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Development and characterization of hydroquinone-loaded nanofiber for topical delivery: effect of chitosan

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ABSTRACT

Hydroquinone (HQ) loaded polymer solution was electrospun for its topical application. Nanofibers were then investigated in terms of stability, drug release, and antifungal activity. The effect of chitosan (CS) was investigated on the viscosity, stability, drug release, and antifungal activity of the developed formulation. Results indicate a significantly stable HQ-loaded nanofiber formulation. The addition of CS caused hydration of the drug delivery system and enhanced drug release but reduced its stability. HQ-loaded nanofiber mat showed significant antifungal activity, however, there was no inhibition zone in samples containing CS.

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Hydroquinone; chitosan; antifungal activity; stability; nanofiber

Introduction

Hyperpigmentation is a cosmetically undesirable skin disorder with great impact on quality of life. It often results due to an excess of melanin level in the skin caused by pregnancy, skin trauma, hormone replacement therapy, and exposure to ultraviolet light or the use of certain drugs such as contraceptives. Hydroquinone (HQ) can interact with tyrosinase to reduce skin pigmentation.[1–3] It has poor stability and a high rate of degradation due to oxidation. This instability causes difficulty in the formulation of a stable product. Discoloration occurs from creamy to dark brown as a result of oxidation.[4]

Electrospinning is a common technique employed for the production of nanofibers with high porosity and surface area to volume ratio. The electrospun nanofiber mat can be employed in various fields including tissue engineering and drug delivery. Fiber formation and morphology depend on the selection of polymer and solvent types which influence surface tension and viscosity of the polymer solution. Viscoelastic forces of the polymer solution help to eliminate bead and enhance fiber formation.[5] Chitosan (CS) is a biodegradable and biocompatible natural polysaccharide widely used for drug delivery.[6,7] Polyvinyl alcohol (PVA) is a nontoxic water-soluble polymer with wide application in various drug delivery systems including electrospinning of nanofibrous structures.[8–12]

This study aims to develop and assess hydroquinone loaded nanofibers for their potential as a topical facial mask. Polymeric solutions were investigated for their viscosity prior electrospinning. We further aim to assess the HQ-loaded nanofibers for their structure, stability, drug release, and

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antifungal activity. The effect of CS on the developed formulation was also investigated in terms of stability, drug release, and antifungal activity.

Materials and methods

Materials

CS (degree of deacetylation >75% and Mw 140,000–220,000 kDa) and PVA (Mw 89,000 ~ 98,000) were obtained from Sigma. HQ and ketoconazole were purchased from Sepidaj Pharmaceutical, Iran. Sabouraud Dextrose Agar was obtained from Merck, Germany.

Electrospinning

Polymer solutions were prepared by dissolving PVA (10% w/v) in distilled water at 80 °C, hydroquinone (5% w/v) was dissolved after temperature cooling to make HQ loaded PVA solution. In another formulation, CS solution (2%w/v) was prepared by dissolving CS and lactic acid (1% v/v) in distilled water and was blended with the PVA solution at a weight ratio of 1:9 to obtain HQ loaded PVA + CS nanofiber samples. For antifungal studies, ketoconazole was used as positive control and was made by blending 0.1% w/v of ketoconazole into PVA (10% w/v) solution after cooling.

The spinning solutions were placed in a 5 ml syringe connected to a blunt 27 G stainless steel needle. The needle was mounted on a syringe pump (Fanavaran Nano Meghyas, Iran) flowing at a constant rate of 1 ml/h and it was connected to positive charge at a fixed voltage of 20 kV (Fanavaran Nano Meghyas, Iran) held at a distance of 15 cm from the collector at room temperature. The electrospun nanofibers were collected on aluminum foil and dried under vacuum for a week prior to usage.

Scanning electron microscopy (SEM)

Morphological appearance and nanofiber diameter were evaluated using a scanning electron microscope (MIRA3, TESCAN, Czech Republic). Samples were sputter coated with gold prior observations. Average fiber diameter was determined by measuring 20 random fibers from SEM micrograph.

