

Research Article

Release of Tetracycline Hydrochloride from Chitosan/ Poly-caprolactone Electrospun Web

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ABSTRACT

In the present research a fibrous comprising of Polycaprolactone (PCL) and Chitosan (Cs) was electrospun from a solvent consisting of acetic acid. PCL concentration was varied from 8% , 15% by fixing Cs concentration as a constant (3%). The composition of mixing was selected as 80:20, 50:50, 20:80 for using them for drug release of tetracycline. The blends were characterized by Fourier transmission infra red spectroscopy (FTIR), UV-visible Spectroscopy (UV), scanning electron microscopy (SEM). From the FTIR spectra the various group present in Cs and PCL blend were monitored. The homogeneity, morphology of the blends were ascertained from SEM. All these results indicate that PCL:Cs with concentration of 8% Pcl and 3% Cs for nanofibrous web would be a proper material for drug release.

Keywords: Tetracycline, Chitosan, Poly-caprolactone, Drug Release, Nano Fibers.

INTRODUCTION

Electrospun has been used as an efficient method to produce nonwoven fibers that are consist of a large network of interconnected fibers and pores. There are numbers of processing methods, such as drawing, Phase separation, self assembly and template directed synthesis and electrospinning are widely used to production micro and nanofibers[1-4]. Electrospinning is simple method, cost effective and powerful technique. The fiber produced by this method have shown unique characteristic, such as a very large surface to volume ratio and a high porosity with a small pore size. Electrospinning has been used for producing of scaffolds from many biodegradable polymers and numerous of synthetic or natural polymers such as Cs and PCL [5-7].

Cs, a (1-4)-linked 2-amino-2-deoxy-D-glucopyranose is made from chitin, one of the most affluence natural polysaccharides. Cs is well known for its nontoxic, biocompatible and biodegradable properties. Also it has several singular properties: it is antimicrobial and inhibits the growth of a wide variety of fungi, yeasts, and bacteria, which can be profitable for using in the field of biomedicine. In addition, it can bind toxic metal ions, which can be profitable for using in air cleaning and water purification applications [8-10].

PCL has various benefits, including mechanical flexibility, low antigenicity, easy processability, and low degrees of choronicoersistence. It is semi-crystalline, hydrophobic polymer its relatively polar ester group and five nonpolar

methylene groups in its repeating unit. PCL is mostly synthesized by ring opening polymerization method from ϵ -caprolactone monomers[11]. However, it can also cause problems related to its high hydrophobicity and low water absorptivity, toxicity, and low cell attachment and proliferation.

The applications of blending the chitosan and PCL based nanofibers in areas such as enzyme filtration, wound dressing, tissue engineering, drug delivery and drug release systems. Polymeric drug delivery systems are able to improve therapeutic efficacy, reduce toxicity and enhance compliance of the patients by delivering drugs at a controlled rate over a period of time to the site of action. Encapsulation of low molecular weight drug substances like tetracycline hydrochloride, ibuprofen, rifampicin, and other molecular into electrospun has already been substantiated by a number of authors[12-21]. Blending two polymers is an approach to develop new biomaterials exhibiting combinations of properties that could not be obtained by individual polymers[22]. Blends made of synthetic and natural polymer can imbibe the wide range of physicochemical properties and processing techniques of synthetic polymers as well as the biocompatibility and biological interaction of natural polymers[23]. This study explored the blending of Cs, a naturally derived polysaccharide, with a synthetic polymer PCL. The hypothesis is that blending Cs and PCL will give a superior biomaterial where the limitations of Cs are complemented by PCL. Cs has generated enormous interest due to its various advantages such as low cost, easy availability, positive charge, biocompatibility and antimicrobial activity. Hence, this blend can be a better biodegradable and biocompatible material to be used in control drug release systems.

Tetracycline HCL, having antimicrobial effect in many of systemic infections that act against gram-positive and gram-negative bacteria, the

intracellular pathogens chlamydiae, mycoplasmas, and rickettsiae, as well as eukaryotic protozoan parasites[24]. It is used to treat urinary tract infections, acne, gonorrhoea, and other conditions. Tetracycline yields a toxic degradation product, 4-epianhydrotetracycline.

