

Nanofibrous Polymers Blend of Fluorouracil loaded Chitosan-Hydroxy Ethyl Cellulose/ Poly Vinyl Alcohol: Synthesis and Characterization

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Abstract

For treating the chronic illnesses, the best choice of drug delivery might be the controlled drug delivery systems. Nanoscaled drug delivery systems like nanofibres, nanoparticles are in the advancement of the controlled drug delivery system. Nanofibres are being a good choice due to its advantages like high drug loading and encapsulation efficiency. The present study is concerned with the synthesis of simple electrospun nanofibres of Chitosan (CHI)/Polyvinyl alcohol (PVA) blended with Hydroxy ethyl cellulose (HEC) which will be preloaded with a model chemotherapeutic agent 5-Fluorouracil (5-FU) before electrospinning. HEC is chosen for its capability of enhancing the nanofiber formation since it is used as the thickening agents in several fields. The various mixture proportions of HEC and PVA blends were tried to form electrospun fibers and characterized using FT-IT, SEM, TGA and UV-VIS (for drug release study). The data output strengthening that HEC might be used to get the optimum nanofibre in the CHI/PVA nanofibre preparation which may be useful for controlled releasing of the drugs like 5-FU for treating colo-rectal cancer like chronic illnesses.

Keywords: Synthesis, nano, analysis, extraction, 5-Fluorouracil

1. Introduction

Controlled drug delivery system is the main focus in the past decade of research in obtaining the best drug delivery system, since it is having many advantages over the conventional drug delivery systems like less toxicity and high efficiency. Due to the past efforts the pharmaceuticals' have developed the varieties of temporal controlled or site-specific drug delivery systems [1]. Apart from the normal micro scale controlled delivery systems, in recent years the formulations with nanoscale sized systems like nanofibres, nanoparticles, micellar polymers and liposomes have attracted the researchers in order to reduce the high dosage of the toxic drugs. In these, electrospun nanofibres were attracted as a new drug delivery device because of possibilities like high loading and encapsulation efficiency along with the capability for providing simultaneous delivery of different therapeutic agents, which is also having more surface area with high porous nature [2-6]. The electrospun nanofibres can be modified easily as different varieties of controlled release systems like delayed, immediate, smooth and biphasic releasing systems [7-9].

The wide ranges of polymers are being used successfully in the electrospinning techniques with the advantages of making nanofibres within the submicron range. These polymers are being a synthetic/natural/blend of proteins which also includes proteins

[10-12]. Among these polymers, natural polymers attracted the wide range of research for various biological applications because of its high biocompatibility and low toxicity. Many natural polymers suited for electrospun nanofibres have been reported earlier [13-14].

Among the various natural polymers, chitosan (CHI) became more versatile because of its more biocompatibility, easy availability, natural mucoadhesive property and by itself an anticancer agent which can be useful for preparing nanofibres through electrospinning techniques [15].

Poly vinyl alcohol (PVA) is a synthetic, semi-crystalline hydrophilic and biodegradable polymeric. Like chitosan, PVA also being with good biocompatibility, non-toxic and non-carcinogenic property [19,20]. Due to its gel forming capabilities with various types of solvents, PVA can be used in the wide ranges of applications like in medical, cosmetic, food, pharmaceutical etc [16]. Hydroxy ethyl cellulose (HEC) is one of the nonionic water-soluble and water-swellable cellulose and it is mostly compatible with most of the water soluble polymers. Since it is having the thickening property it is been used in paints, paper finishes etc. [17].

In this present work, the conventional electrospinning technique is used for producing CHI-PVA nanofibres which was previously loaded with 5-FU a model drug useful for colorectal cancer and also blended with HEC in order to increase the efficiency of nanofibre formation. The HEC blended, 5-FU loaded CHI-PVA nanofibres formed are studied for the controlled release characteristics by *in vitro* method.