Viscosity measurement

Solution viscosity was measured using a viscometer with a spindle L3 configuration at room temperature at a speed of 135 rpm with a 2 min acquisition time (Fungilab, Evol series, Spain, $n = 4$). The data is displayed as mean ± standard deviation for each composition.

Fourier transform infrared spectrophotometry (FTIR)

Chemical structure of the electrospun fibers was characterized using spectrophotometer (PerkinElmer Spectrum) in the spectral region of 400–4000 cm^{-1}. The Spectrum software version 10.03.02 was used for data collection.

In vitro *drug release*

In vitro drug release studies were performed by the total immersion method. Briefly,

10 mg of nanofiber mats were placed in a 10 ml of phosphate buffer solution (pH 7.4) incubated at 25 °C or 32 °C and shaken at 110 rpm (GFL-3031 Rontgen, Germany). At given intervals,

1 ml of release medium was collected and replaced with the same amount of fresh buffer. Drug analysis was performed using spectrophotometer at 289 nm using spectrophotometer (Cecil CE 7200, UK).

Loading capacity was calculated according to the following equation:

$$\%\ Nanofiber\ loading\ capacity\ =\ \frac{Q_t}{W_t} \tag{1}$$

Where Q_t is the amount of drug embedded in the nanofiber mat and W_t is the weight of the nanofiber mat.[13]

Stability studies

Freshly prepared electrospun nanofiber samples were placed in tight container and stability of HQ was assessed under normal conditions in room temperature (25 °C) and in the fridge (2–8 °C) for 140 days. Aqueous HQ 2% w/v samples were prepared as control. Drug content was measured spectrophotometrically and all experiments were performed in triplicates.

Zone of inhibition testing for antifungal activity

Disc diffusion tests were carried out by growth of clinical candida albicans 11035/7 aerobically in Sabouraud Dextrose Agar at 35 °C or 24 h. Electrospun nanofiber discs (6 mm) were incubated at 35 °C for 24 h on agar surface inoculated with fungi suspension with turbidity of 0.5 McFarland standard. The inhibition zone around the discs was measured in millimeters (mm) using a ruler. Ketoconazole loaded PVA was used as a conventional efficient drug for positive control and plain PVA samples were employed as blank (negative control).

Hydroquinone (HQ) quantification

HQ was quantified by a spectroscopic method. A calibration curve was obtained by analyzing HQ samples (Cecil, CE 7200, UK) concentrations in 289 nm.

Statistical analysis

Experimental measurements were carried out in triplicates and the data are expressed as mean ± standard deviation. Differences between variables were examined using one-way ANOVA, and post hoc analysis was performed using Tukey's test. *P*-value of $p < .05$ was considered statistically significant.

Results

Fiber morphology and solution viscosity

The morphology of the electrospun nanofibers is demonstrated in Figure 1. Fibers were formed without bead or drug crystals and had a smooth and uniform surface. Solution viscosity and its effect on electrospun fiber morphology were investigated and the results are tabulated in Table 1. Pure PVA had sufficient viscosity (812.1 ± 98.3 cP) for fiber formation at the specified electric voltage with a mean fiber diameter of 537.24 ± 52.5 nm. As expected, the addition of CS increased solution viscosity (5805 ± 243.54 cP) and fiber diameter (628.09 ± 32.41 nm) but was insufficient to change fiber shape. The results were also supported by the representative image of fiber morphology and the effect of high viscosity on the formation of large fiber diameter. The introduction

