

Research Article**Study of anti-cancer drug release (tamoxifen) of the nanofibers
made of poly-caprolactone -chitosan****Zahra Saeidi^{*1}, Ali Ashjarian¹
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ABSTRACT

Breast cancer is a kind of cancer that begins than breast tissue. That in a kind of it, breast skin is quite involved. Than symptoms of the breast cancer is a dimple on the skin and peeling of skin and on the chest and..Natural polymers in terms of the environmental characteristics and their compatibility with the human body have vast applications in the industry: cosmetics, wound dressing, delivery of medicine scaffold, and so on.In this study, the cancer drug (tamoxifen) used to complete the PCL-chitosan nanofibers made of a biocompatible polymer packag that deliver drugs will be examine. For this purpose, first the nanofibers made of PCL-chitosan-containing drugs (tamoxifen) with optimal concentration of the drug was produced by electrospinning. The surface morphology of nanofibers microscopy (SEM) were studied. Using infrared spectrometer instrument (FTIR) evaluated the drug in nano-fibers. The effects of drug release and antimicrobial from nanofibers with standard (AATCC100) measurement and determined. According to antimicrobial test the nano-fiber manufacturing against Gram-positive and Gram-negative bacteria, Escherichia coli and Staphylococcus Cocos considerable influence along. Based on the results of tests it was found that nano-fiber PCL-chitosan-containing drug with a ratio of 70-30 and a concentration of 1 gr and 0.3 gr, speed And release better itself show compared to concentrations and the other ratios. The test results of drug release indicate a total release rate was high.

Keywords:-electrospinning, poly-caprolactone, chitosan, tamoxifen, nano-fiber**1. INTRODUCTION**

Cancer is mean a range of diseases along with uncontrolled proliferation and invasion of other organs. Cancer cells are through the blood or lymphatic system be transferred to other parts of the body [3,2,1]. Several treatments is used for cancer, including chemotherapy, radiation therapy, hormone therapy, immunotherapy, surgery Each of them, due to the treatment of

cancer cells have cause complications in the patient's body The most important of these effects is poisoning the healthy cells in the bone marrow, gastrointestinal and.... can be cause organ damage, like liver, kidneys, heart, lungs, etc. That's why researchers are trying to deliver drugs directly to cancer cells. Therefore, be studied about nanotechnology and biodegradable

polymers and natural non-harmful for human body [5,4]. Raw material of chitosan is chitin, after cellulose is, the most abundant natural polymer. Chitin is the hard substance with crystalline structure and is white, and the base material is variety of animals and lobster shells of also The outer cover snails and insects and fungi, also can be found in the cell wall, chitin is a polysaccharide extraordinary alkaline, chitosan derived of chitin, which is chitosan derived from acetylation of chitin. Chitosan substance is non-toxic and biodegradable in nature, but its solubility is low and has poor reactivity [6].

PCL is a semi-crystalline polymer, water repellent (hydrophobic). is also superior mechanical properties (mechanical flexibility), good biocompatibility, process flexibility easy, low melting point and non-toxic product of the destruction. it has also biocompatibility and biodegradability due to lower rates used in medicine, in other words use to restore the hard tissue and soft tissue the body[5].

One of the drugs used in chemotherapy in breast cancer and melanoma is tamoxifen [7].

And tamoxifen is a drug in SERM groups (Modulator Selective Receptor Estrogen). These drugs have similar effects to estrogen in some tissues (agonists) and has similar effects in other tissues relative or block the effect of estrogen (antagonists). Tamoxifen is a SERM that prescribed for treatment of cancer responsive-hormone. And in this context as antiagonists of endogenous receptor activation by estrogens prevent. Tamoxifen metabolism is in the liver and have gut-liver cycle. Remove the two-stage drugs that 4-7 hours after administration of the first stage and second stage lasts 7 to 14 days, The desirable therapeutic effect is achieved 4-10 weeks after starting treatment drug, predominantly in the metabolites excreted through the bile and feces [10,9,8].

Shalumon and colleagues in a study in 2009 to examine the chitosan-polyester PCL electrospun **Table (1)** samples without medication

nanofibers using a mixture of formic acid \ asetone's, He chitosan-poly blend of PCL in a ratio of 50:50, 75:25, 25:75 in order to get a good nanofibers. could produce nanofibers as an excellent scaffold for biomedical applications present [11]. Lan Zheng and colleagues in a study in 2011 to evaluate the antitumor activity of PEG-poly-PCL micelles with their doxorubicin against melanoma doxorubicin of used. and highly effective anti-cancer agents in this study, by combining it with PCL micelles made of polyethylene glycol and evaluation concluded that Antitumor activity and toxicity in the body obviously improved [12]. Novin nikhbakhsh and colleagues in a paper in 2011 to investigate the effect of tamoxifen in the treatment of idiopathic gynecomastia (male breast cancer) addressing, In this study, patients who had idiopathic gynecomastia tamoxifen after obtaining the consent of 10-20mg \ day were treated for 6 months, The results showed that tamoxifen is an effective treatment for gynecomastia And due to the reduced size of the breasts and the bouncing it is recommended tamoxifen to treat gynecomastia [13]. So this research with using nanotechnology and natural nanofibers to study tamoxifen release.

