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Mucoadhesive Electrospun Nanofibers of Chitosan/Gelatin Containing Vancomycin as a Delivery System

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Mucoadhesive oral delivery systems have been of interest to researchers because of improving bioavailability and patient compliance by increasing concentration gradient. This study aimed to investigate local delivery of vancomycin using against gram-positive bacteria. Vancomycin is not absorbed from the gastrointestinal tract, so it will be given by injection. Also, slow infusion of vancomycin in blood is so vital to reduce its severe side effects and is excreted through the urine. Controlled dose of vancomycin can reduce drug resistance. Here in, a blend of chitosan and gelatin loaded with vancomycin was electrospun. The obtained nanofibers were characterized by FTIR, UV and SEM. The swelling and stability of the nanofibers were evaluated. *In vitro* release of the vancomycin was measured over a 72-hours period by total immersion method. The average diameter of drug-loaded nanofiber was 384 nm. The release behavior of the optimum fibrous mat conform Higuchi kinetic model.

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Introduction

Post- antibiotic era has been started all over the word. It increased antimicrobial resistance (AMR) minor and curable bacterial infection has become dangerously fatal. Unfortunately, for various classes of produced antibiotics, at least one mechanism of resistance has been reported [1].

Vancomycin (VAN) is a first-line treatment usually prescribed for complex skin infections, bloodstream infections, endocarditis, bone and joint infections, and meningitis caused by *Methicillin-resistant Staphylococcus aureus* (MRSA) [2]. Although it is an effective agent, AMR to Van have been extended rapidly in most countries. Different trends have been reported to control AMR to Van and reduction of dosage is a prophylactic method. Various drug delivery systems (DDS) such as mucoadhesive DDS has been preferred to control drug release. Advantages of mucoadhesive DDS can be summarized as I) localizing a high concentration of the drug which leads to developed drug absorption and bioavailibity. II) Predicting the release rate and absorption of the drug passing from texture's membranes. III) Avoiding relative drug metabolism caused by the effect of hepatic first-pass [3].

Mucoadhesive DDS was made of biocompatible Electrospun Nanofibers that significantly improved controlling of drug release. Electrospinning is the best method for producing Nano fibrous patches, because the mentioned method is versatile, inexpensive, and flexible.

According to electrospinning principles, a charged electrode is placed in polymeric solutions and another electrode with opposite charge is connected to the collector. Afterward an electrical potential is applied between polymeric drops and collector. When electrostatic forces overcome surface tension of the polymeric drop, first jet is formed. The jet is unstable due to repulsive forces that make the jet longer and narrower to receive fibers with nano-size diameter. Simultaneously the existence solvent in polymeric jet evaporates in which solid polymeric fiber collects on the surface of the collector. The Morphology of nanofibers is controlled by different electrospinning parameters such as solution conductivity and concentration, polymer molecular weight, viscosity, applied voltage, feed rate, and etc. Polysaccharide nanofibers such as chitosan (CS) have been intensively investigated because of their biocompatibility and biodegradability features [4]. Since chitosan is a liner polysaccharide it is considered as FDA GRAS (Generally Recognized as Safe) made up of N-acetyl glucosamine with rigid D-glucosamine units. It contains many amino groups (pK_a = 6.2-7.0) and is water- soluble in aqueous acid [5]. Blending of natural polymers such as chitosan and gelatin (Gel) will introduce interesting biomaterial with high potential application. The interactions between the molecules of chitosan and gelatin will affect their characteristic. Van is a glycopeptide antibiotic used for the treatment of infections caused by methicillin-resistant staphylococci. It has a molecular weight of approximately 1500 Da, water soluble and poorly absorbed from the gastrointestinal tract or mucosal tissue [6]. Mucoadhesive material such as CS and Gel will improve mucosal absorption of various drugs such as Van [7, 8].

In the present work, a new mucoadhesive CS-Gel base patch of Van was prepared. The obtained nanofibers were investigated by SEM, and FTIR analysis. Drug release properties of the mat were study by total immersion method and UV spectrophotometer.

Materials and Methods

Materials

Chitosan (low molecular weight, Sigma Aldrich Co., USA), Polyethylene oxide (PEO) (600 KD_a, Across Co., USA), Glacial acetic acid (98%, Sigma Aldrich Co., USA, Grade: Reagent Plus®), NaOH (98%, Sigma Aldrich Co., USA, Grade: Reagent), KH_2PO_4 (Merck, Germany, Grade; ISO), Vancomycin (Jaber Ebne Hayyan Pharmaceutical Co., Iran) were used.

