

ELECTROSPINNING OF CYCLODEXTRIN FUNCTIONALIZED CHITOSAN/PVA NANOFIBERS AS A DRUG DELIVERY SYSTEM

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Abstract A new type of nanofibrous structure from chitosan bearing carboxymethyl- β -cyclodextrin (CS-g- β -CD) as a novel drug delivery system was synthesized by grafting carboxymethyl- β -cyclodextrin (CM β -CD) onto chitosan (CS) in the presence of water soluble 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as the condensing agent and *N*-hydroxysuccinimide (NHS). Defect free mats containing CS-g- β -CD have been fabricated using electrospinning of an aqueous solution of poly(vinyl alcohol) (PVA)/CS-g- β -CD blends. The morphology and diameter of the electrospun nanofibers were examined by scanning electron microscopy (SEM). The average fiber diameter was in the range of 130–210 nm. SEM images showed that the morphology and diameter of the nanofibers were mainly affected by weight ratio of the blend at constant applied voltage. The results revealed that increasing CS-g- β -CD content in the blends decreases the average fiber diameter. It was observed that the PVA/CS-g- β -CD nanofibrous mat provided a slower release of the entrapped drug in compare to PVA/CS nanofibrous mat.

Keywords: Chitosan; β -cyclodextrin; Nanofibers; Drug delivery.

INTRODUCTION

Cyclodextrins (CDs) are a family of cyclic oligosaccharides composed of 6, 7, or 8 D(+)-glucose units joined by R-1,4-glucosidic linkages. The most important known CDs are α , β and γ -CDs which consist of six, seven and eight D-glucose units, respectively. CDs are applied in drug delivery systems to enhance the solubilization, stabilization and controlled adsorption of drugs due to their capability of forming complexes in the inner cavities^[1–2]. CDs, with lipophilic inner cavities and hydrophilic outer surfaces, are capable to form inclusion complexes with drugs of adequate size. During the complex formation with drug molecules, no covalent bonds exist between CDs and its guest, so complexation can be considered as a dynamic process. As drug carriers, CDs allow the solubilization, stabilization and transport of hydrophobic drugs together with several pharmacological benefits such as the reduction of unwanted side effects^[1–10].

Chitosan (CS) is a bioactive polysaccharide prepared by the *N*-deacetylation of chitin, a natural polysaccharide present in shells of crustaceans such as crabs and shrimps. CS is widely used in pharmaceuticals because of its attractive biocompatibility, biodegradability and antimicrobial activity^[11]. Grafting CD molecules onto CS may lead to a molecule carrier that possesses the cumulative effects of inclusion, size specificity and transport properties of CD and attractive properties of CS^[5, 6, 12].

Electrospinning is found to be a versatile and cost-efficient technique for producing multi-functional nanofibers with diameter in the range of nano to a few microns using electrically driven jet of polymer solution or melt. When the diameters of polymer fiber materials are shrunk from micrometers to submicrons or

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nanometers, it provides several amazing characteristics such as large surface area to volume ratio, flexibility in surface functionalities, and superior mechanical performance compared with any other known form of the material. Several authors have explained details of the electrospinning process; for the sake of brevity only a few are mentioned in the list of references^[13–16]. Morphology and diameter of electrospun fibers depend on a number of parameters such as polymer type, conformation of polymer chain, viscosity (concentration) of the solution, conductivity, polarity and surface tension of the solvent, applied field strength, distance between the capillary and collector and feeding rate^[13, 14].

