DEVELOPMENT OF ANTIBACTERIAL ELECTROSPUN CHITOSAN/POLY(VINYL ALCOHOL) NANOFIBERS CONTAINING SILVER INCORPORATED HAP NANOPARTICLES

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Abstract

Chitosan/poly(vinyl alcohol) (CS/PVA) nanofibers with a diameter in the range of 60-80 nm were produced by electrospinning method. The effect of CS/PVA blend ratio and process parameters on the morphology of fibers was investigated by SEM analysis to select the optimum ratio in order to continue the following steps of the study. Silver ion (Ag^+) incorporated hydroxyapatite nanoparticles were added to the blend solution. The produced nanofibers are characterized by SEM, FTIR, DSC and antibacterial analysis. It was observed that the generated nanofibers exhibited antibacterial efficiency against E.coli.

1 Introduction

The electrospinning method for the fabrication of polymer nanofibers has gained increasing research interest during the last decades with the development of nanotechnology. The outstanding properties of polymer nanofibers compared to microfibers arise the potential applications based on nanofibers [1,2]. The flexibility, surface functionality and large surface to volume ratio make them potentially applicable in various applications, e.g. in filtration, catalysis, biomedicine, protective or smart clothing, optical devices, membranes, sensors and as reinforcements in composite materials [1]. Electrospinning method is a simple, cost effective, easily controlled technique and it allows drawing nanofibers from pure and blended, natural and synthetic, organic and inorganic polymers. A typical electrospinning system consists of a syringe pump, a high voltage supplier and a grounded collector. The syringe is filled with the polymer solution and the syringe pump is useful to drive the solution with a constant rate. A droplet at the tip of the needle is formed and when the electrical forces exceed its surface tension the electrically charged jet is elongated into the direction of the collector. The nanofibers are formed with the evaporation of the solvent.

The properties of formed nanofibers are influenced by many parameters including process parameters (applied voltage, feed rate, collection distance, needle tip), polymer molecular

chain length, concentration, viscosity, solvent, conductivity, temperature, presence of additives etc.. [1, 3]

Chitosan (CS) is a naturally occurring nontoxic and biodegradable biopolymer and it is derived from the second most abundant polysaccharide chitin [4]. Chitosan is a polycation, the copolymer of N-acetyl-Dglucosamine and D-glucosamine. The D-glucosamine content is dependent on the degree of deacetylation (DDA) of chitin to chitosan. Due to its unique properties of binding toxic heavy metal ions and its ability to inhibit the growth of a wide variety of fungi, yeasts and bacteria much attention has been paid to the utilization of chitosan, especially in the biomedical field. The antimicrobial properties of chitosan are a result of the polycationic nature of chitosan arising from the protonation of $-NH_2$ groups on the chitosan backbone. Poly(vinyl alcohol) (PVA) is water-soluble, biocompatible and biodegradable synthetic polymer has advantageous chemical and physical properties, like chemical resistance, high melting point and biological compatibility [1,5]. This non-toxic, inexpensive material is commercially available in a broad range of degrees of polymerization and hydrolysis.

The usage of polymer blends improves the properties and functionality of nanofibers. The formation of nanofibers from neat chitosan is a challenging process because of its high viscosity [6]. The electrospinning of chitosan/PVA blends were studied by many reserchers [1,5,6,7,8]. Antimicrobial functionalisation of nanofibres could be a solution to inhibit the uncontrolled growth of microbes. The antibacterial capability of nanofibers provides the usage of them in special applications e.g. in air filters and wound pads as they combine permeability with selective barrier properties. The high surface area-to-volume ratio also increases the antibacterial efficiency of nanofibers.

In this study, silver ion doped antibacterial hydroxyapatite based nanoparticles were incorporated into the CS/PVA polymer solutions. The effect of process parameters, blend ratio and additive concentration on the fiber formation were investigated by scanning electron microscopy (SEM), Fourier Transform Infrared (FT-IR), Differential Scanning Calorimetry (DSC) and antibacterial tests.

