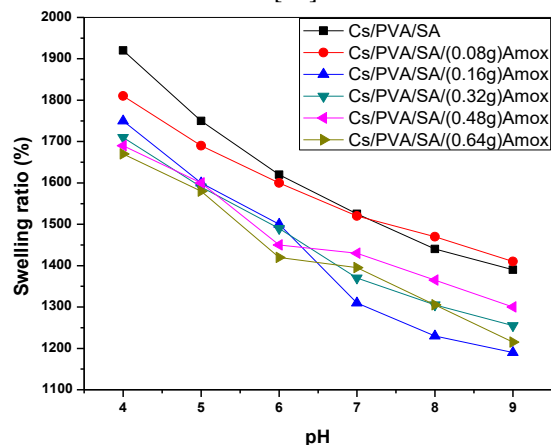


Figure 4. Swelling ratios of (Cs/PVA/SA) blend with different content of Amox against various pH values.

The swelling response of samples with various concentrations of the antibiotic drug (Amox) as a function of various (pH 4, 5, 6, 7, 8 and 9) showed in **Figure 4**. This figure shows that as the amount of (Amox) drug increase in the blend, swelling observes high at (pH 4). So, the system swells mostly in the acidic medium compared to the neutral or basic medium and explains based on the protonation of the amino groups in the system. This response may be due to the increased ionic strength of the swelling condition and reduce osmotic pressure between the external condition and the blend system. More explanations, the swelling ratio (SR) of the blend with various content of Amox decrease in acidic conditions (pH 4 -6) due to the lack of ionization regarding the carboxyl groups (-COOH). At neutral condition (PH 7), the swelling ratio also decreases but slightly changes appear at (Cs/PVA/SA/0.48g/g Amox) and (Cs/PVA/SA/0.64g/g Amox) due to the presence of the carboxyl functional group (-COOH) in the side chains of each polymer and drug which ionized to carboxylate (CH_3COO^-) and the electrostatic repulsion caused to some extent swelling behavior. At basic conditions (pH 8-9), the swelling ratio also drops due to ionization (deprotonation) of (-COOH) into (-COO). So, at neutral medium the blend with different content of drug slightly incorporated water in different forms around pores, hydrogen-bonded groups which gave slightly increase in swelling. Furthermore, the system can realize a large quantity of blood from the wound due to good absorption of water. At the same time, it can adhere to the wound surface and clot a ruptured wound [17, 18]. Interestingly, the crosslinking efficiency increase by

increasing the pH which maintains the compact structure of the ternary blend [19].

Amoxicillin with a ternary blend is the top candidate for wound healing and dressing applications and can determine swelling degree which permits to predict exudate ability. The variation of the swelling ratio of (Cs/PVA/SA) system at various values of pH (4, 5, 6, 7, 8 and 9) as a function of the different concentrations of Amox drug (0, 0.08, 0.16, 0.32, 0.48 and 0.64 g/g) represented in **Figure (5)**. At (pH 4- 6), the swelling ratios of the system decrease by increasing the concentration of Amox drug. At (pH 7), no noticeable changes in swelling ratio of the system with (0, 0.08 g/g) of Amox drug, sudden high dropping in swelling ratio reach to (1312%) which appear at concentration (0.16 g/g) of Amox drug then the swelling ratio return to increase especially at the concentration (0.48 g/g) of Amox drug and rise to (1430%). At (pH 8- 9), the swelling ratios dramatically decrease in swelling ratio of the system at the same concentration of Amox drug (0.16 g/g) which reach to (1228%, 1187%), then return to increase especially at the concentration (0.48 g/g) of Amox drug. This noticeable behavior occurs due to the increased ionic strength of the swelling condition. The conclusion from this figure, when the pH increased the swelling decrease due to increase cross-linked, the rigidity of network and compact structure of the blend due to

intra/inter molecular interaction then, the percent of solubilized and released amoxicillin reduces. This preserves Amox within the blend for prolonged periods (prolong time action of the drug) and get the ability to determine water uptake. Moreover, the swelling ability decreases due to the crosslinking degree hinder the disentanglements of the chain and intermolecular motion in the polymer blend [19, 20].

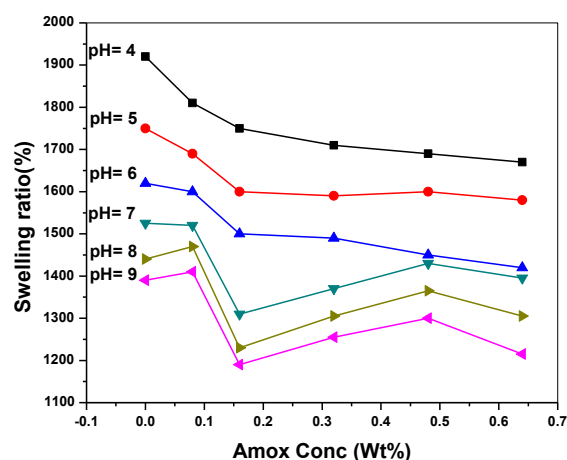


Figure 5. Swelling ratios of (Cs/PVA/SA) blend with various pH values versus various concentrations of Amox drug.

Table 2. Antibacterial studies of tested samples.

No.	Sample	IZD (mm)	A.I. %	IZD (mm)	A.I. %	IZD (mm)	A.I. %	IZD (mm)	A.I. %	IZD (mm)	A.I. %
1	S ₀	NA	----	2	8.7	2	8.3	NA	----	3	11.1
2	S ₁	NA	----	4	17.4	2	8.3	3	13.0	5	18.5
3	S ₂	5	19.2	9	39.1	6	25.0	7	30.4	8	29.6
4	S ₃	8	30.8	11	47.8	13	54.2	12	52.2	14	51.8
5	S ₄	11	42.3	17	73.9	15	62.5	16	69.6	19	70.4
6	S ₅	15	57.7	19	82.6	20	83.3	18	78.3	23	85.2
Ampicillin		26	100	23	100	24	100	23	----	NA	----
Colitrimazole		NA	----	NA	----	NA	----	NA	----	27	100

IZD (inhibition zone diameter), A.I. (activity index)

4. CONCLUSIONS

Different amoxicillin content (0.08, 0.16, 0.32, 0.48 and 0.64 g/g) have been mixed within chitosan/ polyvinyl alcohol/ sodium alginate (300 ml) blend. The effect of increasing the amoxicillin content on the different properties of the ternary blend has been investigated. Increasing the amoxicillin content within the ternary blend enhanced their antibacterial activity against both 2g (+), 2g (-) bacteria and one fungus. Whereas, C. Albicans resulted unique inhibition zones values at all amoxicillin

concentrations included within the ternary blend. The swelling of the samples was greatly influenced by the concentration of incorporated amoxicillin and the degree of crosslinking. Lowest swelling was noted in the samples due to high cross-linked between blend and antibiotic drug which get drug to prolong time action during wound healing which is advantageous for tissue engineering and wound healing applications.

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