2. MATERIALS AND METHODS

Poly-caprolactone (Mw 80kDa) was purchased from Sigma-Aldrich. Chitosan was obtained from orbital Co., acetic acid and other chemical materials such as sodium hydroxide (NaOH) and potassium phosphate monobasic (KH_2PO_4) were purchased from Merck Company (Germany). Tetracycline was provided by Hakim Pharmaceutical co. (Iran).

2.2.1. Electrospinning

Electrospinning was carried out using 10,15% w/v solution of PCL in 90% acetic acid as solvent and 3% w/v solution of Cs in 80% acetic acid with ratios of 80:20, 50:50, 20:80. The drug concentration was in the range of 0.25% and 0.5% w/v with respect to the polymer used. The resulted clear solution was transferred to a 5-ml syringe pump with a right angle-shaped needle attached to it and the applied positive voltage was in the range of 18-20 Kv. The resulting fibers were collected on a grounded aluminium plate. The distance between the needle tip and the grounded target was 10 cm.

2.2.2. Scanning Electron Microscopic (SEM) studies

The blending of the Cs-PCL composites containing different concentrations were characterized using SEM (Kato Tech CO., LTD, Japan). The powered specimens were placed on aluminium specimen mounts with double-sided adhesive electrically conductive carbon tape. The specimen mounts were then coated with gold for 30 sec in a sputter coater. The coated specimens were then observed on the SEM using an accelerating voltage of 24 kV at the Cs-PCL composite blends.

2.2.3. Fourier transmission infra red spectroscopy (FTIR)

The FTIR spectrum of the Cs-PCL blend was obtained using a Bruker-TENSOR27 type FTIR spectrophotometer.

2.2.4. In vitro drug release studies

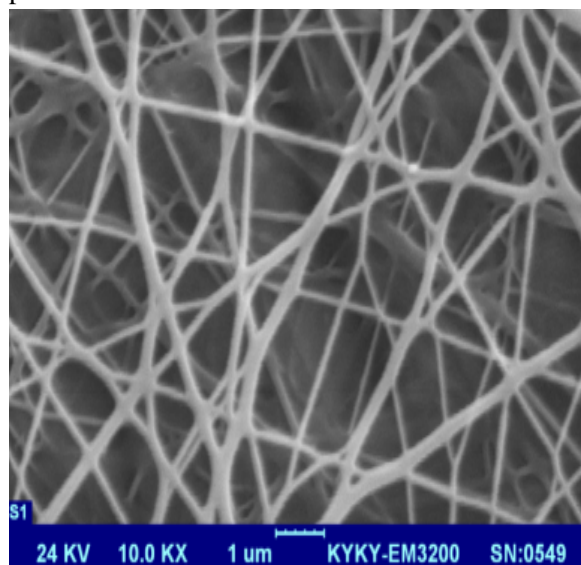
The medicated electrospinning nanofibers webs were cut into about $2.5 \times 2.5 \text{ cm}^2$ pieces. The samples were accurately weighed, and then both sides of the web were rinsed with 200 ml of distilled water to wash out the superficial drug. Then, the samples were placed in 20 ml of phosphate buffer solution (pH 7.4) at 37 °C. At predetermined time intervals, the nanofibers sample was taken out from the incubation buffer and then buffer solution was added them. The amount of released drug was determined spectrophotometrically using a Bruker-TENSOR 27 spectrophotometer (German). The UV of Tetracycline in buffer solution was determined at $\lambda_{\text{max}} = 380 \text{ nm}$ and converted to the Tetracycline concentration according to the calibration curve of Tetracycline in the same buffer. The results were reported as an average of three determinations. The drug release system was developed for the purpose of releasing, activating and targeting the drugs at the right time period, dose and place. The biodegradable polymer can contribute largely to this technology by adding its own characters to the drugs. In this connection, some polymers such as PLA, PCL, etc., are commonly used as these polymers can be prepared in the moderate conditions, has a similar stiffness of the body and has an appropriate biodegradability and low crystallinity enough to be mixed well with many kinds of drug.