2 Materials and testing methods

2.1 Materials

Poly(vinyl alcohol) 72000 (Fluka, 97.5-99.5 % hydrolyzed), chitosan (Aldrich, coarse ground flakes and powder form, >75 deacetylated, viscosity range of 800-2000 mPaS) and acetic acid (Aldrich) were used as received.

2.2 Synthesis and characterization of the antibacterial silver ion-incorporated hydroxyapatite (HAP)

HAP powder was synthesized by aqueous chemical method. Silver ions dispersed in pure water were mixed with calcium hydroxide. Orthophosphoric acid was added to the solution in

order to control the pH of the solution to form a stoichiometric hydroxyapatite structure. The precipitate formed was sequentially filtered, dried and sieved.
The phase of the nanoparticles were monitored at room temperature by X-ray difractometer (XRD, Rikagu Rint 2200) using the CuKα (1.542 A^o) radiation and 2θ scan rate of $2^o min^{-1}$ at 40kV, 30mA. Grain size and powder morphology of powders were investigated by SEM (Zeiss Evo 50EP). The antibacterial tests of synthesized particles were done by Halo method. *E.coli* colonies were cultivated in nutrient broth at 37 °C for 24 hours in shaking incubator. Formed colonies were diluted to a concentration of $2x10^5$ CFU/ml. The nutrient agar was poured on to the powder sample in petri dish so that a thin layer was formed on the surface. After incubation a period of 24 hours at 37 °C, the growth behavior of bacteria colonies were observed.

2.3 Preparation of Electrospun Nanofibers

The PVA solution (8 wt%) was prepared in distilled water at 80°C under magnetic stirring and subsequently cooled to ambient temperature before electrospinning. Chitosan is dissolved in 2 wt% acetic acid solution. The concentrations of CS/PVA solutions were expressed in wt/wt %. Ag^+-HAP nanoparticles were added to the PVA solution in weight ratios of 0.1, 0.3 and 0.5 wt. %.

Electrospinning was performed at environmental conditions. The electrospinning setup used consists of a syringe with a metal needle, a pump for controlling feeding rate and a high voltage d.c. power supply connected to the grounded collector. As the collector a grounded metal plate with a fixed aluminum foil flat was used. The electrospinning voltage was kept between 12-14 kV. The tip-to-collector distance was varied between 5-10 cm. The feeding rate of the polymer ranging between 0.01-0.08 ml/min was tried. The optimum process parameters were selected by investigating the morphology of fibers by using SEM (Zeiss Supra 50VP). The thermal analyses were conducted by DSC- TA Instruments Q 2000 in N_2 atmosphere using ca. 10 mg sample. The samples were heated at a heating rate of 10°C/min from 20°C to 200°C. The FT-IR measurements were performed by the KBr method. For contact tests, the samples and the culture of *E.coli* colonies were contacted for 18-24 hours at 25°C. Thereafter it was cultivated to the nutrient agar and the bacterial growth was observed.

3 Results and Discussion

X-ray diffraction analysis of the synthesized powder in Figure 1 shows the intensity peaks corresponding to JCPDS (0090432-0090169) for hydroxyapatite.

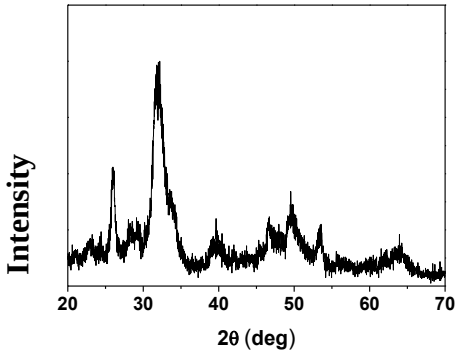

Figure 1. The XRD plot of HAP nanoparticles

SEM images presented in Figure 2a and Figure 2b show that the synthesized HAP consist of irregular agglomerates. Essentially these agglomerates include spherical particles with a size ranging between 80 and 100 nm.