2. MATERIALAND METHODS

Chitosan product of Sigma-Aldrich company, poly caprolactone product from the laboratory Mehrazma, tamoxifen produced by Iran hormone company and acetic acid 80% and 90%.

a. Electrospinning conditions

Electrospinning voltage of 18.9 at a distance needle to collector of 15 cm.

2.2Analysis method

Chitosan at a concentration of 0.3g and 0.5g and PCL with two concentrations of 1g and 1.5g in a solution of acetic acid is prepared and were mixed together to the ratio of 30-70, 20-80, 50-50, 70-30, 80-20 And then was a electrospinning.(table1)

Materials and methods	Poly(caprolacton) 1g	Poly(caprolacton) 1.5g	Chitosan 0.3g	Chitosan 0.5g	Aceticacid	Distance needle to collector	Feed rate	Voltage	Temperature
sample									
S1	80%		20%		10 cc	15 cm	0.00	18.6	28c
S2	20%		80%		10 cc	15 cm	0.01	19.8	35c
S3	50%		50%		10 cc	15 cm	0.00	18.9	33c
S4	70%		30%		10 cc	15 cm	0.00	18.5	28c
S5	30%		70%		10 cc	15 cm	0.01	19.9	36c
S6		20%	80%		10 cc	15 cm	0.01	20.3	35c
S7		80%	20%		10 cc	15 cm	0.00	18.8	29c
S8		50%	50%		10 cc	15 cm	0.00	19.4	33c
S9		30%	70%		10 cc	15 cm	0.01	20.1	36c
S10		70%	30%		10 cc	15 cm	0.00	19.3	29c
S11	20%			80%	10 cc	15 cm	0.01	22.5	39c
S12	80%			20%	10 cc	15 cm	0.00	18.7	28c
S13	50%			50%	10 cc	15 cm	0.00	20.6	33c
S14	70%			30%	10 cc	15 cm	0.00	19.5	29c
S15	30%			70%	10 cc	15 cm	0.01	20.8	36c
S16		80%		20%	10 cc	15 cm	0.00	19.0	30c
S17		20%		80%	10 cc	15 cm	0.01	21.9	38c
S18		50%		50%	10 cc	15 cm	0.00	20.4	36c
S19		70%		30%	10 cc	15 cm	0.00	19.7	30c
S20		30%		70%	10 cc	15 cm	0.01	22.3	34c

Table (2)Samples with medication

Materials and methods	Poly(caprolacton)1g	Poly(caprolacton)1.5g	Chitosan 0.3g	Chitosan 0.5g	Tamoxifen	Aceticacid	Distance needle to collector	Voltage	Temperature
sample									
S1	80%		20%		15 mg	10 cc	15 cm	18.6	28c
S4	70%		30%		15 mg	10 cc	15 cm	18.5	28c
S12	80%			20%	15 mg	10 cc	15 cm	18.7	28c

To identify the optimal sample of 20 samples taken from the device electron microscopy (SEM) was used. (Figure1) For the detection of chain link PCL- chitosan infrared spectrometer(FTIR) was used.(Figure3)

Then identify three examples of optimal concentrations of PCL-chitosan 80-20% and 1g-0.5g, PCL-chitosan 80-20% and 1g-0.3g, PCL-chitosan 70-30% and 1g-0.3g with drug were combined and do electrospun again.(table 2) for the detection sized of Nano-fibers with drugs electron microscopy(Figure2) and To determine the link between drugs and nano-fiber infrared spectrometer was used.(Figure4) Antimicrobial tests with two *Escherichia coli* and *Staphylococcus* were performed with standard AATCC100.(Figure5) And drug release using absorption spectrophotometer, after charting standard the drug in PH=5.5 and PH=7, Of each sample, choic two parts in to phosphate buffer with PH=5.5 and distilled water with PH=7 were placed.

Then every 60 minutes using an absorption spectrophotometer and the values obtained taken from samples absorption was recorded. And this practice has continued to until absorption zero.(Graph2)

2.3 Tools electron microscopy, infrared spectroscopy and absorption spectrophotometer

Scanning electron micrographs by electron microscopy of MV2300 Camscan model at Tehran University, Faculty of Metallurgy.

Infrared spectrometer of Model pectromone Manu facturing pekin elmer Co. Absorption spectrophotometer testing by Cary 100 UV-Visible Spectrophotometer.