Apparatus

The electrospinning equipment was made of PARS NANO RIS CO. of Iran. The following instrument utilized for characterizing the nanofibers. The

scanning electron microscope (Philips XL30 microscope) at an accelerating voltage of 10 kV and Fourier transform infrared spectroscope (Bruker - USA) used for chemical bonds investigation. Release of VAN was investigated by UV-Vis (Agilent 8453) spectrophotometer. For optimum the electrospinning condition microscopic images was captured by 400X, EUROMEX Co., Netherlands.

Preparation of Nanofibers

CS and PEO with the weight percent ratio of 1.57: 4.23 were added to acetic acid–water- as 15.7: 78.49 (stirred at room temperature for 24 h). In addition, a solution of 15Wt% gelatin in distilled water was prepared separately (stirred at room temperature for 12 h). Different ratio of CS: Gel as 50: 50, 30: 70, and 20: 80 were mixed for 24 h at ambient temperature. Van and glutathione as a mucoadhesive agent was directly added to the best mixture include 5 % and 0.25 % of dry weights of polymers (CS-Gel) for electrospinning. It was stirred for 5 h at 25 ˚ C and it was electrospun under mentioned conditions. For electrospinning, 1 ml syringe with 23 gage needles placed in a digit pump with accuracy of 1.0 ml per minute. In addition, a rotary collector was placed in front of it. The CS-Gel solution was electrospun under different electric fields include 7, 12, 17, and 22 kV onto an aluminum sheet wrapped around the rotating cylinder (width and OD of the cylinder ≈ 10 cm; rotational speed ≈ 2.1 rpm and the distance between collector and syringe was 15 cm). The pump injection was 0.7 ml/h and the spinning time was 1 h for each sample.

Crosslinking

Here, Gluteraldehyde (GTA, 50 % wt) was used to improve the water stability and mechanical property of the nanofibrous mat. Therefore, the mat was placed in a vacuum desiccator at the vicinity of GTA vapor for two different residence times as 2 and 24 h at 37 °C. Finally, the water stability of the textural was analyzed by soaking in water for 72 hours in distilled water.

Swelling Study of CS-Gel Nanofibers

The swelling test of the Van loaded mat was estimated after immersing it in 10 ml distilled water (at 37 ˚C) for 60 sec. Then the mat was putted on filter paper for about 15 min. The weight of the mat before and after wetting was measured by a five-digit scale respectively as W_0 and W_t. Swelling index calculated by the following formula:

$$\text{Index of swelling} = [(W_t - W_0) / W_0] \times 100 \quad (1)$$

In vitro Release Study

In vitro release of Vancomycin from nanofibrous mat was investigated by the immersion method. About 50 mg of nanofibers was placed in a dialysis bag (12000DaSERVA, MWCo), then 2 ml PBS (pH = 7.4) was added to each bags. This part acted as the donor solution. The bag immersed in 50 ml of receptor medium (phosphate buffer saline 0.2 M, pH 7.4) and incubated at 37°C under magnetic stirring (about 400 rpm). At specified time intervals, 1 ml of medium was taken and was replaced with the same volume of fresh buffer. For drug concentration assay, the taken samples were analyzed at 283 nm using UV-Vis spectrophotometer [9].

For In vitro release kinetics evaluation, several approaches can be used such as analysis of variance, model-independent and model-dependent approaches. In this study, model-dependent approaches were used for comparison of dissolution profiles. The liner regression of the data was obtained by Microsoft Excel software.

In model-dependent approaches, release data were fitted to kinetic models including the zero-order (Eq. 2), first-order (Eq. 3), and Higuchi model (Eq. 4) release equations to find the equation with the best fit [10].

$$C = K_0 t \quad (2)$$

$$\text{Log } C = \text{Log } C_0 - Kt/2.303 \quad (3)$$

$$Q = Kt^{1/2} \quad (4)$$

In (Eq. 2) and (Eq. 3), C_0 is the initial concentration of drug, k is the first order rate constant, and t is the time. In (Eq. 4) Q is the

amount of drug released in time t per unit area and K is the Higuchi dissolution constant [10].

Results and discussion

Figure 1 shows the microscopic images of nanofiber captured using light microscope. When the ratio of Gel was increased, the spin ability of the solution was improved. The blend of CS and Gel makes a polyelectrolyte complex through formation of hydrogen bonds between amine (-NH_3^+) of CS and carboxylic acid (-COO^{2-}) of Gel. These interactions undermine the rigid forces of two polymers and make them ionize-able which CS will be more dissolvable and e-spin able [6]. With increasing applied voltage from 7 to 22 KV a decrement in fiber formation is observed that is due to an increment in surface charges and repulsion of positive charges. The optimum fiber which has the higher percentage of CS and higher fiber density was obtained in the ratio of 70/30 of CS/Gel and 17 KV voltage was applied.