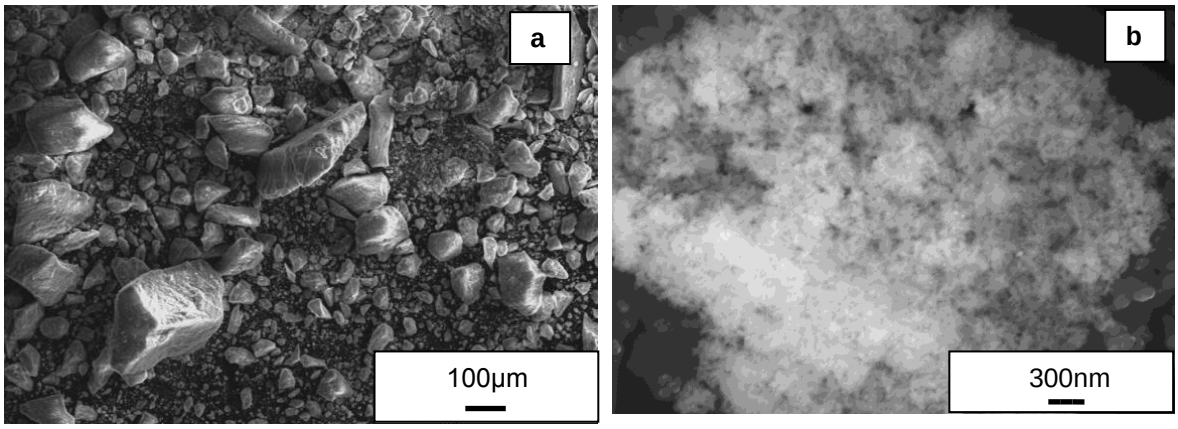

Figure 2. Low (a) and high (b) magnification SEM images of synthesized antibacterial nanoparticles

In Figure 3 the antibacterial efficiency of Ag^+ ion doped and undoped HAP particles were investigated. Dense bacteria colonies were observed around the undoped HAP whereas no colonies were detected on the surface of Ag^+-HAP particles. The reason was the release of silver ions and inhibition of bacterial growth around the sample.

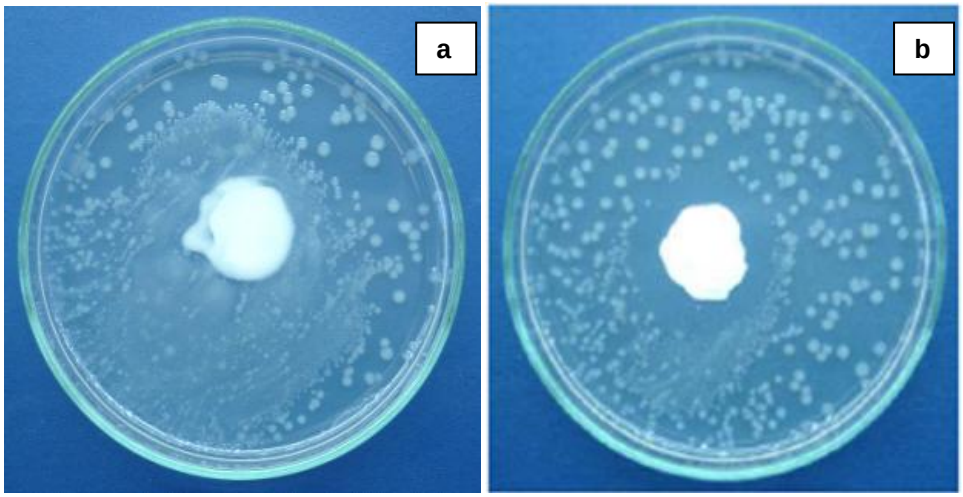

Figure 3. Antibacterial test of the Ag^+ ion a) undoped and b) doped HAP nanoparticles