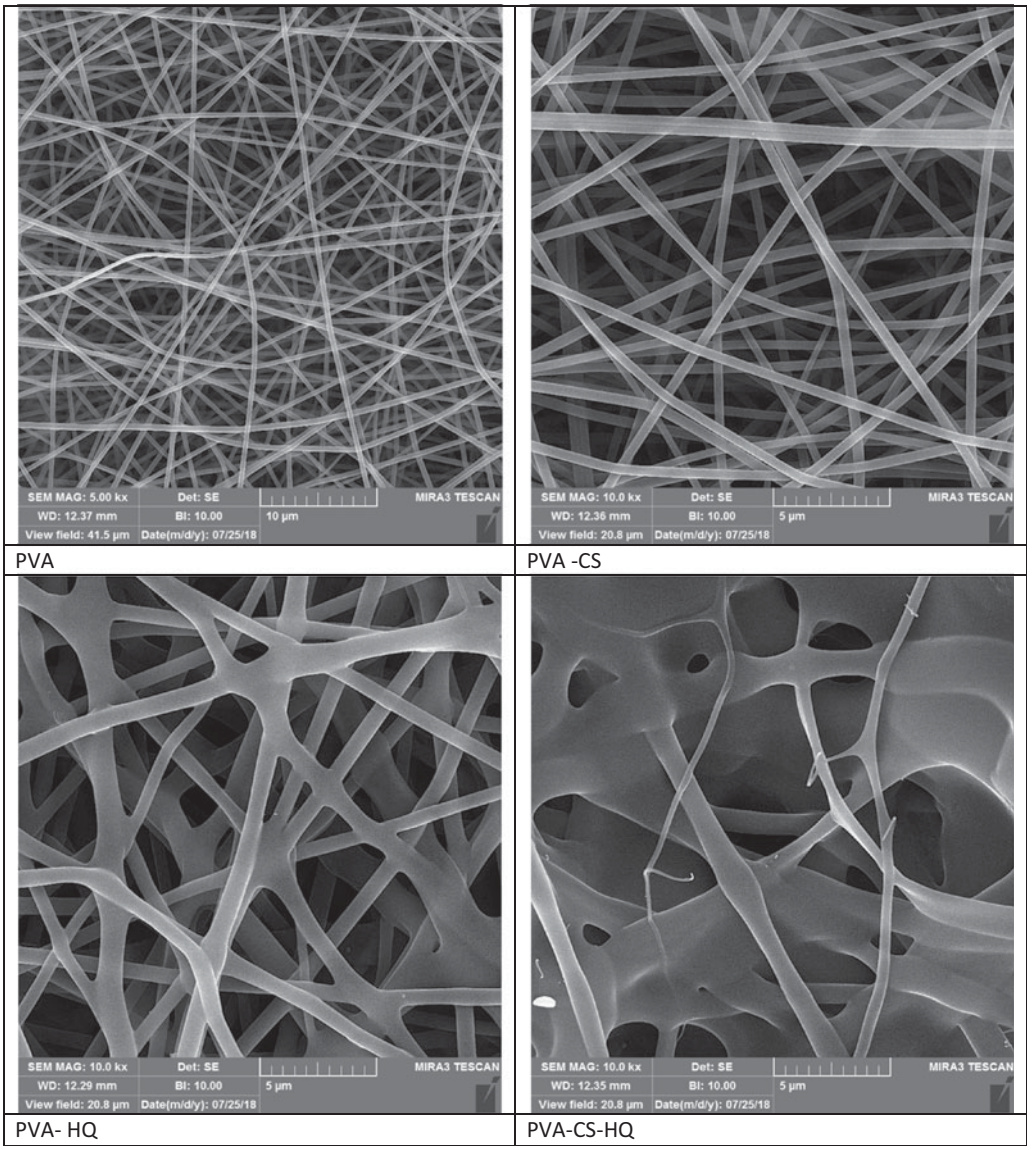

Figure 1. SEM images of (a) pure PVA, (b) PVA + HQ, and (c) PVA + HQ + CS.

of HQ in PVA samples increased solution viscosity to 1130 ± 68.5 cP and increased fiber diameter to 778.81 ± 46.51 nm. Polymer solution of PVA + CS + HQ had enhanced viscosity of 6765 ± 147.8 cP and resulted in fibers with diameter of 845 ± 35.63 nm. It is observed that fibers containing both CS and HQ seem hydrated due to the hydrogen bonding of the two molecules.