3. RESULTS AND DISCUSSION

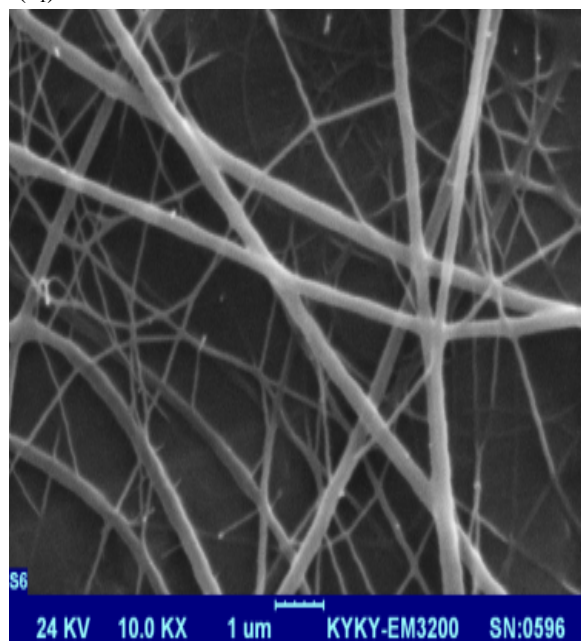
3.1. Preparation and characterization of nanofiber nonwovens

For the morphology study, the influence of the polymer concentration on the resulting nano fibers was examined. Two different emulsions

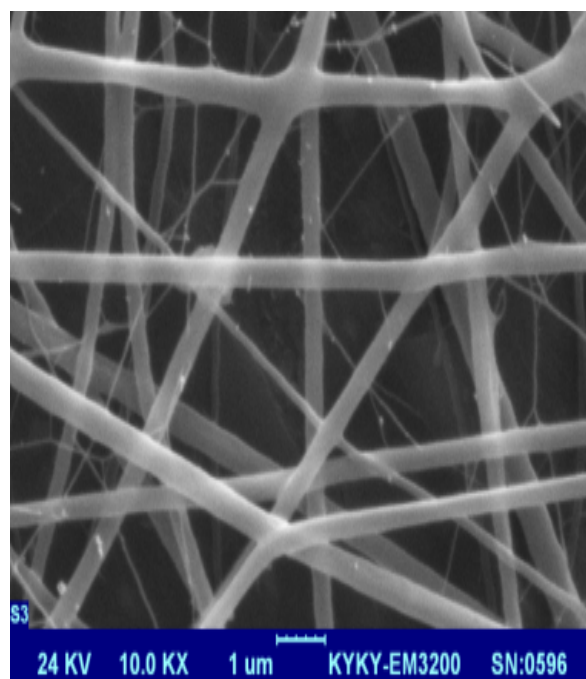
with a concentration ranging from 8,15% PCL and 3% Cs in the organic phase were electrospun. With increasing polymer concentration of PCL the uniformity of the fibers and the mean fiber diameter increased. The presence of PCL in the blend leads o reduction of hydrophilicity of the blend and preserves webs integrity in aqueous media. Higher physical properties of blend webs are because of the presence of PCL.



(a₁)

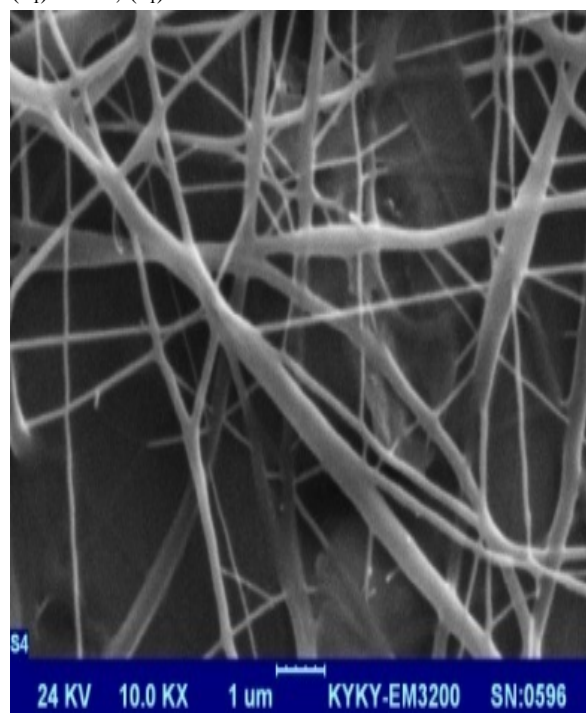


(b₁)