Chitosan/cyclodextrin nanoparticles were prepared by Krauland *et al.*^[3] and their potential for the association and delivery of macromolecular drugs was evaluated. They showed that the release profiles of the associated macromolecules were highly dependent on the type of molecules and their interaction with the nanomatrix. In their results, insulin was very fast released whereas heparin remained highly associated to the nanoparticles for several hours^[3]. Uyar and coworker^[4] studied electrospinning of cyclodextrin functionalized polyethylene oxide (PEO) nanofibers. They found that presence of the CDs in the PEO solutions facilitate the electrospinning of bead free nanofibers from the lower polymer concentrations. This behavior is attributed to the high conductivity and viscosity of the PEO/CD solutions. Synthesis and drug release properties of hydroxypropyl chitosan-graft-carboxymethyl β -cyclodextrin microparticles were investigated by Prabakaran^[5]. They concluded that hydroxypropyl chitosan-graft-carboxymethyl β -cyclodextrin provided a slower release of the entrapped ketoprofen than chitosan. Chitosan bearing β -cyclodextrin nanocomplexes for controlled protein release was synthesized by Zhang *et al.*^[6]. Prabakaran^[7] has synthesized thiolated carboxymethyl chitosan-*g*- β -CD as a drug delivery carrier. Recently Gonil and coworkers^[10] reported synthesis of a novel water-soluble quaternized chitosan containing β -CD and evaluated its antimicrobial activity^[12]. Nanofibers of water soluble non-ionogenic polymers such as PVA^[17] have been successfully electrospun, whereas CS derivatives are hardly processable in order to be electrospun into fibrous structure. Electrospinning of CS and its derivatives is feasible using a second flexible polymer such as PVA^[18] or poly(vinyl pyrrolidone)^[19]. PVA was used as a non-ionogenic polymer partner due to being a non-toxic, water-soluble, biocompatible and biodegradable synthetic polymer for biomedical field applications^[18, 20].

According to the previous research^[1, 2], CDs are the agents with capability of entrapping drug molecules. On the other hand, CS is a biopolymer, which is able to react with CDs. It is postulated to combine the strengths of the CDs and CS *via* grafting to each other. To the best of our knowledge, no attempt has been made to fabricate nanofibers containing chitosan-graft- β -cyclodextrin (CS-*g*- β -CD). For this purpose, an attempt has been made to fabricate a biodegradable nanofibrous mat of PVA/CS-*g*- β -CD blends *via* electrospinning technique. Finally, the control release of salicylic acid, as a drug model, from the PVA/CS-*g*- β -CD electrospun fibers was investigated.

EXPERIMENTAL

Materials

Chitosan (CS) (viscosity average molecular weight 20 kDa, degree of *N*-deacetylation 86%) and β -CD (molecular weight 1135 Da) were purchased from Acros Organics Company. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), poly(vinyl alcohol) (PVA) (M_w = 72 kDa, degree of hydrolysis 98%), salicylic acid and methanol were purchased from Merck.

Synthesis of CM β -CD

CM β -CD was prepared according to a method reported in the literature^[7, 12]. Briefly, a mixture of β -CD (5.675 g, 5 mmol) and sodium hydroxide (5 g, 0.125 mol) in 20 mL of distilled water was treated with 0.4725 g (5 mmol) of chloroacetic acid at 50 °C for 5 h. Then the reaction mixture was adjusted to pH 7 by adding HCl. After neutralization, the obtained product was precipitated with excess amount of acetone and dried at 40 °C under vacuum.

Synthesis of CS-g- β -CD

The coupling reaction of CM β -CD onto CS was carried out in water at pH 7.0, in the presence of EDC and NHS. A solution of CM β -CD (1.193 g, 1 mmol) in 40 mL of distilled water was first activated with 0.192 g (1 mmol) of EDC and 0.115 g (1 mmol) of NHS for 30 min. With this mixture, 0.181 g (1 mmol) of chitosan dissolved in 10 mL of 1% (V/V) acetic acid was added dropwise. The reaction was carried out at room temperature under constant stirring. After 8 h, the product was dialyzed against deionized water using a dialysis tubing (molecular weight cutoff 2 kDa) for 3 d.