FTIR

Figure 2 represents the chemical structure of nanofiber formulations and the possible interaction between the components. The FTIR spectra of the blank PVA nanofiber mat exhibit characteristic absorption band at 3307, 2937, 1734, 1431, and 1093 which are attributed to the

O–H, C–H, C=O, CH–O–H, and C–O resonance respectively.[10] The infrared spectrum of PVA + CS blend show characteristic peak at 849 cm^{-1} and 1092 cm^{-1} which correspond to the

Table 1. Composition and rheology of polymeric solutions used in nanofiber production

Formulation	Composition %w/v			Viscosity(cp)	Viscosity Torque	Speed(RPM)	Spindle
	PVA	CS	HQ				
A	10%	–	–	812.1	91.9%	135	L_3
B	10%	2%	–	5805	87.6%	90	L_4
C	10%	–	2 %	1130	94.7%	135	L_3
D	10%	2%	2 %	6765	90.7%	90	L_4

saccharide structure and an N–H bending characteristic peak at around 1597 cm $^{-1}$. Other typical bands of –OH and –CONH- stretching vibration of CS are seen at 3273 cm^{-1} and 1651 cm^{-1}, respectively.[11,12]

HQ hydroxyl functionalities appear at 3326 cm^{-1}. The intensity of the –OH group has broadened and reduced due to the interaction between the –OH group from HQ and PVA counterparts and shifted to lower wave number upon addition of HQ. This may suggest formation of a hydrogen bond due to an interaction between the hydroxyl groups from CS and PVA counterpart. The disappearance of the peak at 3326 cm^{-1} is due to the interaction between the –OH groups from HQ and PVA counterparts. Absorption bands at 1961 cm^{-1} to 1712 cm^{-1} confirm the presence of aromatic ring system of HQ. Skeletal vibration of aromatic ring appears at 1635 and 1516 cm^{-1}. The intense band at 2856 cm^{-1} is due to the C–H stretching vibration. The adsorption at 1374 cm^{-1} and 1353 cm^{-1} indicate O–H bending vibration. C–O stretching vibration is seen at 1095 cm^{-1}. Strong absorption at 831 cm^{-1} and 760 cm^{-1} relates to C–H bending.[4]

Drug release

Total HQ content was determined in all nanofiber formulations. The drug loading capacity was measured 5% in PVA + HQ formulation and 4.4% in PVA + CS + HQ formulations. Cumulative HQ release from various formulations at 32 °C and 25 °C is shown in Figure 3. In the first 2 h, almost 80% of the contained HQ was released from all samples. Similar drug release profile pattern was observed for all samples. All formulations exhibited an initial fast release which may correspond to the desorption of the drug on the surface layer of the mat followed by a slower drug release which might be due to the degradation of the polymer and drug release from the matrix.[13] Drug release was slightly higher at 32 °C when compared to that at 25 °C, however, the difference is not significant.

Stability studies

Stability of HQ was assessed under normal conditions in room temperature, RT, at 25 °C and in the fridge temperature, FT, at 2–8 °C for 140 days and the results are represented in Figure 4. All control samples were sealed tightly and protected away from light to prevent additional drug degradation. Results indicate that at the end of 140 days, control samples underwent >10% degradation at RT, however, storage at FT slightly increased the stability of control samples and reduced its degradation rate. It may be speculated that the degradation of control samples occur due to the atmospheric oxygen and hydrolysis in aqueous condition. Nanofiber formulations were able to preserve almost 100% of their drug content without any degradation at both studied temperatures. CS containing nanofibers signify drug degradation which may be due to water uptake and the reaction of CS with water molecule.[6]