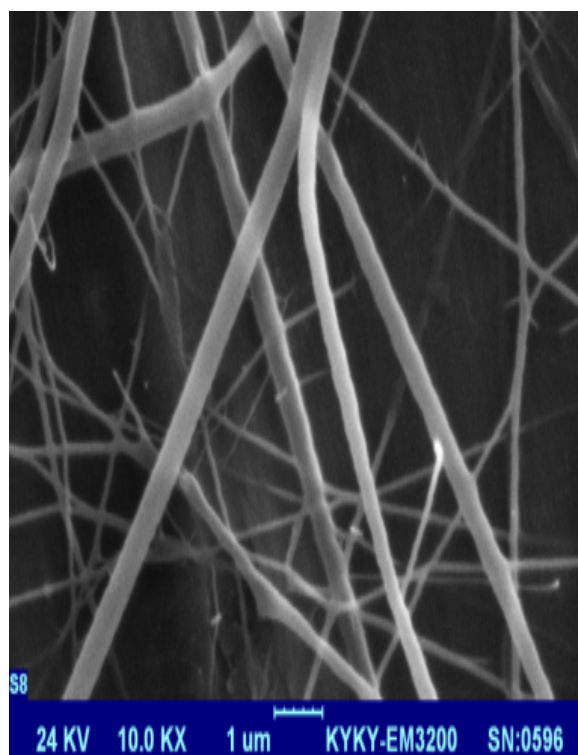


(c₁)

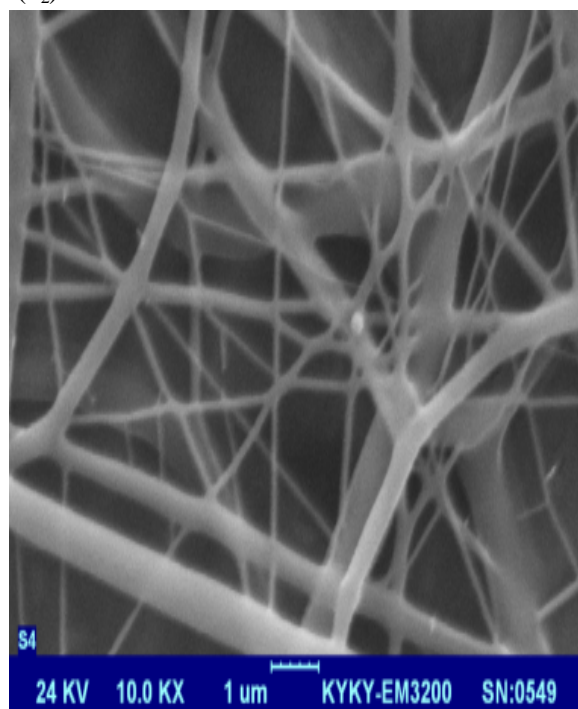
Fig. 1. SEM photographs of electrospun PCL:Cs, 8%:3% containing 0.25% w/v tetracycline: (a₁) 80:20, (b₁) 50:50, (c₁) 20:80.



(a₂)



(b₂)



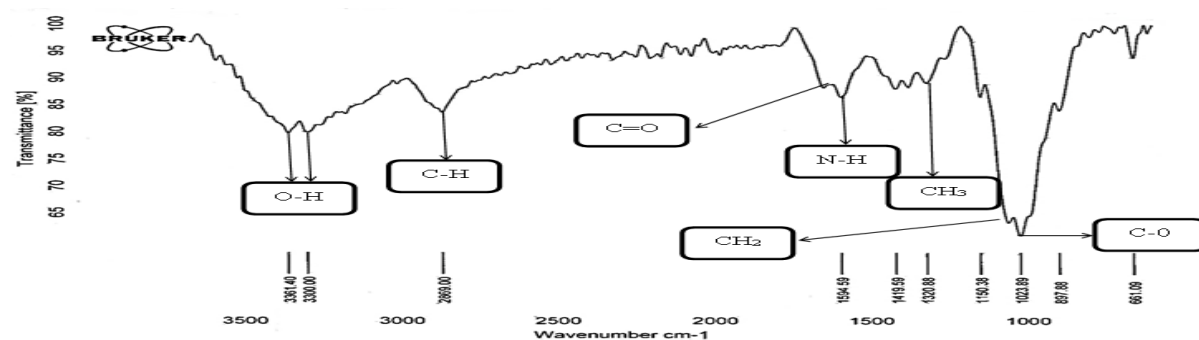
(c₂)

Fig. 2. SEM photographs of electrospun PCL:Cs, 15% : 3% containing 0.25% w/v tetracycline: (a₂) 80:20, (b₂) 50:50, (c₂) 20:80.