Measurement of the Apparent Amount of Grafting

The apparent amount of grafting was determined according to the procedure described by Zhang *et al.*^[6]. Briefly, 15 mg of CS-g- β -CD was hydrolyzed in 10 mL of sulfuric acid (0.5 mol/L) for 10 h at 100 °C. The hydrolysate was diluted to 50 mL and the concentration of glucosyl unit determined with a spectrophotometer at $\lambda = 490$ nm. The apparent amount of grafting was calculated using Eq. (1),

$$Q = \frac{C \times 50}{180 \times 7 \times w} \quad (1)$$

where Q is the apparent amount of grafting ($\mu\text{g CD/mg CS-g-}\beta\text{-CD}$), C is the concentration of glucose unit ($\mu\text{g/mL}$), and w is the weight of CS-g- β -CD (mg). The apparent amount of grafting for the product was 49 $\mu\text{g CD/mg CS-g-}\beta\text{-CD}$.

Preparation of Electrospinning Solutions

CS-g- β -CD solution was prepared by dissolving CS-g- β -CD in 1% acetic acid at room temperature with gentle stirring in order to prepare 3% (W/V) homogeneous solution. Afterward PVA was dissolved in distilled water at 70 °C with vigorous stirring for 1 h to make 9% (W/V) homogeneous solution. Then, the CS-g- β -CD solution was mixed up with PVA solution in the weight ratios of (PVA/CS-g- β -CD) 100/0, 90/10, 80/20, 75/25, 70/30 and 50/50. The mixture was stirred for further 1 h before electrospinning.

Salicylic acid in concentration of 5% (W/W) on the weight of PVA/CS-g- β -CD was dissolved in a small amount (2 mL) of methanol and was added to (PVA/CS-g- β -CD) solution and was stirred for 20–30 min before electrospinning.

General Procedure for Electrospinning Process

About 2 mL of PVA/CS-g- β -CD solution was put into a 5 mL syringe with a stainless steel needle (inner diameter: 0.7 mm) attached at the open end. The syringe was fixed horizontally on the syringe pump (New Eva Pump System Inc., USA) and the solutions were electrospun by using a standard high voltage power supply (Gamma High Voltage Research, USA), which is capable of generating DC voltages in the range of 0–30 kV. For the electrospinning process a feed rate of 1 $\mu\text{L/min}$, an applied voltage of 17 kV and tip-to-collector distance of 15 cm were used, at normal laboratory condition (*i.e.*, about 22 °C).

Stabilization of Electrospun (PVA/CS-g- β -CD) Fibrous Mats Containing Salicylic Acid

Due to the water solubility of the PVA/CS-g- β -CD mats, before evaluation of drug release, samples of electrospun PVA/CS-g- β -CD (90/10, 75/25) mats were stabilized by methanol for 24 h and were then dried overnight at room temperature. For the purpose of comparison, casted film with the same composition was also treated with methanol in the same way as for electrospun fibers. The amount of salicylic acid released during stabilization was determined by UV spectrophotometer at $\lambda_{\text{max}} = 303$ nm for each stabilized sample.

Drug Release Study

The release of salicylic acid from the stabilized electrospun mats was determined by placing a known mass of the material (10 mg) in 10 mL phosphate buffer (PB) of pH 7.4 and was shaken at 37 °C and at room temperature (20 °C). The salicylic acid release was monitored by monitoring the absorbance at $\lambda_{\text{max}} = 303$ nm as a function of time.

Characterization Methods

FTIR spectra were recorded using a Nicolet Magana IR560 Fourier-transformed infrared spectrometer. Conductivity of each blend solution was measured by an electric conductivity meter (Sartorius professional meter pp-50). Shear viscosities of the spinning fluids were measured at 22 °C with the aid of a Brookfield viscometer (DVII+, USA). Absorbance measurements were carried out in a UV-Visible spectrophotometer model Cintra 10. Calibration curve of absorbance against different concentrations of salicylic acid was obtained at 303 nm. A scanning electron microscope (SEM) XL-30 (Philips Co. HOLLAND) was used to study the morphology and surface texture of the fabricated nanofibers. Measurements of about 100 random points on the fibers were used for determining fiber diameter distribution and the average fiber diameter.