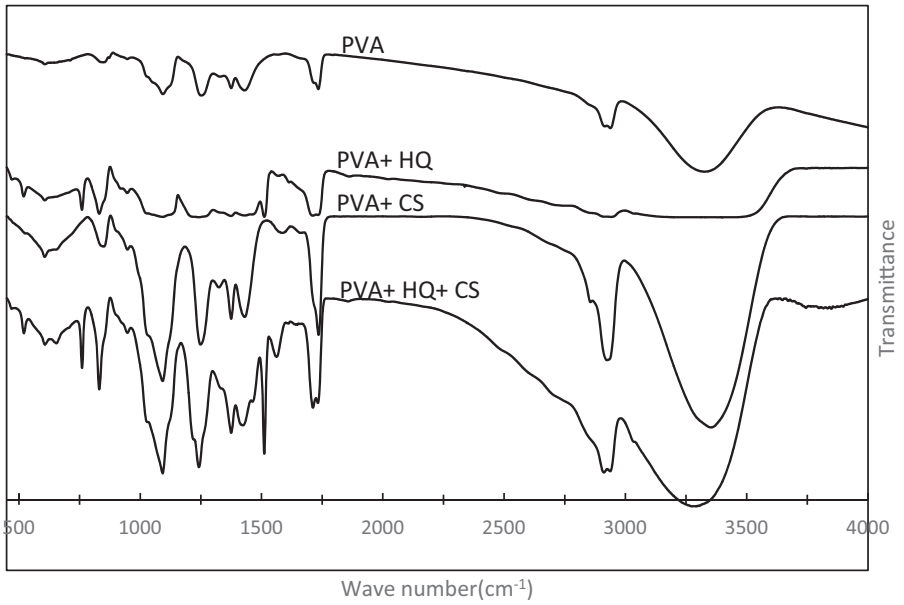

Figure 2. FTIR spectra of nanofibers.

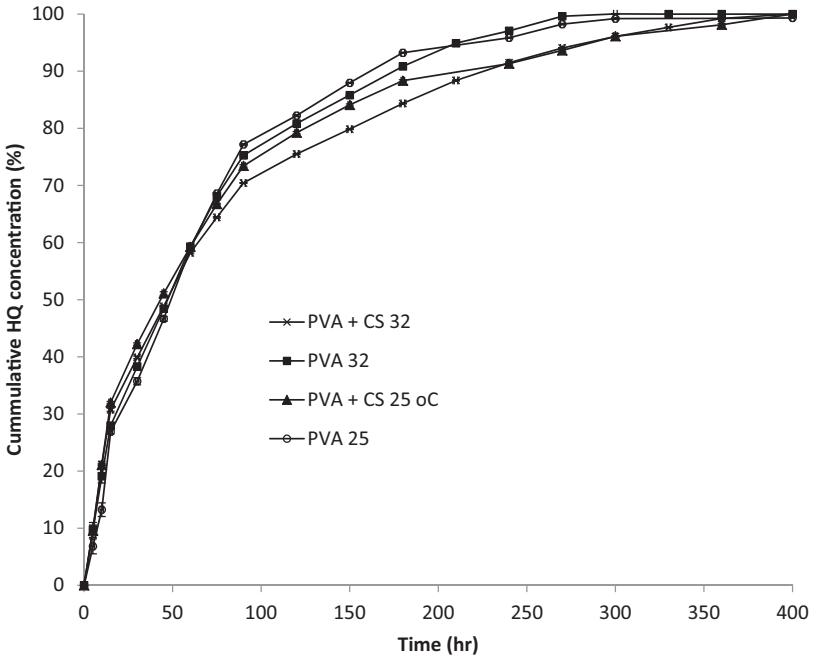

Figure 3. Drug release profile of HQ nanofibers at 32 °C and 25 °C.

In vitro *antifungal assay*

The *In vitro* antifungal activity of HQ-loaded nanofibers was measured against *Candida albicans* by the disc diffusion method and the zone of inhibition measurement are presented in Figure 5. There was no antifungal activity from PVA and PVA + CS samples (results not shown). Similar