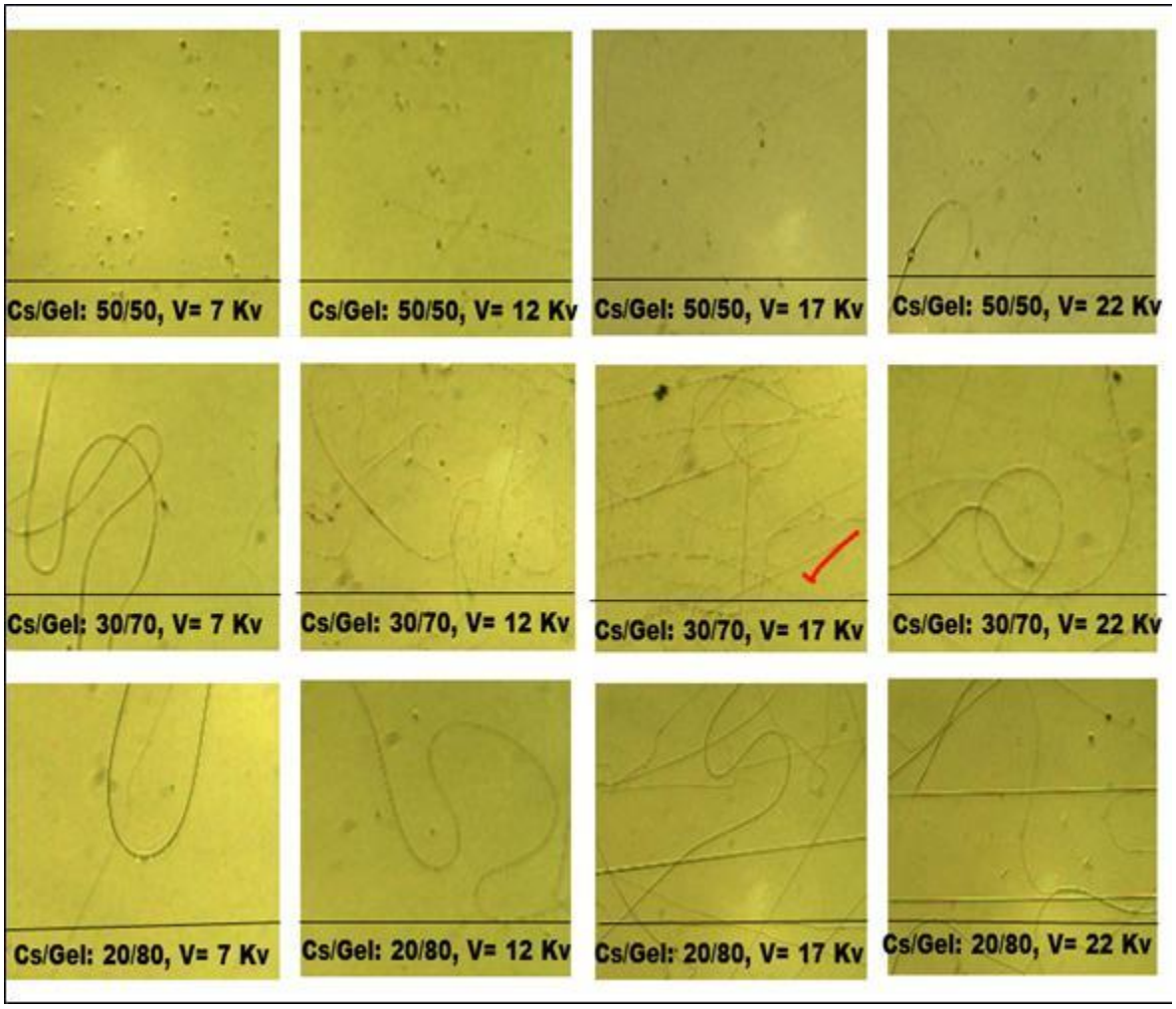

Fig. 1. Microscopic images of Cs/Gel nanofibers (400X).

The SEM images of the optimum sample before and after addition of Van were investigated. The average diameter of the nanofibers without Van was 184 nm, so it was 384 nm after loading Van to initial solution (Figure 2). In addition, fiber morphology rarely changes to ribbon like structure in which is due to electrical conductivity of CS/Gel solution (Figure (2- A, B). In ribbon like

structure, the cross section of fiber changes from circular shape to hyperbola and the perimeter is like the first jet [11].