RESULTS AND DISCUSSION

Characterization of CS-g- β -CD

The Grafting of CM β -CD onto chitosan was confirmed by FTIR spectroscopy. The FTIR spectrum of chitosan is shown in Fig. 1. The absorption band at 3415 cm^{-1} represents OH stretching; a peak at 2920 cm^{-1} confirms the aliphatic C—H stretching. The absorption band between 1220 and 1020 cm^{-1} could be assigned to the free primary amino group ($-\text{NH}_2$) in the chitosan. The peak at 1650 cm^{-1} represents the acetylated amino group, which indicates that the sample is not fully deacetylated. Finally, the peak at 1385 cm^{-1} represents the $-\text{C}-\text{O}$ stretch of primary alcoholic groups. The FTIR spectrum of β -CD in Fig. 1 shows a strong $-\text{OH}$ absorption band at 3392 cm^{-1} and a strong $-\text{C}-\text{O}-$ band around 1029 cm^{-1} as characteristic bands of β -CD. Figure 1 shows a strong peak at 1720 cm^{-1} in the FTIR spectrum of CM β -CD, which could be assigned to the stretching vibration of carbonyl group of CM β -CD. This peak is not observed in β -CD and confirms the synthesis of CM β -CD. In the Fig. 1, the characteristic peak of the β -pyranyl vibration of chitosan at 894.9 cm^{-1} and the characteristic peak of the α -pyranyl vibration of CM β -CD at 945.7 cm^{-1} both appeared in the FTIR spectrum of CS-g- β -CD confirming the grafting of CM β -CD to chitosan. Furthermore, in the FTIR spectra of CS-g- β -CD, the relative intensity of the 3425 cm^{-1} band of CS-g- β -CD is much higher than the absorption band of OH stretching of chitosan. This could be attributed to the presence of more OH groups in CS-g- β -CD that resulted from the coupling of CM β -CD.