The SEM images shown in Figure 4 summarize the effect of blend ratio, flow rate and spinning distance on fiber formation. Bead-less, uniform fiber formation was improved with the decrease in electrospinning rate. The increment in the chitosan content of the blend reduced the diameter of the fibers. The addition of chitosan results in a higher charge density on the jet surface because of the ionic character of chitosan [5]. Higher elongation forces imposed on the jet lead to the reduced diameter of the blend fibers.

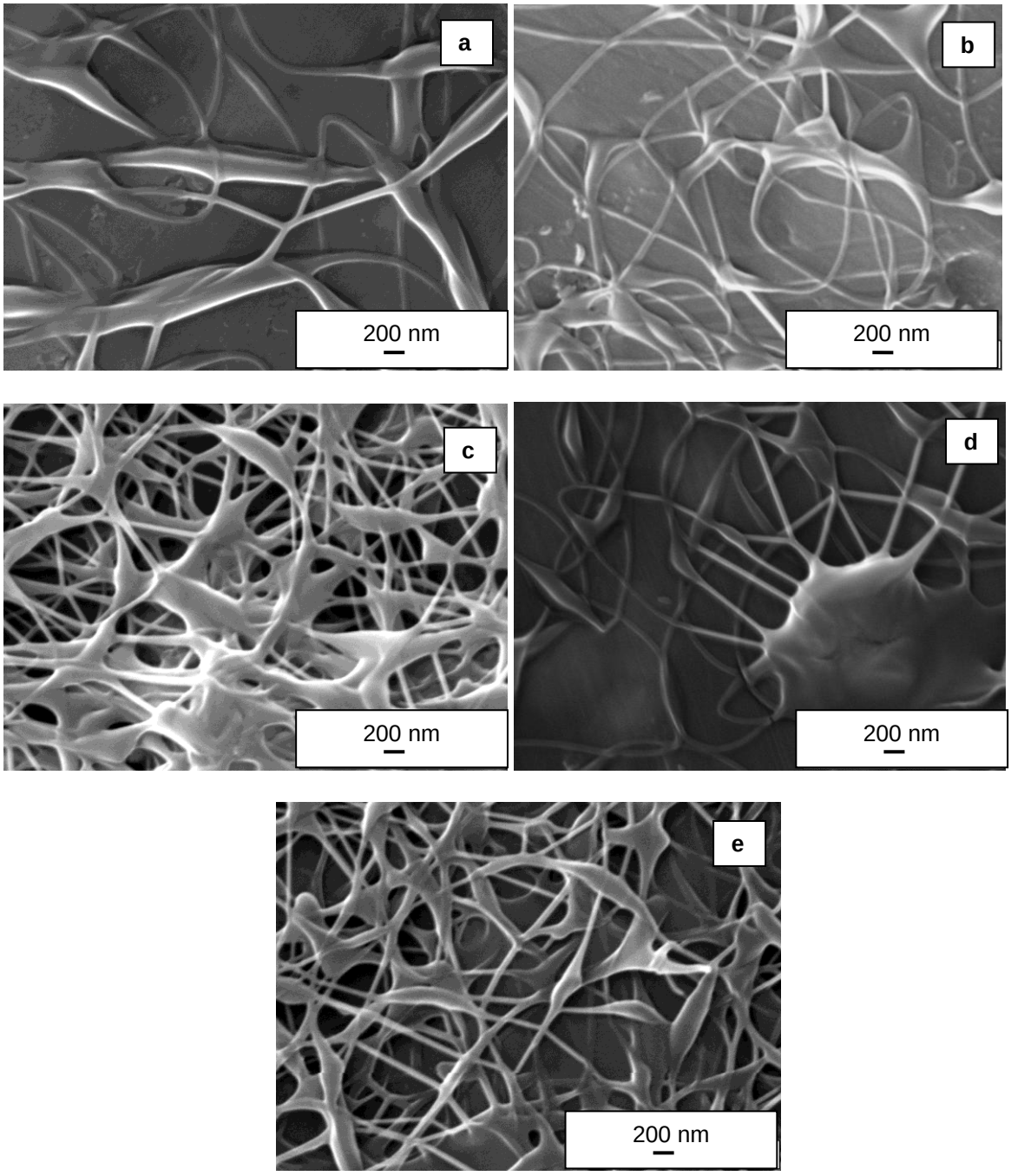

Figure 4. SEM photographs of CS/PVA nanofibers at different blend ratios, flow rates and spinning distances (d): a) 10/90, 0.03 ml/min, d= 10 cm; b) 10/90, 0.08 ml/min, d= 10 cm; c) 10/90, 0.01 ml/min, d= 5 cm; d) 10/90, 0.01 ml/min, d= 10 cm; e) 25/75, 0.01 ml/min, d= 10 cm.

Figure 5 shows the SEM image of neat CS/ PVA nanofibers and Ag^+ ion incorporated HAP/CS/ PVA nanofibers. Under the same electrospinning conditions the HAP-additive nanofibers seemed finer when compared to neat ones. This is because Ag^+ ion accumulated charges in the solution, thus affecting the charge density of the solution and also the fiber diameter. No microcracks were seen on the surface of fibers.