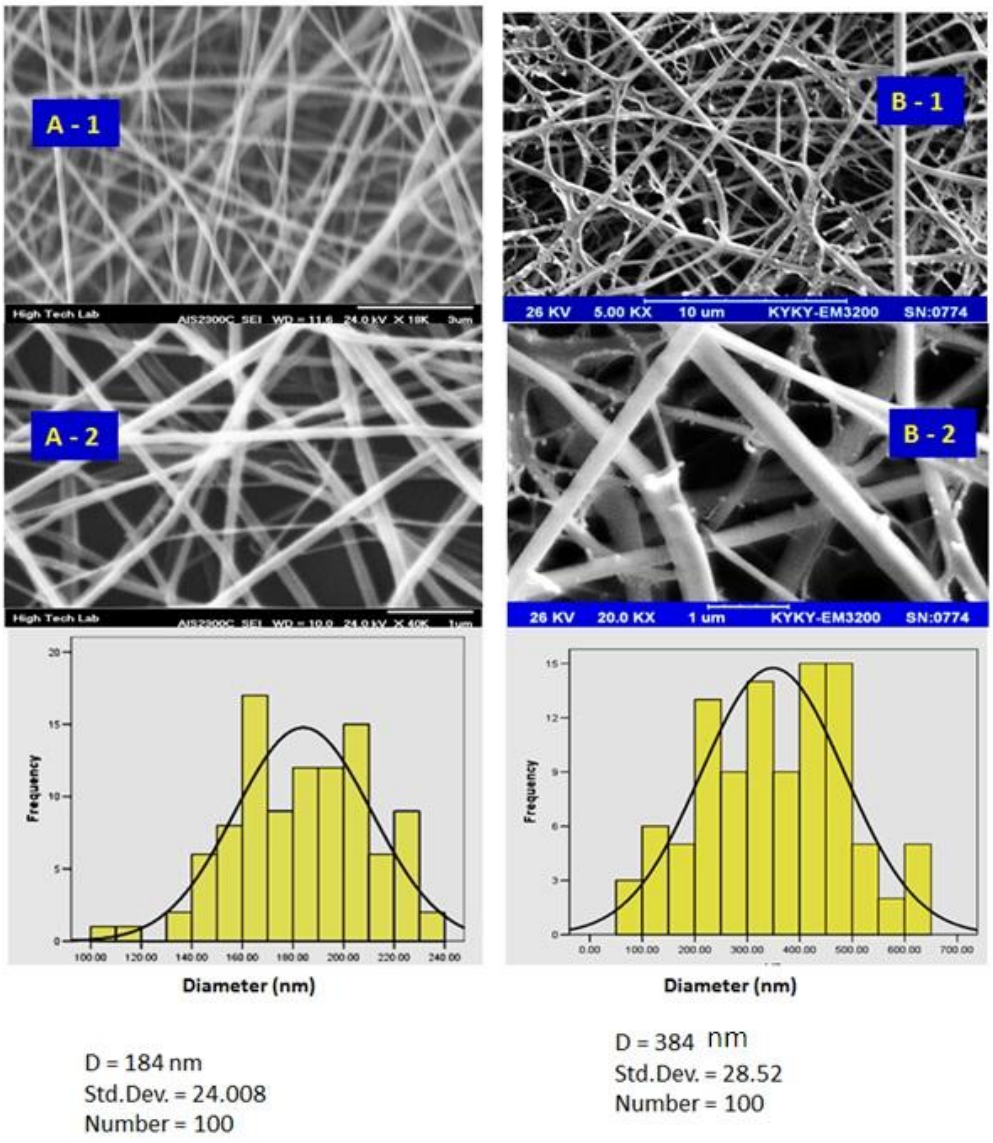

Fig. 2. SEM micrographs of Van free fibers (A-1, A-2), Van loaded fibers (B-1, B-2).

Another phenomenon, which could be indicated by SEM images, was spider like structure that is also related to ionization of polymeric solution [12]. After addition of Van, spider like structure is more obvious that is due to negative and positive charges dispersed on the surface of the fiber. During electrospinning process, the thinner fibers (with average diameter of 53 nm) were formed. Figure 3 shows that phenomenon schematically. It is constructed of electron donor (OH ،-C=O), electron donate (NH-) groups and CS molecules which possess negative charges during ionization. Consequently, the density of the electron donates groups is higher. After dissolving of Van in polymeric solution with hydrophilic acetic acid (dielectric constant = 2.6) as solvent, density of negative charges will increase [13].