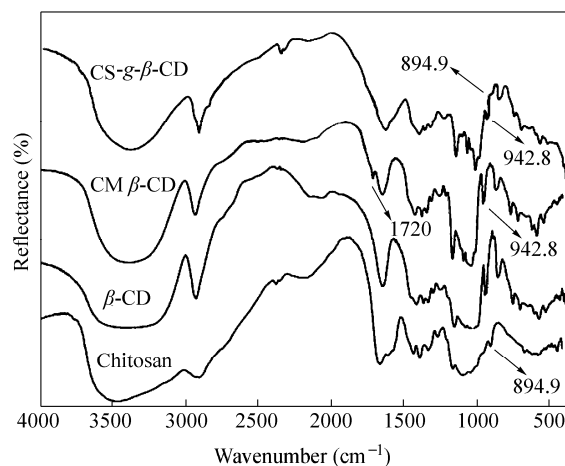


Fig. 1 FTIR spectrum of chitosan, β -CD, CM β -CD and CS-g- β -CD

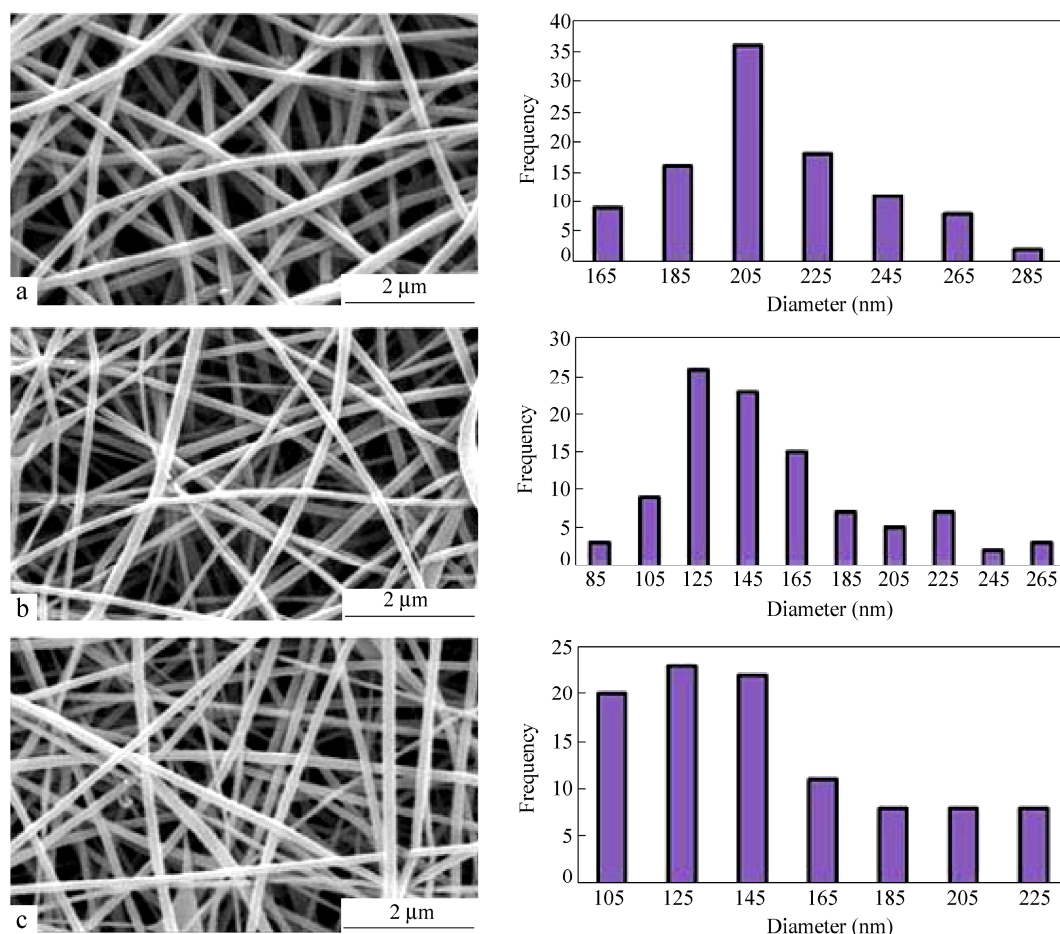
Preparation of Nanofibers, Effects of Electrospinning Parameters

Electrospinning of PVA/CS-g- β -CD blend solutions: effect of blend ratio

Due to the viscosity of CS-g- β -CD our attempts to obtain fibrous structure from the pure CS-g- β -CD solutions with a concentration of 3% (W/V) were unsuccessful and only nanoparticles were collected on the target plate

instead. Viscosity is an important rheological property to assess spinnability of the polymer solutions. Generally, there is a critical viscosity for a solution to be electrospun; below this viscosity, chain entanglements would be insufficient to stabilize the coulombic repulsion within the ejected jet, leading to the formation of sprayed droplets.

In order to increase viscosity of the electrospinning solution, PVA was selected as a suitable non-ionogenic partner for the preparation of blend nanofibers containing CS-*g*- β -CD. A series of experiments were carried out using various amounts of the CS-*g*- β -CD (10%–50%) in the blend. In the electrospinning, cylindrical continuous defect free fibers were obtained and no formation of defects along the electrospun fibers in the whole studied range of blend ratios of PVA/CS-*g*- β -CD was observed. Figure 2 depicts the SEM images and average diameter distribution of the electrospun nanofibers. The results showed that diameters of the electrospun fibers of PVA/CS-*g*- β -CD blends were in the range of 130–210 nm, depending on the electrospinning conditions. It can be clearly seen that composition of the spinning solutions had a significant effect on the average diameter and size distribution of the fibers. With the increase in CS-*g*- β -CD content, a decrease in the average diameter of the fibers was observed at a constant applied field. This observation could be attributed to viscosity and conductivity of the solutions, which are more important parameters affecting the solutions spinnability and the morphology of electrospun fibers.