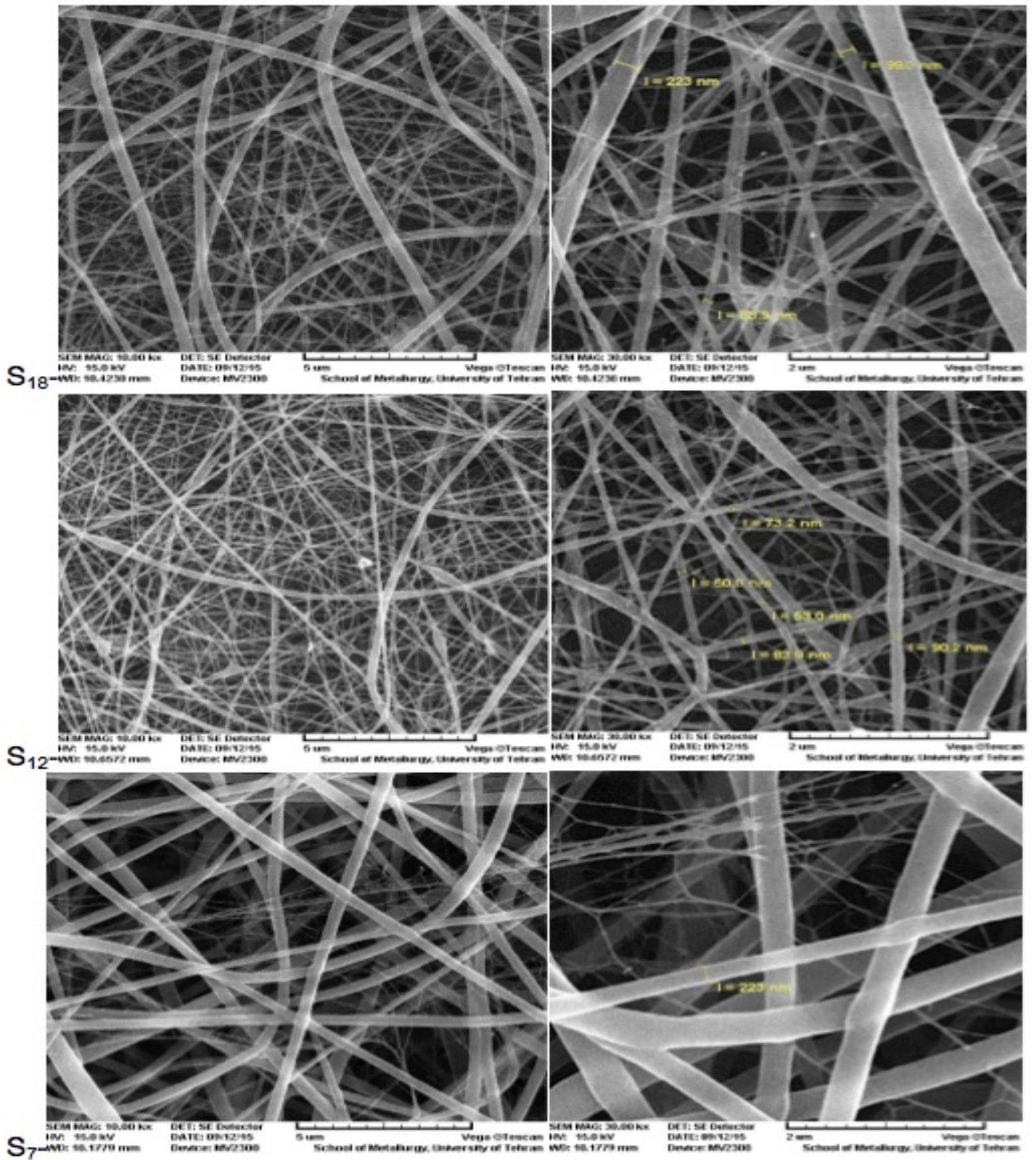
3. RESULTS

3.1 scanning electron microscope test

PCL-Chitosan with 20 different weight ratio were mixed together and electrospinning Using a scanning electron microscope (SEM) optimized samples were identified and selected. (Fig1)

average diameter of nanofibers is 84.86 nm. Samples S_{12} , S_4 , S_1 as examples of the top 20 were selected.

The samples were prepared again, this time with the drug was electrospun, Photo scanning electron microscope of nanofibers containing drug have shown that increasing the diameter of nanofibers containing drug and nano-fibers without drug. The mean diameter of nanofibers containing the medication is 159.55 nm (Fig. 2).