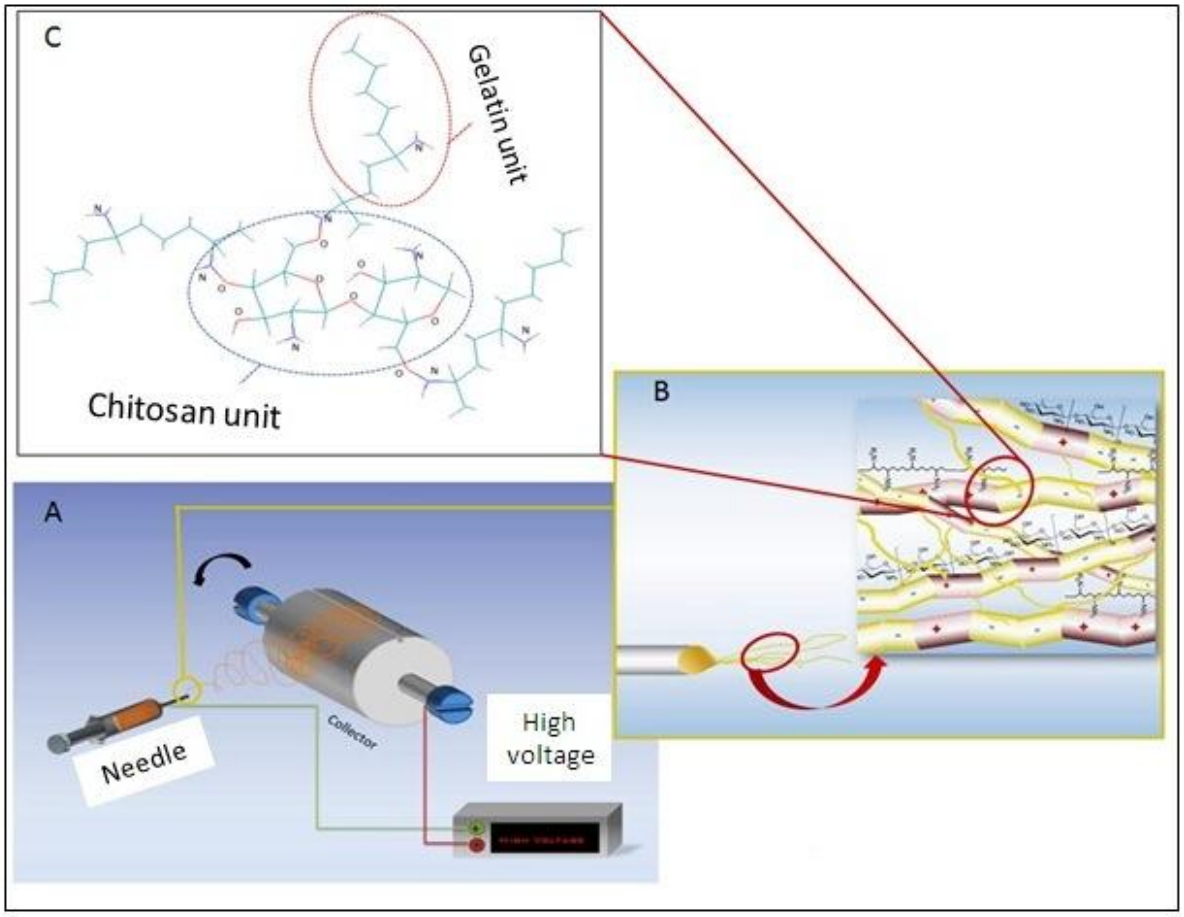

Fig. 3. Schematic of electrospinning method (A) spider-like network formation (B), schematic of CS/Gel complex chemical structure.

Figure 4 shows the FTIR spectra of Gel, CS, and Gel-CS. Due to similar functional groups of CS and Gel, similar absorption bands are detected. Amine groups of CS are overlapped by absorption of OH groups at the range of 3200-3500 cm^{-1}. The major characteristic peaks at 898 and 1153 cm^{-1} are related to the saccharide structure as a repeating unit of CS. Also two peaks at 2850 and 2916 cm^{-1} identified –OH, $–CH_2$, and $–CH_3$ groups. The peak at 1639 cm^{-1} and 1543 cm^{-1} in pure CS, can be interpreted as amide groups (CN=O) and type II amide(N-H) respectively. NH- and OH- at aliphatic groups are detected at 1581 and 1423 cm^{-1}. The peaks at 1381 cm^{-1} shows type III amides. At CS spectrum, the broad peak at 1083 cm^{-1} indicates the C–O stretching vibration. The sharp peak at 1381 cm^{-1} could be assigned to the CH_3 symmetrical deformation mode.