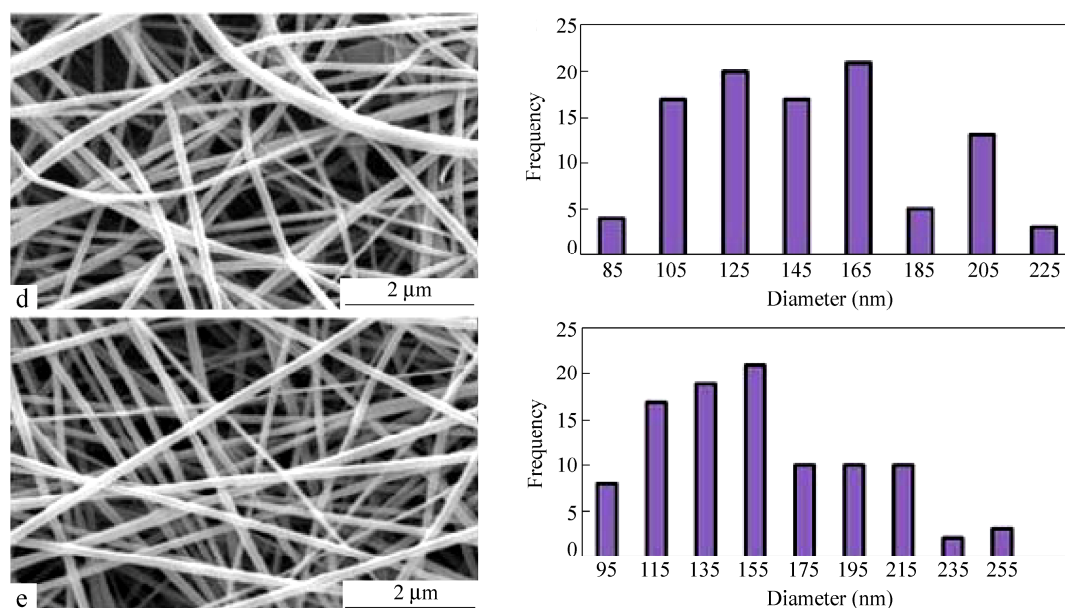


Fig. 2 SEM micrographs and average diameter distribution of the fibers prepared from PVA/CS-*g*- β -CD blend solutions: PVA/CS-*g*- β -CD = 90/10 (a), 80/20 (b), 75/25 (c), 70/30 (d) and 50/50 (e) (Applied voltage = 17 kV, spinning distance = 15 cm.)

Figure 3 shows shear viscosity and electrical conductivity of the electrospinning solutions by varying PVA/CS-*g*- β -CD blend ratio. This figure shows that the shear viscosity of the PVA/CS-*g*- β -CD blend solutions decreases whereas the electrical conductivity of the solutions increases with an increase in CS-*g*- β -CD content.