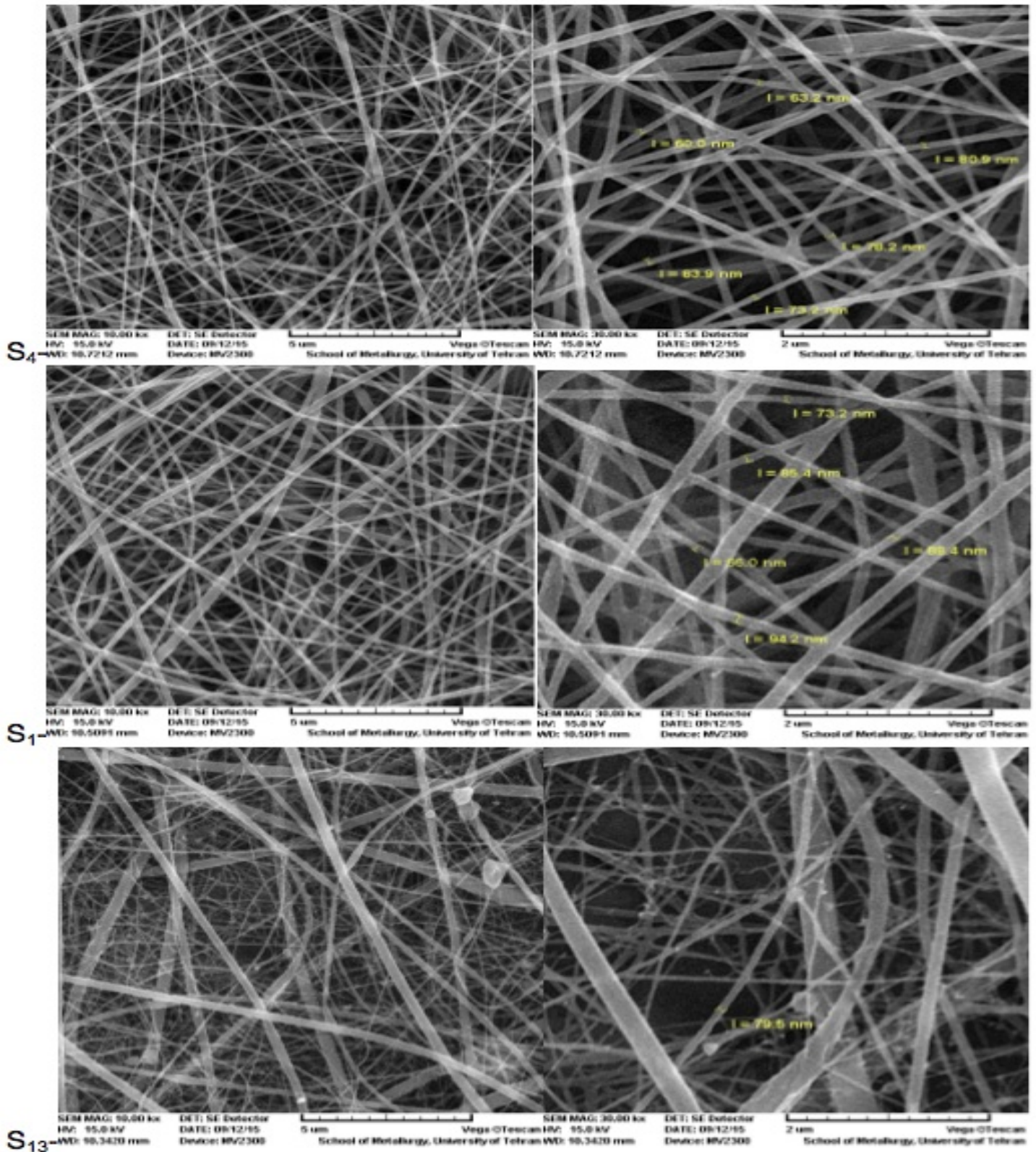


Fig (1). Scanning electron microscope image S₁₈- 50 % PCL 1.5g, 50% CH 0.5g S₁₂- 80 % and 20 % PCL 1g CH 0.5g S₇- 80%PCL 1.5g, 20% CH 0.3g S₄- 70 % PCL 1g and 30% CH 0.3g S₁- 80 % and 20 % PCL 1g CH 0.3g S₁₃- 50 % and 50 % PCL 1g CH 0.5g.