2. Experimental

2.1. Materials

Chitosan-low molecular weight (Sigma-Aldrich, South Korea), Hydroxy Ethyl Cellulose (Samchun pure chemical Co.Ltd, South Korea), PVA (Moiwol[®] 20-98, Molecular weight 1,25,000) (Sigma-Aldrich, South Korea), 5-Fluorouracil (Sigma-Aldrich, South Korea). All other chemicals used in this experiment were in Analytical grade.

2.2. Preparation of Composite Polymers/HEC Solution Containing 5-FU

The synthesis is carried out through sol-gel route followed by electrospinning. The typical ideal procedure is as follows. Initially 6 ml of 10% Poly vinyl alcohol (PVA) solution was added with 2 ml of 5% Chitosan (CHI) solution and allowed to stir well for 20 minutes by using the magnetic stirrer. To the above mixture 0.5 ml of 5% 5-Fluorouracil (5-FU) was added and stirred well for 10 minutes in order to load the drug into the above mixture. Again, 1.5 ml of 1% Hydroxy ethyl cellulose (HEC) was added to the above mixture and allowed to disperse completely and subjected to electrospinning.

2.3. Electrospinning

The stable mixture solution was subjected to electrospinning by using the NanoNC (ESR-200R2D, eS-robot). The drug containing CHI-PVA-HEC blend was filled into a glass syringe with the flat end metal needle with an inner diameter of 0.7mm. Then the 15 kV current was applied, with the injection volume of 1 mL/Hr. The rotating collector with the aluminium foil wrapped was placed with the distance of 10 cm from the needle tip and rotated at the rate of 25 RPM. The electrospun mat was removed carefully and dried under vacuum for the complete removal of the solvent and subjected for further studies.

2.4. Characterization of 5-FU Containing CHI-PVA-HEC Nanofibrous Blend

The morphology and diameter of the drug loaded composite nanofibres were observed using a scanning electron microscopy (SEM, JEOL JSM 5600, TOKYO, JAPAN). Before subjecting to analysis, each sample was sputter coated with gold and the nanofibres were analyzed in different positions.

The 5-FU, CHI-PVA-HEC composite nanofibre containing drug were characterized by using FT-IR (Fourier-transform infrared spectroscopy, NICOLET-200, Thermo, USA), using KBr pellets.

The stability of 5-Fluorouracil and the 5-FU containing CHI-PVA-HEC blended nanofibres were indexed by using thermo-gravimetry (TGA, Scinco Co. Ltd, Seoul, Korea), in air atmosphere at 10°C/min.

2.5. Drug Entrapment Efficiency

The drug entrapment efficiency of the composite nanofibres were calculated by subjecting the drug-loaded nanofibres for drying at 45°C for one hour in a hot air oven, and the known area (1 X 1 cm) of dried nanofibre mat was dissolved in water. Then the drug content in the solution was examined through UV spectroscopy and this content was compared with the amount of drug which was loaded during the electrospinning process of these fibres as mentioned in the (18) and this was given as equation number (1).

$$\text{Entrapment Efficiency (\%)} = \frac{\text{mass of maximum drug released}}{\text{mass of total drug added}} \times 100 \quad (1)$$

2.6. Degree of Swelling

The degree of swelling was carried out separately for CHI-PVA-5-FU and CHI-PVA-HEC-5-FU nanofibres by using 7.4pH phosphate buffered saline, at 37°C for different time periods of 1,3,5,7,9,11 and 13 hours. The following equation was used for identifying the degree of swelling (18)

$$\text{Degree of Swelling} = \left(\frac{M - M_d}{M_d} \right) \times 100 \quad (2)$$

Where, M is the weight of the swollen nanofibres, which was wiped to dry by using the filter paper, M_d is the mass of dried sample which was immersed in the buffer solution. Degree of swelling was measured by drying the swollen nanofibres in an oven at 40°C until it attain the constant weight.