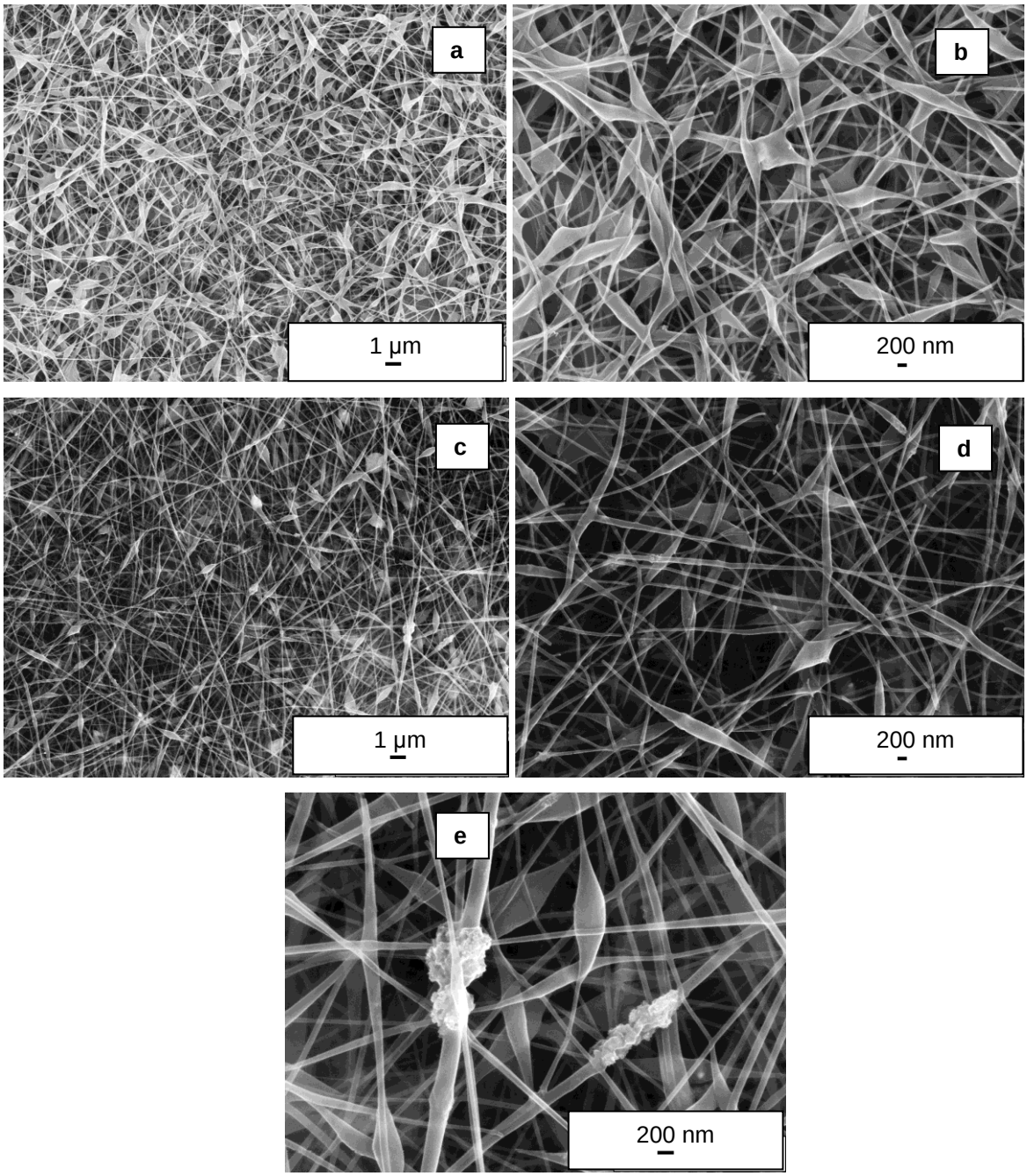

Figure 5. SEM photographs of a) Neat CS/PVA nanofibers x 10000, b) a) Neat CS/PVA nanofibers x 30000 c) CS/PVA nanofibers containing 0.5 wt% HAP x 10000 d) CS/PVA nanofibers containing 0.5 wt% HAP x 30000 e) CS/PVA nanofibers containing 0.5 wt% HAP x 50000

Figure 6 represents the DSC curve of neat PVA and CS, PVA/CS blend, HAP nanoparticles containing PVA/CS blend. The thermograms of most polysaccharides show a broad endothermic peak that is associated with evaporation of water at about 100 °C. Instead of melting they degrade at high temperatures. Neat PVA