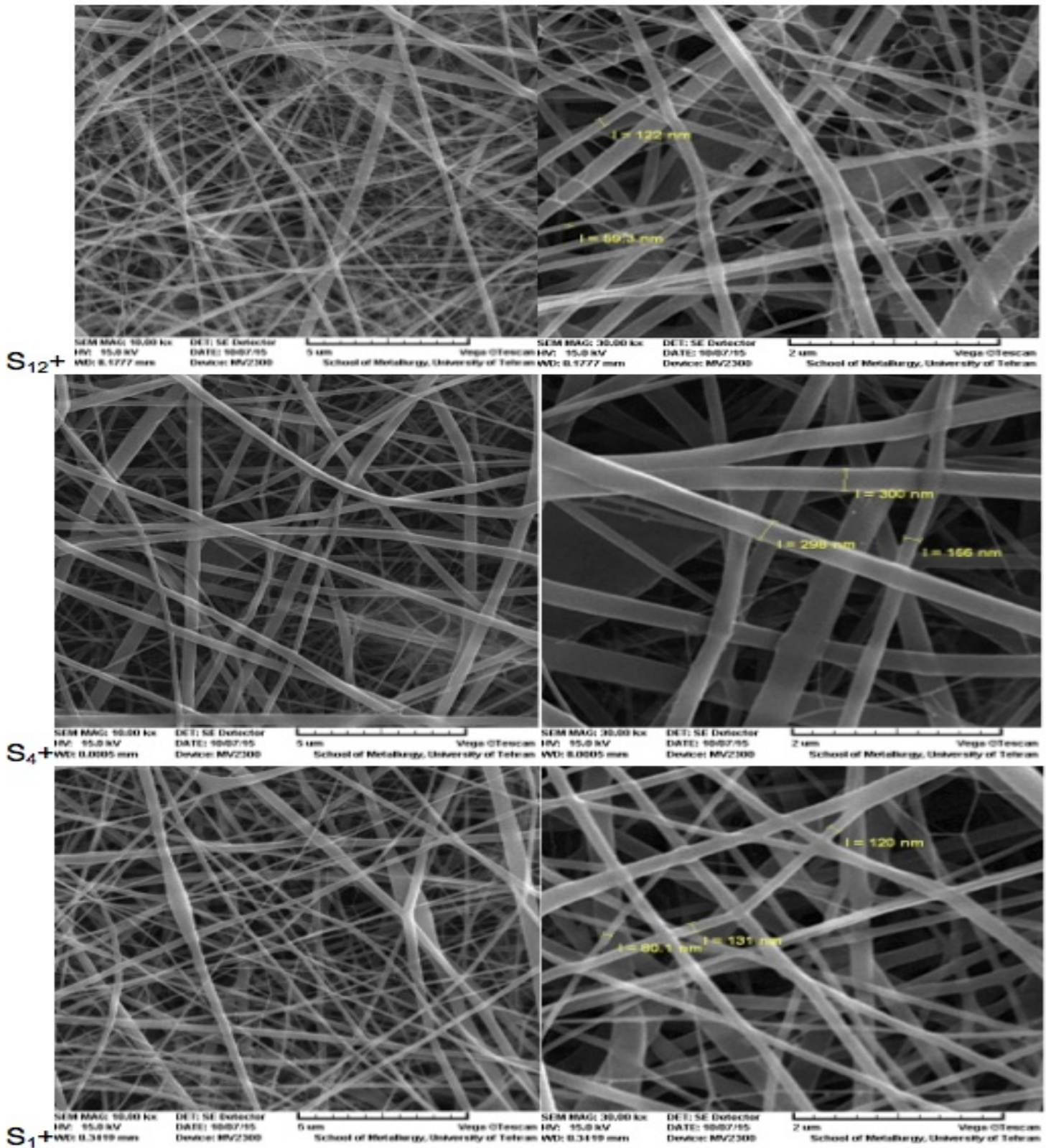


Fig (2). Scanning electron microscope image of nanofibers containing medicines and (+) means nanofibers containing medicine.