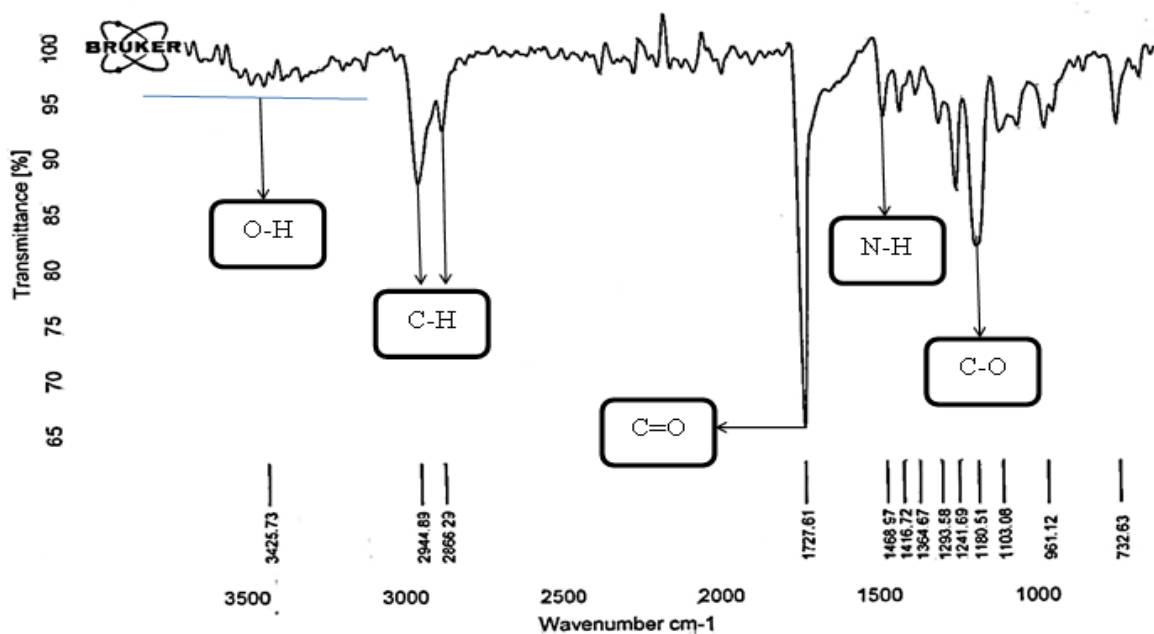
3.2. Fourier transmission infra red spectroscopy (FTIR)

Cs is an amino glucose characterized by a small proportion of amide groups via an amide linkage with acetic acid. In the IR spectrum, powder chitosan exhibited broad peaks at 3000 and 3361 cm^{-1} , which is assigned to the O–H stretch vibrational frequencies. A peak at 1594 cm^{-1} is that of free N–H and hydrogen bonded. Further, in the C–H stretch region of FTIR spectrum, the higher intensity peak at 2944 cm^{-1} is assigned to the asymmetric and the lower intensity peak at

2869 cm^{-1} is assigned to the symmetric modes of CH₂. In addition, the characteristic band due to CH₂ scissoring, which usually occurs at 1727 and 1180 cm^{-1} was also present in the sample. The bands of O–H functional groups have been created in 3300 cm^{-1} . Tetracycline (C₂₂H₂₄N₂O₈) is expected to have many functional groups of C–H that can be seen in this spectrum. In fig. 2. (d) There are not any significant changes in comparison with (b). The peaks in 2866 and 2945 cm^{-1} related to the groups are the presence of tetracycline.