sample showed an exothermic peak at about 195 °C. However, for blends the endothermic curve broadened and shifted to the lower temperature. The CS content in the blend leads to decrease in the crystallinity of blends.

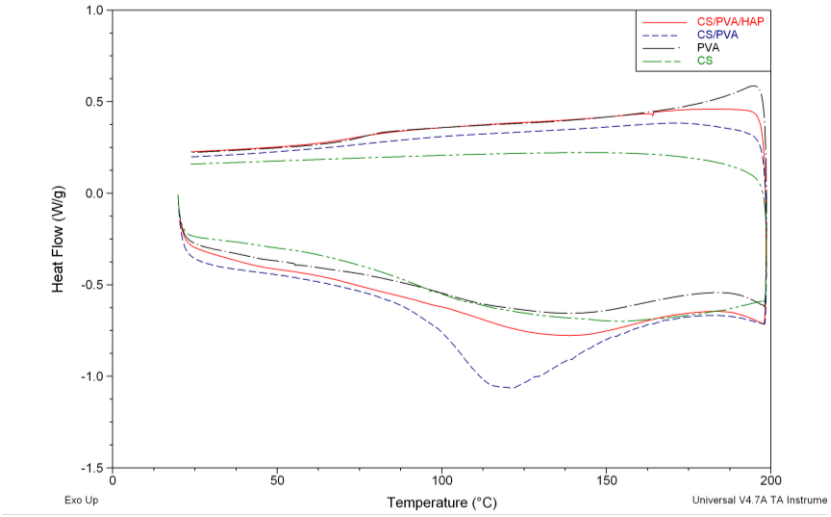

Figure 6. The DSC thermograms of PVA, CS, CS/PVA blend, CS/PVA/HAP blend

Figure 7 shows FT-IR spectra of neat PVA, CS and PVA/CS blend. As compared with FT-IR spectra of neat CS the peak at 1255 cm-1 (assigned to –OH bond) disappeared in the CS/PVA spectra. This was probably caused by the formation of hydrogen bond between CS and PVA molecules.