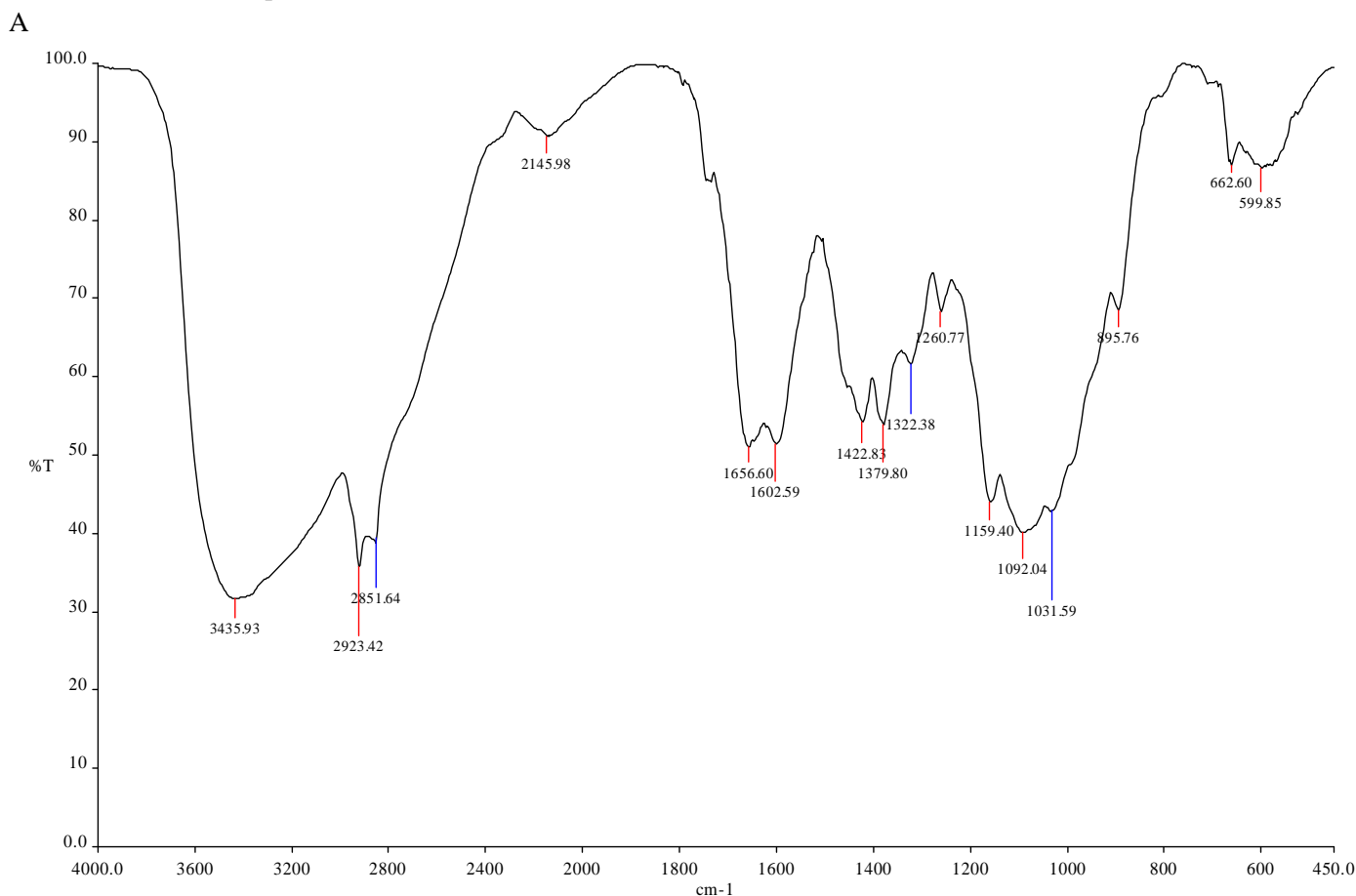
3.2 Infrared spectroscopy test

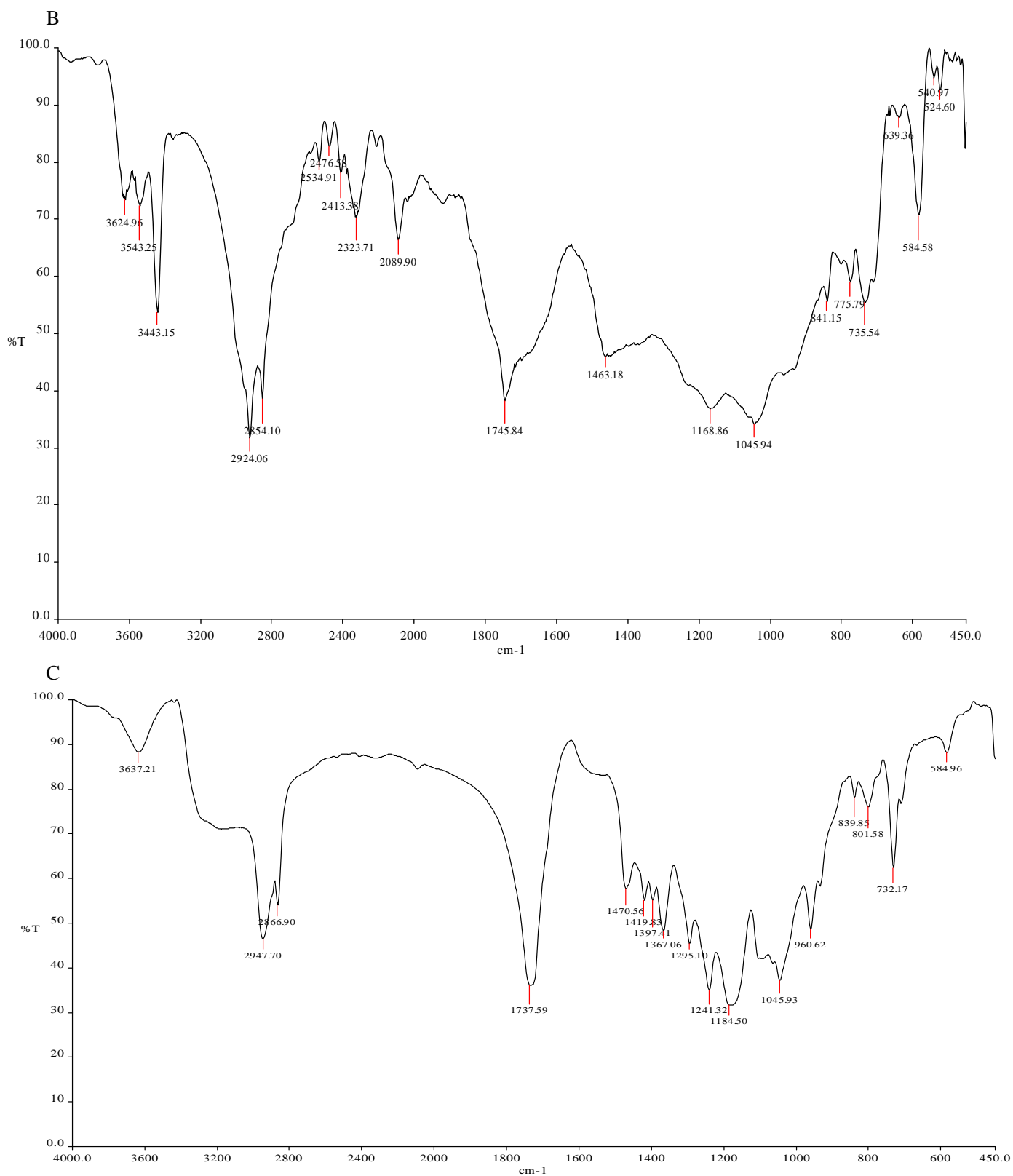
In FTIR, all the stretching and bending vibrations are found to be well matching with the theoretical values. The representative spectrum is shown in Fig. 3. In CS, the broad peak at 3421 cm^{-1} was due to N-H and hydrogen bonded O-H stretching.