Pure gelatin (Figure 4-B) shows a peak at 1639 cm^{-1}, which corresponds to peptide bonds (C=O).

The peak at 1539 cm^{-1} of Gel spectrum is related to C-N-H bending. The peak at 1458 cm^{-1} shows C-H bending. Two peaks at 2850 and 2920 display stretching vibration of C-H. Peptide bond of stretching N-H is detected at 3460, 3414, and 3240 cm^{-1}.CS-Gel spectrum shows a sharp peak at 1107 cm^{-1} related to saccharide ring of CS. Two different peaks at 1655 and 1538 cm^{-1} can be related to stretching C=O in N-acetyl group and ammonium ions (NH_3^+). The result suggests

polyanionic– polycationic interactions between gelatin and chitosan [13].

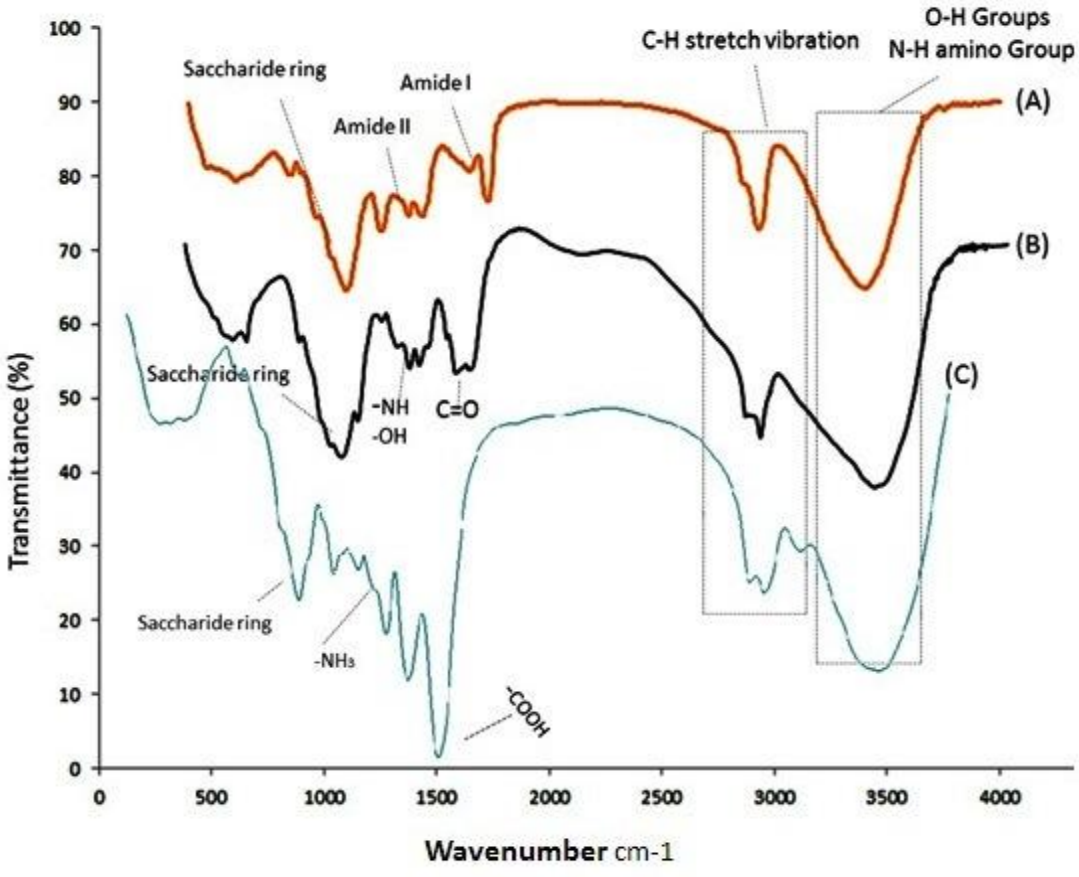

Fig. 4. FTIR spectra of CS (A), Gel (B) and CS/Gel drug loaded nanofiber (C).

Water stable nanofibers resulted from reaction of free amine groups of lysine or hydrolyzing of residual amino acids in the polypeptide chains of the polymers with aldehyde groups of GTA. During crosslinking reaction, the mat color changed to light yellow. The mat was shrunk because of establishing imide groups (CH=N) between free amine of Gel and CS and aldehyde groups of GTA [13].