(a)



(b)

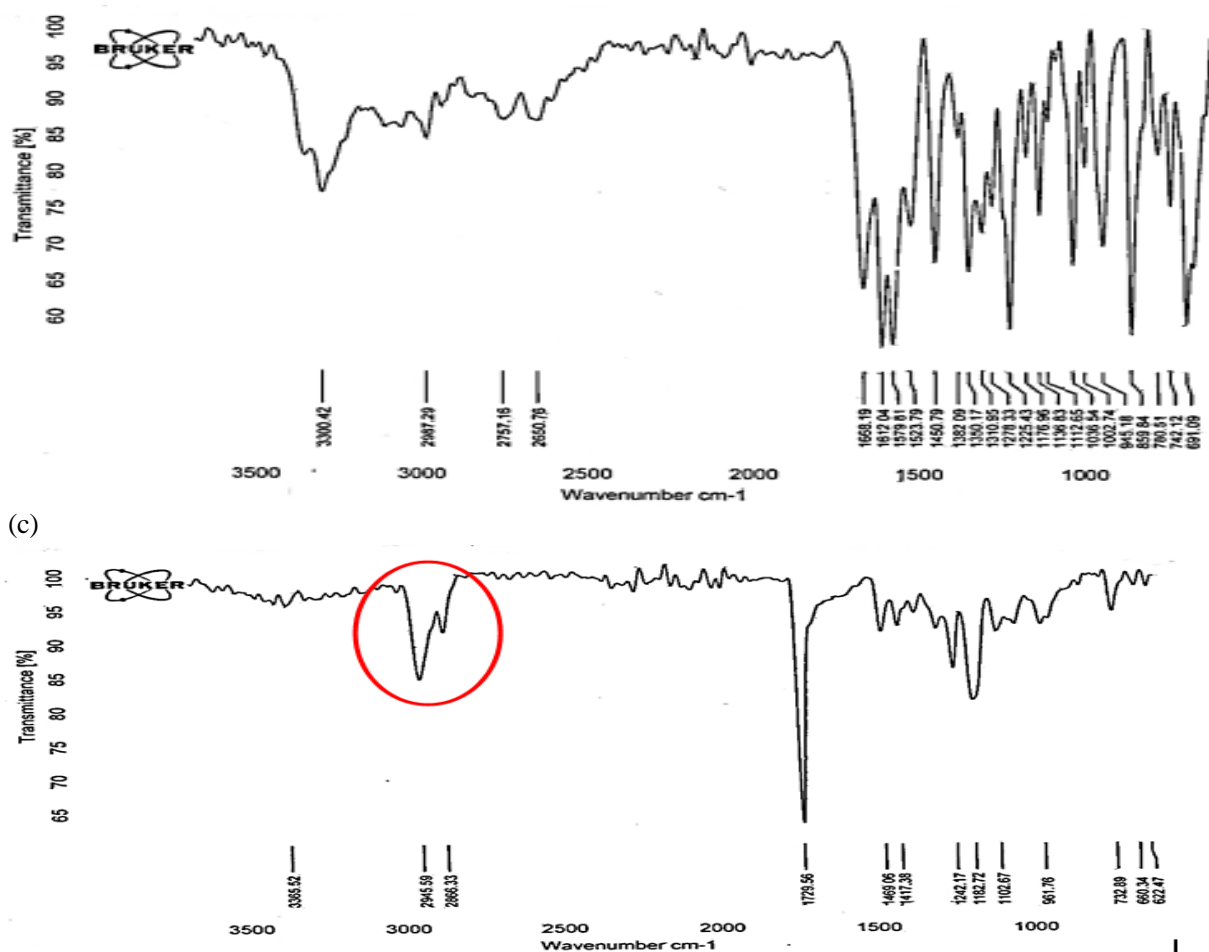
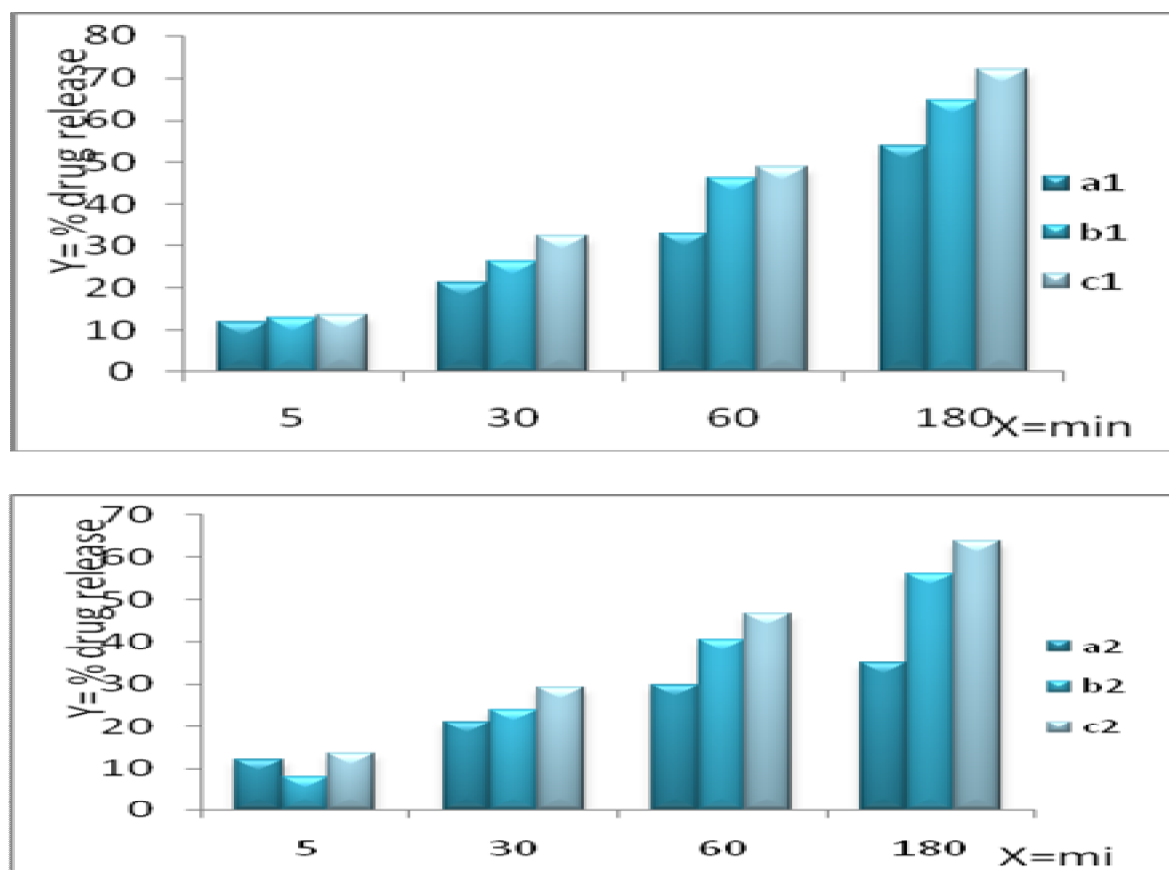