The peak at 2922 cm^{-1} was due to the asymmetric bending of C-H group. The N-H and -C-O-C peaks were observed at 1648 and 1100 cm^{-1} , respectively. In PCL, the peaks at 2929 and 1729 cm^{-1} represent the characteristic peaks for C-H and ester carbonyl groups, respectively. In PCL/CS scaffold, the O-H stretching has shifted to the lower frequency side and a sharp peak at 3437 cm^{-1} was observed. A slight bending at 1727 cm^{-1} is due to the presence of carbonyl group in PCL and another peak at 1166 cm^{-1} is due to C-

O-C group. From all these results, it is clear that the combined electrospun scaffold contains both CS and PCL (Sarmila, Abhishek, Rajashree, Phani, & Nayak, 2009)[11]. New link areas $2944, 2869, 1727, 1180$ indicating the presence of chains is polycaprolacton(fig3).

From the FTIR of Tamoxifen Citrate Pure drug (Figure 4) we could interpret 4 major peaks of functional groups Alcohol O-H Stretching, Alkane C-H stretching, Alkene -C=C- stretching and C=C ring stretching. Their frequency is $3200-3500\text{ cm}^{-1}$, $2800-3000\text{ cm}^{-1}$, $1700-1740\text{ cm}^{-1}$ and $1400-1500\text{ cm}^{-1}$ respectively[14]. The new range created in 3290 which corresponds to that of the OH and in 2948 for the CH and 1732 -C = C- that the drug is in nanofiber layer prove.





Fig(3). Infrared spectroscopy FTIR A- spectrometry infrared chitosan B- spectrometry infrared PCL C- PCL-chitosan nanofibres infrared spectroscopy