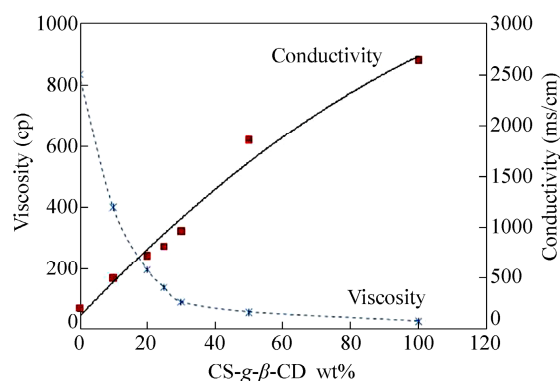


Fig. 3 Viscosity and conductivity of PVA/CS-*g*- β -CD blend solutions

In the electrospinning process, the viscosity and conductivity of the solution are two important parameters that have significant effects on the morphology of the resulting electrospun fibers. In general, high viscosity and high conductivity facilitate the electrospinning and yield bead-free nanofibers^[21]. The results show that viscosity of the PVA solution is higher than that of the CS-*g*- β -CD solution; confirming a decrease in viscosity of the blend solution with an increase in CS-*g*- β -CD content (Fig. 3). Therefore, the stretching of jet during electrospinning is more effective at higher CS-*g*- β -CD content. As a result, the fibers diameters decrease with the addition of CS-*g*- β -CD content in the blends. Furthermore, it was found that the conductivity of the blend solutions increased with an increase in CS-*g*- β -CD content. This could be attributed to the formation of a higher charge density on the surface of the ejected jet during electrospinning. As the charges carried by the jet increase,

higher elongation forces are imposed to the jet under the electrical field. Thus, the higher CS- β -CD content causes higher charge density on the ejected jet and decreases in the diameters of the final fibers. This is in agreement with the findings of Son *et al*^[22].

In vitro drug release

When an electrospun PVA mat is immersed in water, the mat shrinks and becomes transparent and gelatinous. Therefore, the electrospun PVA/CS- β -CD mat was stabilized towards disintegration in water by simple soaking in methanol. Soaking the drug containing electrospun PVA/CS- β -CD in alcohol preserves the integrity of the mat when it is immersed in water. However, there is loss of the drug entrapped in the fibers in the first few hours of alcohol treatment. To evaluate the total content of salicylic acid in the stabilized samples, a known amount of the mat or film (10 mg) was immersed in methanol. During this treatment the amount of drug lost in alcohol was determined for each sample by UV spectrophotometer at $\lambda_{\text{max}} = 303$ nm. The results are summarized in Table 1 and used in drug release study in buffer solution.

Table 1. Salicylic acid content for electrospun mats and films made from PVA/CS- β -CD after sonication for 24 h at 60 °C

Polymer code	Found after stabilization (mg salicylic acid)/(g polymer)	Calculated
PVA/CS- β -CD(90/10)-spun	39.98	50
PVA/CS- β -CD(75/25)-spun	41.08	50
PVA/CS- β -CD(90/10)-film	30.89	50
PVA/CS- β -CD(75/25)-film	33.58	50

The release profiles of the salicylic acid drug in buffer solution (pH 7.4) from electrospun fiber and cast film of PVA/CS- β -CD and PVA/CS at room (20 °C) and body temperature (37 °C) are shown in Figs. 4 and 5, respectively. Generally, this study suggested that salicylic acid release profile of PVA/CS- β -CD presented a slow and steady release into the medium and equilibrium was reached after 24 h. In contrast, PVA/CS nanofibers, which have less ability to form inclusion complexes, released salicylic acid much faster (most of the drug was released within 7 h). The slow release of drug from PVA/CS- β -CD electrospun fiber and cast film could be attributed to host-guest complex formation between salicylic acid and CM β -CD. There are different factors involved in the complex formation of CS- β -CD with salicylic acid such as hydrophobic-hydrophobic interactions between the hydrophobic moiety of the guest molecules and the CD cavity^[5].

Figures 4 and 5 show that the electrospun fibers of PVA/CS- β -CD (90/10) released about 45% of its drug content within 2 h, and then it released about 80% of drug content during 24 h at room temperature (Fig. 4), whereas 52% and 86% of the drug content was released at 37 °C within the same period of time (Fig. 5). Cast films from PVA/CS- β -CD (90/10) showed a total release of about 88% of the drug content at 20 °C and about 92% of drug release at 37 °C after 24 h. Comparison between the electrospun mat and cast film showed faster and more drug release at both 20 and 37 °C for casted films.