Fig. 2. FTIR spectra of (a) Cs, (b) Pcl-Cs blend (50:50), (c) Tetracycline, (d) Pcl-Cs-Tetracycline

3.3. In-vitro drug release

Despite the increasing ratio of Cs, diameter of nanofibers can be more thick than others, but because of the Cs is hydrophile, the release of drug is more.

release of tetracycline from electrospun mats of PCL:Cs, 20:80, 50:50 and 80:20 was studied and it was found that ecelctrospun PCL:CS, 80:20 had more release than others and then 50:50 and 20:80 in two different concentration, so the lower concentration of PCL had less diameter of nanofibers and have had more release. the simplicity of the electrospinning process and the wide selection of polymers that can be processed by this means suggest that electrospun polymers matrices may have broad applicability in controlled release technology. hence, the release of tetracycline was shown to be affected by the hydrophobicity of the PCL and diameter of nanofibers.



4. CONCLUSION

In this study Pcl:Csnanofibers scaffolds were electrospun successfully. Cs is a natural biodegradable polymer whereas PCL is a synthetic biopolymer. The blending of the two polymers has been carried out varying the proportion of PCL so that the composite can be a better drug carrier .hence, the presence of PCL in the blend reduce the hydrophilicity of the blend. The preparation of tetracycline hydrochloride loaded nanofiber nonwoven based on PCL and Cs via electrospinning and their characterization and the morphology of the blend have been studied using SEM. From the FTIR spectra the different pendant group present in the nanofibers have been ascertained and for studing the drug relese, spectrophotometer have been used. So, we were able to modify the release profiles by blending the PCL with hydrophilic polymers such as Cs.

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