2.7. In Vitro Drug Release Studies

Drug release was evaluated by cumulative percentage release in the UV-VIS spectrometry at 262 nm for around 20 hours. The known area (2 cm X 2 cm X .020mm) of drug loaded composite nanofiber sample mat was incubated at 37°C in 20 ml of phosphate buffered saline solution (PBS, pH 7.4) and gently shaken by using the thermostatic shaking incubator. Specific volume of drug released solution was withdrawn and replaced with phosphate buffer in the regular interval of time. The release of drug was extended up to 20 hrs.

3. Results and discussion

3.1. Scanning Electron Microscopy (SEM)

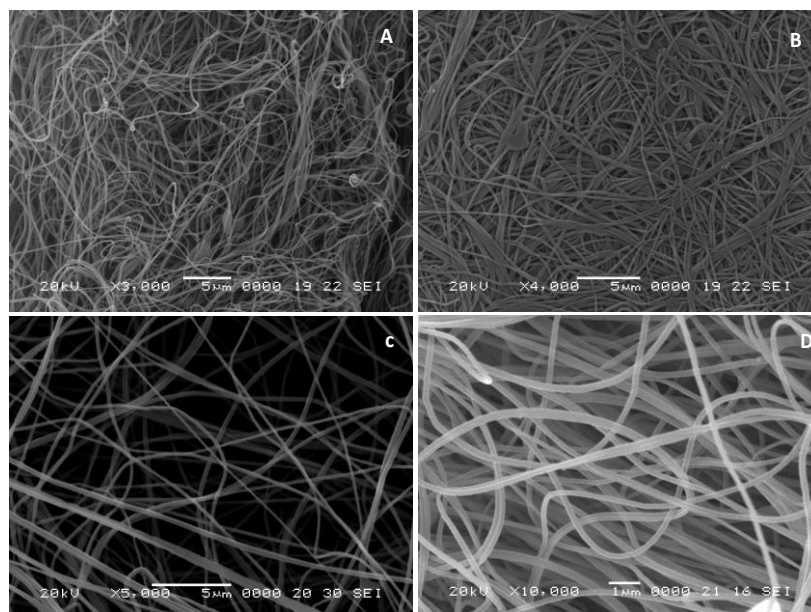


Figure 1. SEM Micrographs of (A) PVA-5-FU, (B) PVA- CHI, (C) PVA-CHI-5-FU and (D) PVA-CHI-HEC-5-FU

The interaction of all the polymers along with the drug 5-FU and HEC were evidenced through the formation of nanofibres which were possibly due to the hydrogen bonding with the water. The morphologies different composite polymeric nanofibres were shown in the SEM micrographs (Figure 1.). The nanofibre formed only by polyvinyl alcohol (PVA) containing 5-Fluorouracil (5-FU) was shown in figure (1A), in which the slightly crystallized 5-FU was evidenced through the white patches across the nanofibres. In the figure (1B) the nanofibres made of PVA and chitosan (CHI) alone was shown which formed the uniform sized nanofibres with almost smooth surface, which was visible even in the higher resolution than the previous figure (1A). The composite nanofibre made of PVA-CHI loaded with 5-FU was shown in figure (1C), in which the slight crystallized 5-FU was visualized with white patches across the composite nanofibre. The composite nanofibre made of PVA-CHI-HEC loaded with 5-FU was shown in figure (1D), where the smooth surface of the nanofibre was visualized in the higher resolution over all other nanofibres. Also the uniform diameter was evidenced in the last figure. This reveals that addition of HEC improves the efficiency in forming the composite nanofibres even though all the components along with 5-FU were hydrophilic.

3.2. FT-IR Spectroscopy

The functional groups and the bonding between polymer and drug were confirmed by characterizing the drug loaded composite nanofiber by FTIR. The FTIR spectroscopy of poly vinyl alcohol (PVA) was shown in the figure 2. Here, a typical strong hydroxyl band of hydrogen bonded hydroxyl group were observed around 3416 cm^{-1} and also a C-H broad alkyl stretching band was observed at 2940 cm^{-1} . The semi crystalline nature of this synthetic polymer is confirmed by the peak around 1096 cm^{-1} .