After crosslinking, the mat was stable more than 3 days. The result of swelling test shows that the average swelling index (after crosslinking 24 h at vicinity of GTA vapor) was 3.88%. While the swelling index (after crosslinking 2 h at vicinity of GTA vapor) was about 50.2%. It can predict to have different release profiles.

Release behavior of Van loaded CS-Gel with different time of crosslinking as 2 and 24 hour is shown in Figure 5 and Figure 6 respectively. As Figure 5 displays about 30 % of Van exited in the first 15 min and stayed approximately stable until 7 h. After 22 h, about 75 % of Van released and received to 87% in 28 h.

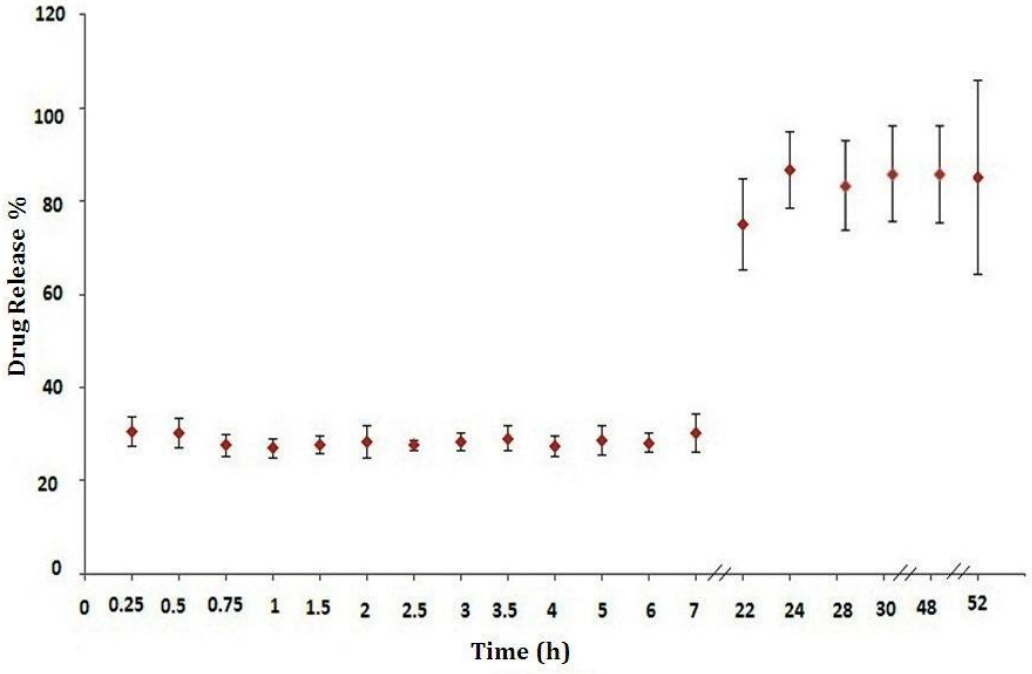

Fig. 5. *In vitro* release profile of Van from the mat cross-linked 2 h at GTA vapor.

Figure 6 presents the release behavior of the mat with same condition, but different time of cross-linking (staying 24 h with GTA vapor). In the first six hour, about 18 % of Van exited the mat. It increased approximately to 29% after 7 h. 40, 59, and 87 percent of Van was released after 22, 48, and 72 hour respectively.

Although comparison the release behavior from CS-Gel mat in the different retention time in GTA vapor, it can be understand that with increasing the time of crosslinking, the release behavior changed from delay to sustained release profile. The overall release and dissolution profile of Van from the nanofibrous mat is described using model dependent method. For this purpose, zero order, first order, Higuchi, and Hixson-Crowell equations were investigated [14].