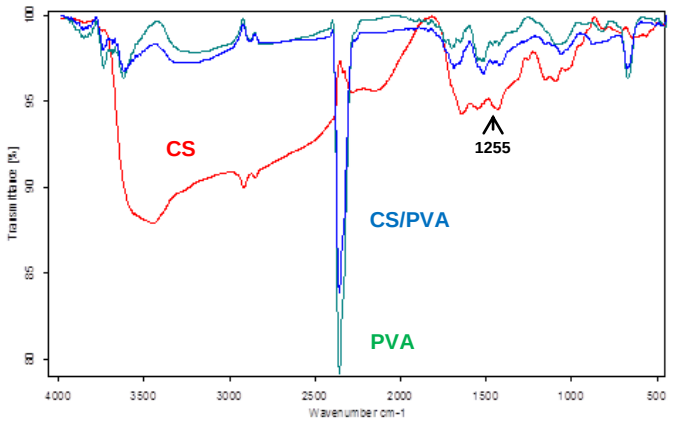

Figure 7. FT-IR Spectra of PVA, CS and CS/PVA blend

In Figure 8 the antibacterial activity of CS/PVA nanofibers with various concentrations of HAP was shown. The antibacterial activity was not seen on the neat CS/PVA nanofibers. The increase of antibacterial activity on samples containing 0.5 wt% was observed in Figure 8b.

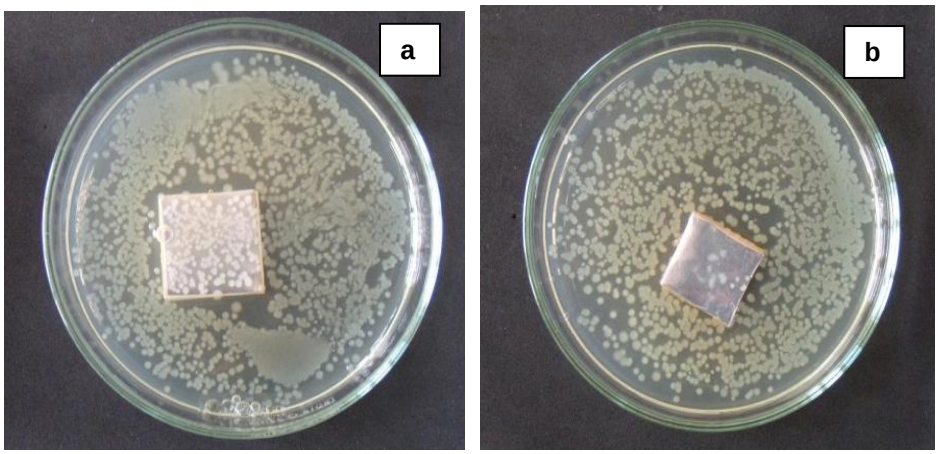

Figure 8. Antibacterial test of the a) neat CS/PVA nanofibers b) CS/PVA nanofibers containing 0.5 wt.% HAP

4 Conclusions

The optimized conditions for nanofibers were determined as 0.01 ml/min flowrate, 10 cm spinning distance and 25/75 (CS/PVA) blend ratio. Uniform, bead-less nanofibers were prepared with fiber diameter 60-80 nm. DSC analysis revealed that the peak of the endothermic curve shifted to the lower temperatures when compared to neat PVA. The antibacterial efficiency of Ag^{+} ion was seen by antibacterial tests. The electrospun antibacterial nanofibers of CS/PVA have the potential to be used in biomedical and filtration applications.

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