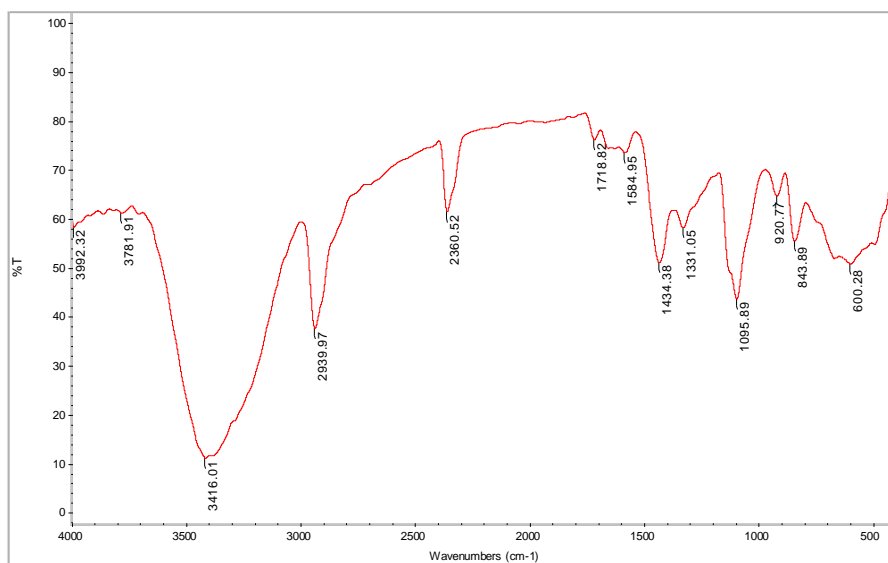


Figure 2. FTIR spectroscopy of Poly Vinyl Alcohol (PVA)

The FTIR spectroscopy of 5-Fluorouracil was shown in figure.3. The free N-H stretching band was observed around 3135 cm⁻¹. Also, C=O and C-N stretching were observed around 1724 cm⁻¹ and 1661 cm⁻¹ respectively. The C-H in plane bending was observed around 1246 cm⁻¹.

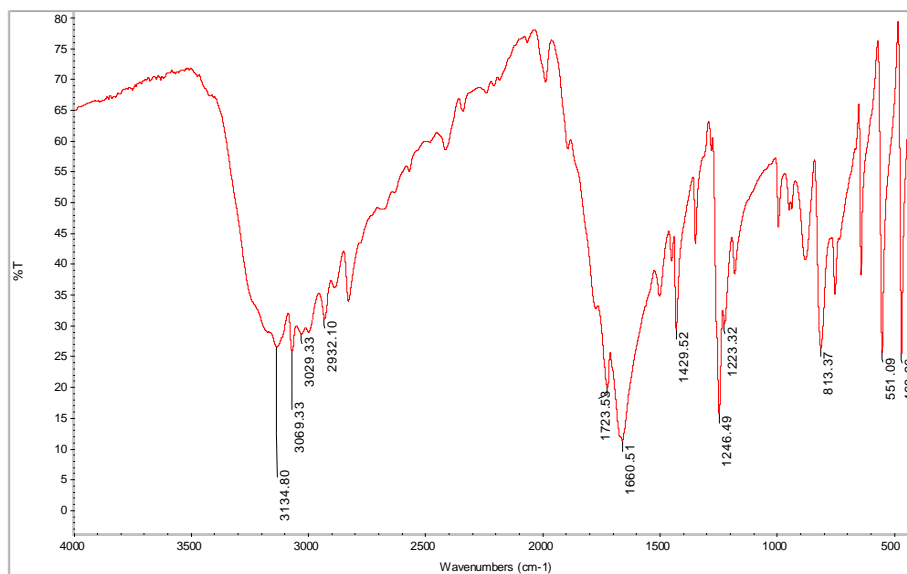


Figure 3. FTIR spectroscopy of 5-Fluorouracil (5-FU)