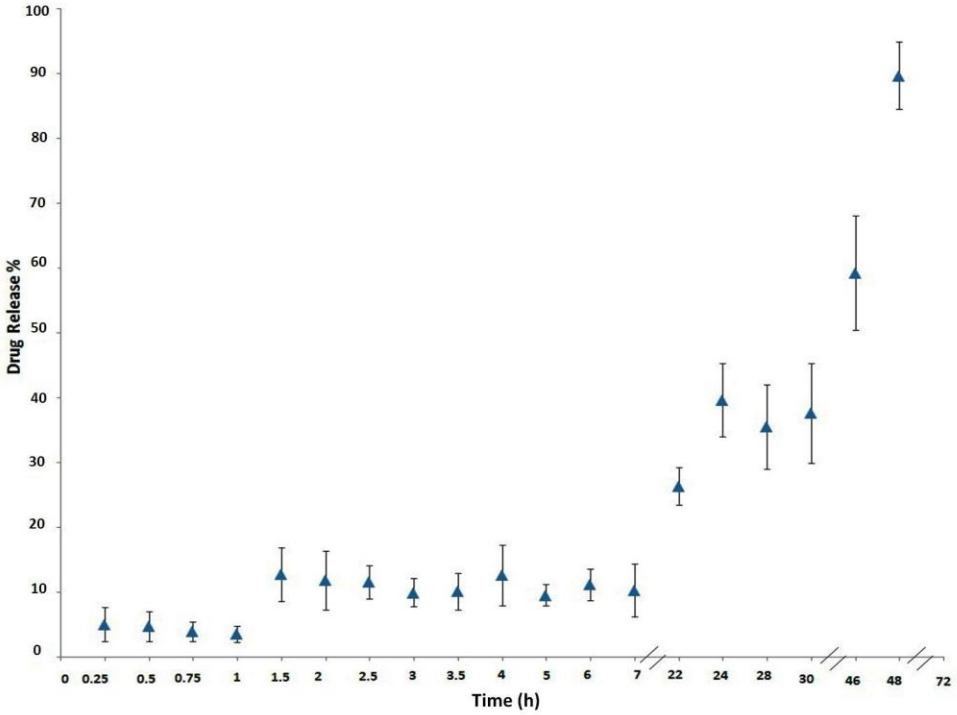

Fig. 6. *In vitro* release profile of Van from the mat cross-linked 24 h at GTA vapor.

Table 1 summarizes the data, obtained from 2 h cross-linking (placed 2 h at GTA vapor). As is shown, according to the regression the best model was Higuchi with R^2=0.7164. In addition, Table 2 shows the data of 24 h cross-linked mat (placed 24 h at GTA vapor). Higuchi (R^2=0.8871) and first order (R^2=0.8649) models were the best models for fitting the data.

Table1. The parameters of model dependent fitting for the cross-linked mat with 2 h at GTA.

Parameter	Zero order model	First order model	Higuchi model
K	-0.0795	31.38	0.5643
R^2	0.6394	0.8649	0.8871

Table 2. The parameters of model dependent fitting for the cross-linked mat with 24 h at GTA.

Parameter	Zero order model	First order model	Higuchi model
K	-0.0201	-0.06149	0.0478
R^2	0.3445	0.4539	0.7164

To find out the mechanism of Van release, first 60% of the release data were fitted in Korsmeyer-Peppas model [15].

Table 3. Interpretation of diffusional release mechanisms from polymeric films.

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fick diffusion	$t^{-0.5}$
0.45<n=0.89	Non-Fickian transport	t^{n-1}
0.89	Case II transport	Zero order release
Higher than 0.89	Super case II transport	t^{n-1}

$$\mathbf{Log\ (M_t/M_\infty) = logk + nLogt} \qquad \mathbf{(5)}$$

Where M_t/M_∞ is the fraction of drug release at time t_n, k is the release rate constant, and n is the release exponent. Table 3 is used to predict the mechanism of drug release.

The calculated data that is shown in Table 4 describes a Fickian release mechanism for Van. It can be stated by Higuchi equation. It confirmed the obtained results from model dependent method.

Table 4. The obtained parameters of Korsmeyer-Peppas model.

Samples	R^2	n	K
24 h cross-linked	0.8919	0.2491	0.3706
2 h cross-linked	0.3838	0.1991	0.2307

Conclusions

The current study investigated a nanofibrous mat of CS-Gel as delivery system for vancomycin, it became water stable using GTA as crosslinking agent. The FTIR results showed a poly-cationic complex between CS and Gel. The average diameter of the Van loaded fibers was 384 nm. Ribbon-like and spider-like network were the dominant phenomenon in the fibers. The effect of crosslinking time on release behavior was study. With increasing the residence time of the mat in GTA vapor, release profile changed from delay to sustained release and swelling index reduced about 120%. It indicated the effect of dissolution on release behavior. The long-term releases model of Van was Higuchi with Fickian mechanism, it is appropriate for antibiotic and mucoadhesive drugs. The infusion time of Van must be so slow to reduce its severe side effects; the proposed system seems suitable to reduce the time Van infusion and increase blood concentration of Van that can lead to increase bio-availability and patient compliance.

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Conflict of Interests

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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