The salicylic acid release profiles from electrospun fiber and cast film of PVA/CS- β -CD (75/25) are also shown in Figs. 4 and 5. At 37 °C (Fig. 5), the amount of released salicylic acid from electrospun fiber within the first 2 h was 46% of its drug content as shown in Fig. 5, while it released 80% of its drug content after 24 h. At 20 °C (Fig. 4), it released 40% of its drug content within the first 2 h. After 24 h, only 75% of drug release was observed. This figure also shows that at 37 °C, the total amount of salicylic acid released from cast film was 89% of its drug content after 24 h (Fig. 5). However, this value of salicylic acid release was reduced to 84% at 20 °C (Fig. 4). Again, the effect of temperature was clear. It increased the amount of released salicylic acid from the cast PVA/CS- β -CD film and nanofibrous mat. Furthermore, decrease in rate and total amount of drug release was observed with an increase in the CS- β -CD content in both film and nanofibrous mat. This observation could be attributed to host-guest complex formation between salicylic acid and CM β -CD as previously mentioned.

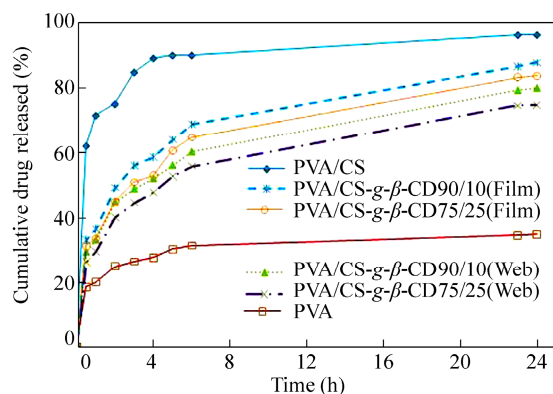


Fig. 4 *In vitro* release profile of salicylic acid from electrospun mats and films made from PVA/CS-g- β -CD in phosphate buffer (pH 7.4) at room temperature (20 °C)

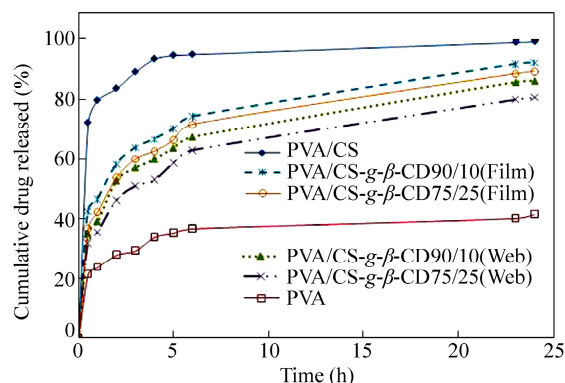


Fig. 5 *In vitro* release profile of salicylic acid from electrospun mats and films made from PVA/CS-g- β -CD in phosphate buffer (pH 7.4) at body temperature (37 °C)

CONCLUSIONS

A novel type of biodegradable drug delivery nanofibrous structure with controlled drug release capability was fabricated. This new structure is based on the delivery of encapsulated salicylic acid as a model drug in the electrospun PVA/CS-g- β -CD nanofibers. The drug release was monitored in phosphate buffer of pH 7.4 at the both body temperature (37 °C) and room temperature (20 °C). Pure CS-g- β -CD solution was not electrospinnable due to the low viscosity of the aqueous CS-g- β -CD solution. However, electrospinning of aqueous solution of PVA/CS-g- β -CD blends was successfully processed, and fibers with a diameter ranging from 130 nm to 210 nm depending on electrospinning conditions were obtained. The results obtained from the drug release experiments showed that as the temperature increased, the released amount of salicylic acid increased. Also the blend ratio of PVA/CS-g- β -CD had an influence on the rate of drug release profile. PVA/CS-g- β -CD nanofibers showed a slower release of salicylic acid in compare to PVA/CS nanofibers. These results suggested that PVA/CS-g- β -CD electrospun mats could be suitable for biomedical applications.

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