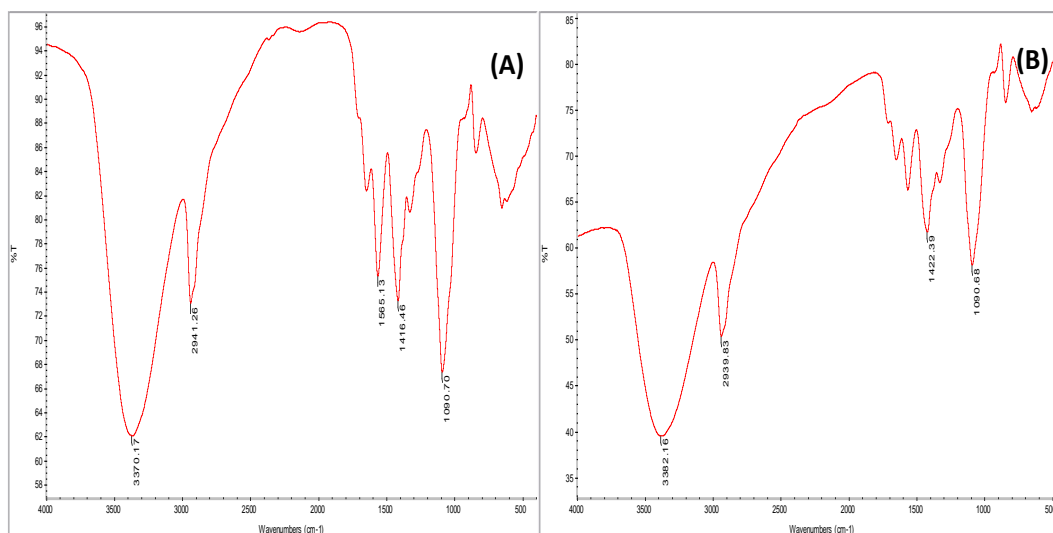


Figure 4. FTIR Spectroscopy of (A) PVA-CHI, (B) PVA-CHI-HEC

In the figure 4 (A) and 4(B), FTIR spectra of PVA-CHI and PVA-CHI-HEC were shown respectively. The spectra clearly depicting the better interaction of PVA with CHI and HEC with minor shiftings from the regular PVA spectrum.