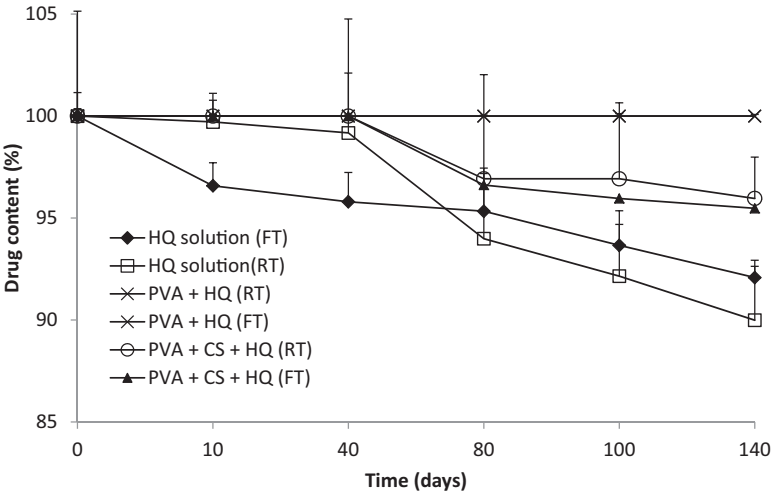

Figure 4. Stability data.

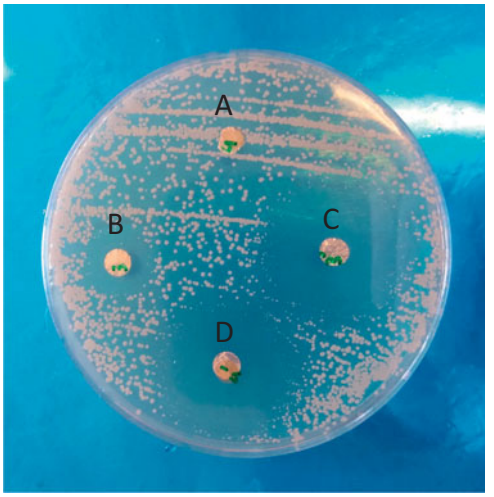

Figure 5. *In vitro* antifungal activity of HQ-loaded nanofibers.

results were observed by Lee and coworkers when CS-loaded nanofiber did not exhibit any zone of inhibition for *P. aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA).[14] On the contrary, the antimicrobial/antifungal activity of chitosan has been reported in another work.[13] Low molecular weight CS has shown inhibitory effect on C. *albicans* ATCC 64550 at a concentration of 5000 mg/ml. While another low molecular and a high molecular weight CS show low activity against other *C. albicans* strain. Contradictory results found in the biological antifungal/antibacterial effect of CS may be due to the use of CS with various Mw, degree of acetylation, polydispersity or variation in the method of sample preparation.[15] Fungal growth inhibition was observed from PVA + HQ nanofiber disks (inhibition zone of 15 mm). The antifungal activity of HQ or its derivatives has been reported before. HQ nanofiber may have the potential to be a promising drug for the treatment of skin candidiasis. Yang and coworkers investigated the antifungal activity of sesterterpene sulfates and halisulfate and they found that it may have been the HQ moiety of the compounds that attributes to the enzymatic inhibition necessary for the

biosynthesis of essential cell constituents in fungal pathogen.[16] Antimicrobial activity has also been detected for HQ moiety of strawberry tree leaves extracted while arbutin did not show any direct antimicrobial activity.[17]

Ketoconazole loaded PVA was able to show a slightly larger inhibition zone (30 mm) when compared to formulations containing PVA + CS + Ket (28 mm). This is in line with the previous findings where no synergism was obtained on the antifungal activity of low molecular weight CS and fluconazole. This may be due to the hydrogen bonding between the drug and CS which might have decreased the antifungal activity of the drug molecule.[18]

Conclusions

This study aims to develop and assess hydroquinone loaded nanofibers for their potential as a facial mask in topical skin delivery. Polymeric solutions were investigated for their viscosity prior fabrication into the nanofibrous structure. Successful formulation of HQ was obtained through electrospinning. HQ-loaded nanofibers were then investigated for their stability and drug release. The newly developed formulations illustrate antifungal activity against clinical *Candida albicans* and a significant increase in stability. Addition of CS to the formulation did not show any antifungal activity but was able to increase drug release.

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