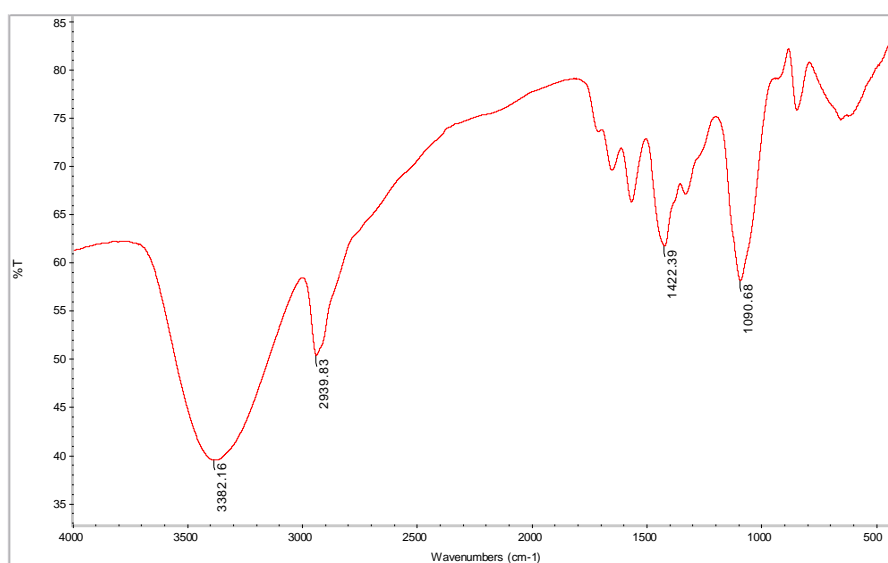


Figure 5. FTIR Spectroscopy of (A) PVA-CHI-HEC-5-FU

The characteristic absorption peaks of 5-FU were not observed in the figure.5, which is suggesting the good interaction of 5-FU with polymer materials along with HEC which is giving way to make the smooth surfaced composite nanofiber. FTIR spectra of the composite nanofibres were clearly suggesting that the addition of Hydroxy Ethyl Cellulose is compatible with the polymers and with 5-FU too, which can be used to increase the efficiency of the nanofibre formation suitable for controlled drug release.

3.3. Thermo Gravimetry Analysis

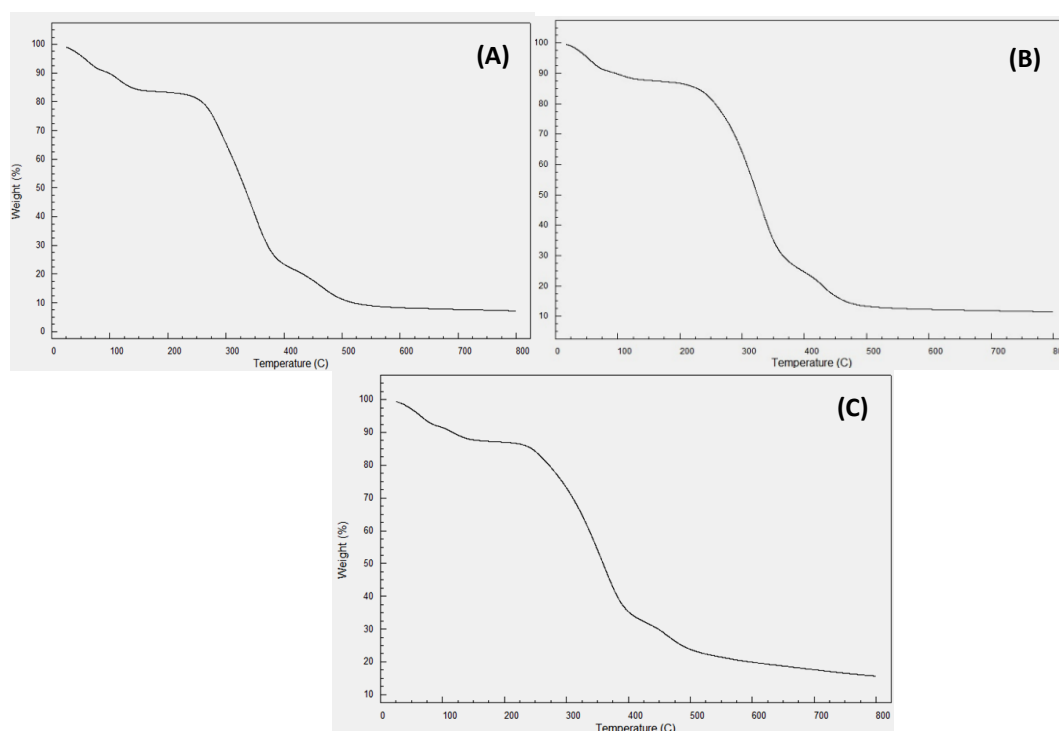


Figure 6. TGA of (A) PVA-CHI, (B) PVA-CHI-HEC and (C) PVA-CHI-HEC-5-FU

The TGA analysis graphs of PVA-CHI, PVA-CHI-HEC & PVA-CHI-HEC-5-FU were shown in figure.6. The figures show the better thermal stability of the composite nanofibres. The weight loss below 100°C is due to loss of adsorbed water. It is followed by a major weight loss around 250°C due to degradation and desorption of the components of the fibers. The decomposition is nearly complete. So, the thermal properties are adequate enough for applications in drug delivery.

3.4. Drug Entrapment Efficiency

The drug entrapment efficiency into the composite nanofibres is mainly relying on the hydrophilicity of drug with the polymer solutions. The non-volatile nature of the drug leads to the higher entrapment into the composite polymers along with hydroxy ethyl cellulose. The entrapment efficiency was calculated by using UV-Vis spectroscopy and was found to be around 95%.

3.5. Degree of Swelling of the Drug Loaded Composite Nanofibres

The degree of swelling of the composite nanofibres will have a vital role in loading and releasing of the drug. Here, in figure 7 it shows the degree of swelling of PVA-CHI-HEC composite nanofibres and 5-FU loaded PVA-CHI-HEC composite nanofibres at various time intervals. The degree of swelling was observed for both the plain and the drug loaded composite nanofibres which was found to be as follows: $109 \pm 3.9\%$ and $101 \pm 4.0\%$ for 1 h, $142 \pm 4.1\%$ and $132 \pm 3.9\%$ for 3 hrs, $160 \pm 4.6\%$ and $151 \pm 4.4\%$ for 5 hrs, $225 \pm 3.6\%$ and $210 \pm 4\%$ for 7 hrs, $202 \pm 4.5\%$ and $191 \pm 4.6\%$ for 9 hrs, $186 \pm 4\%$ and $171 \pm 5\%$ for 11 hrs, $153 \pm 4.4\%$ and $136 \pm 3.6\%$ for 13 hrs respectively. The results clearly depicting the decrease of swelling behavior of the drug loaded composite nanofibres made of PVA-CHI-HEC. This may be due to the hydrophilicity of the drug as well as HEC, which may lead to the hydrogen bonding with the polymeric chains

consequently reduces the swelling. Among all the swelling percentage, the maximum was observed at 7th hour and subsequently the swelling was reduced. This may be due to the degradation of polymeric composites after 7 hours which may get leached into the buffer solution.

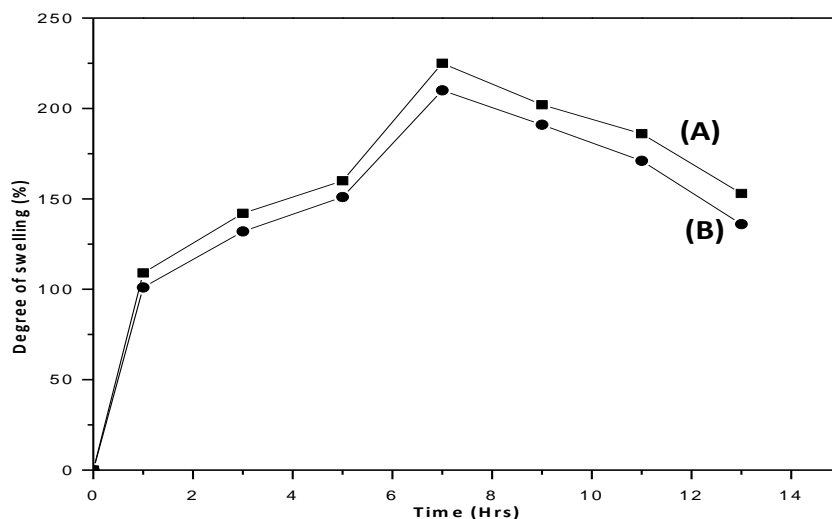


Figure 6. Degree of Swelling of (A) PVA-CHI-HEC and (B) PVA-CHI-HEC-5-FU

3.6. *In Vitro* Release of Drug

Drug release was evaluated by cumulative % release in the UV-VIS spectrometry at 262 nm for around 20 hours and shown in figure 8. The known area of drug loaded nanofiber mat (2 cm X 2 cm X .020mm) was incubated at 37C in 20 ml of phosphate buffered saline solution (PBS, pH 7.4) and gently shaken. Specific volume of drug released solution was withdrawn and replaced with phosphate buffer in the regular interval of time The total release of drug was around 73% which was extended upto 20 hrs which was indicating that this electrospun nanofibrous material might be useful for controlled release of drug for the diseases like colorectal cancer.

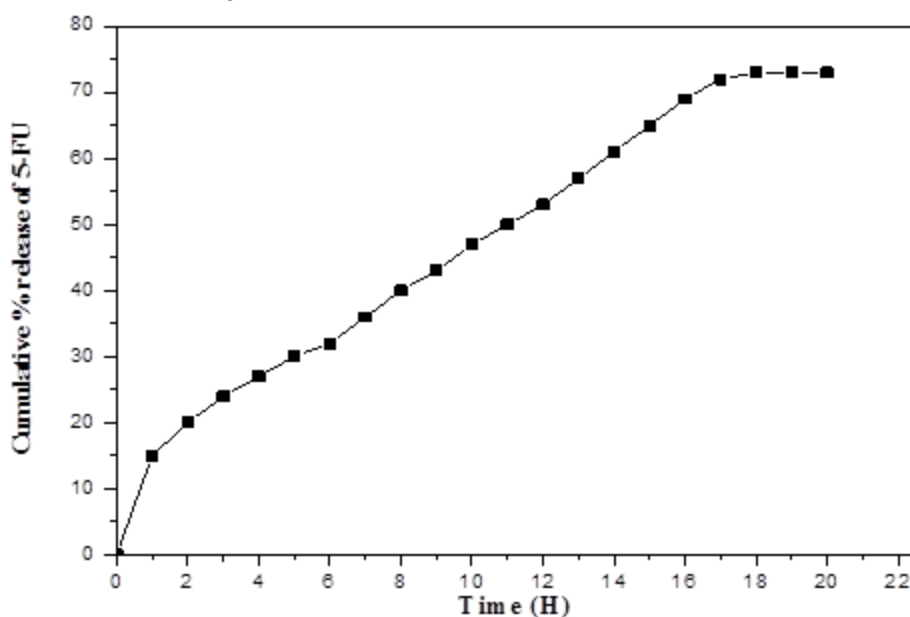


Figure 7. Drug Release Pattern of the 5-FU Loaded Composite Nanofibre

4. Conclusion

A simple method to prepare a multi polymer blend electrospun nanofibre material was developed with the use of Polyvinyl Alcohol-Hydroxy Ethyl Cellulose-Chitosan – loaded with 5-Fluorouracil (an anti-cancer drug especially suitable for colorectal cancer). Here, polyvinyl alcohol acts as a synthetic biodegradable polymer along with the chitosan a natural biodegradable polymer. Both of these will be with better output while making the blended polymer solution suitable for controlled release drug delivery.

Hydroxy ethyl cellulose, pharmaceutically best used as a glident and thickening agent which is mixed with the PVA-CHI blend for forming the ideal nanofibres loaded with 5-Fluorouracil, an anti-cancer drug. Most of the hydrophilic drug can be easily formed in this manner and the release characteristics can be studied in this method. The releasing of the drug was also delayed around 18 hours. Since the drug releasing is slow this material may be useful for releasing drug in the Gastro intestinal region especially during the colorectal cancer like diseases. The carrier is not harmful which was proved in lot of previous works.

In the present study, only the hydrophilic drug is used for the evaluation, in the future hydrophobic drugs will be used for controlled drug release evaluation. Study of influence of various glident and thickening agent contents on the uptake and release characteristics was not examined now, but it will be thoroughly investigated in the future works.

Acknowledgments

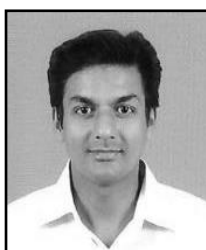
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