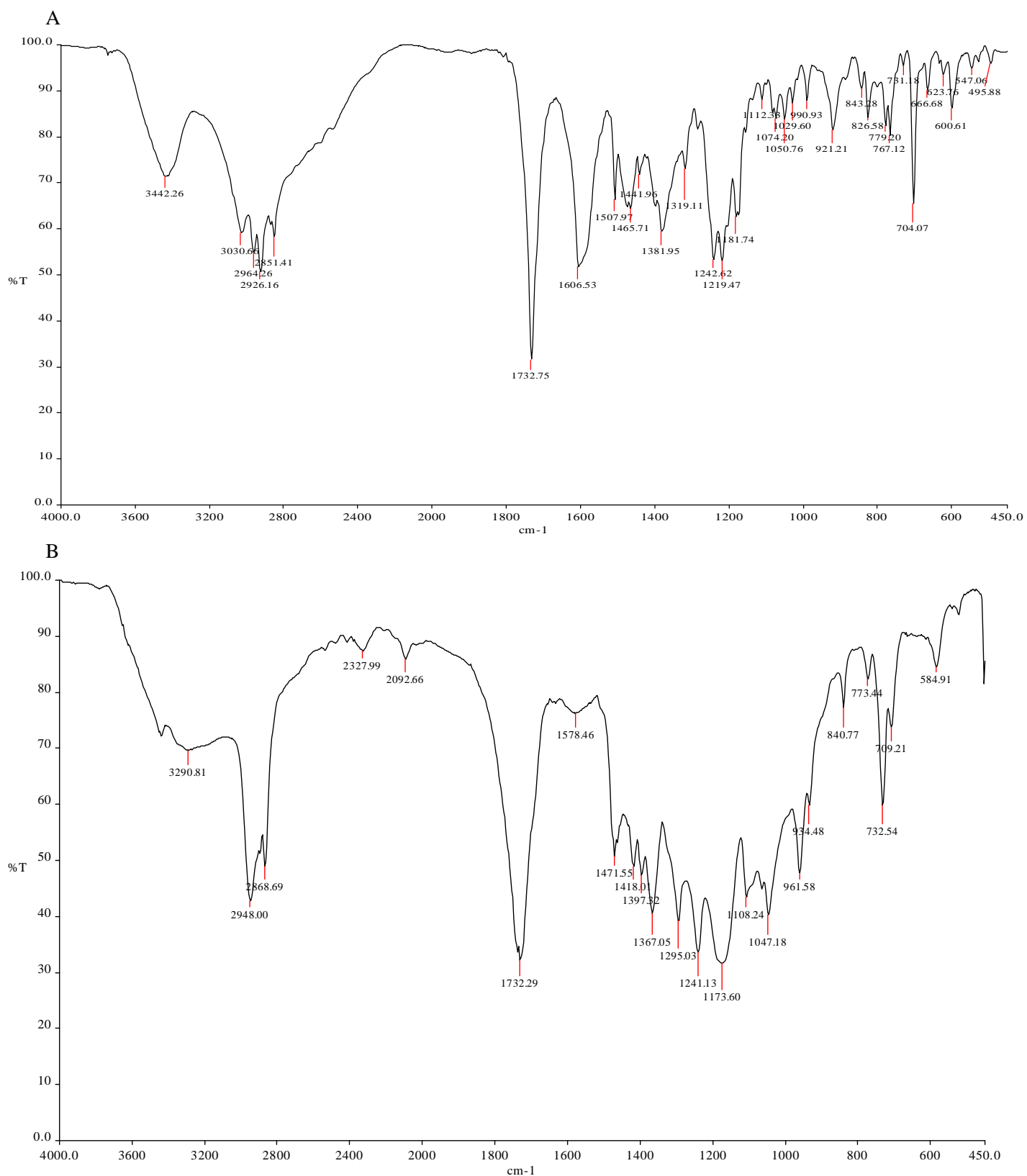


Fig (4). infrared spectrometry A- infrared spectrometry, tamoxifen B-infrared spectrometry nanofibres containing the drug.

3.3 Antibacterial testing

Antibacterial testing of nanofibers containing drug was done on the bacteria *Escherichia coli* and *Staphylococcus*. Antimicrobial percentage is calculated according to the formula 1 and its results is shown as a graph (Figure 5). Nanofibers containing drug better than no drug is shown

therapy against both bacteria, As you can see in the graph in Figure 5 in *E. coli* 3.7% better treatment shown than the *Staphylococcus* And even in the nanofibers containing drug when PCL-Chitosan 80-20 volume percent when the PCL 1g and CH 0.5g are up to 98 percent have been treated against these bacteria.

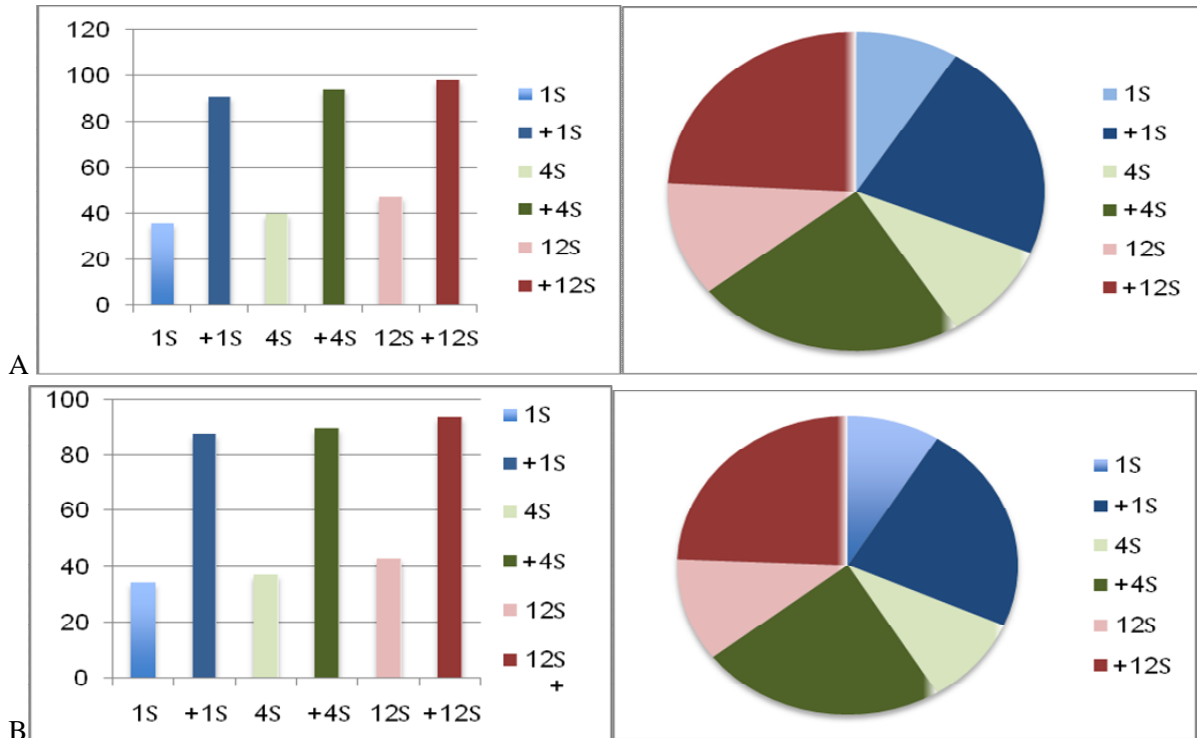


Fig (5). Antibacterial test A- *E. coli* and B-*Staphylococcus*.

3.4 Drug release

The first graph in the two standard drugs for drug discovery PH = 7 and PH = 5.5, respectively, related to the body and skin, respectively, and were drawn, Then any three of nanofibres containing the drug 2 select And in the two

environments were mentioned in body temperature And each time it was absorbed And it was continued until the drug leave in fiber, In the early hours of his release drug has shown good And gradually ended after 6 hours (Fig 6).

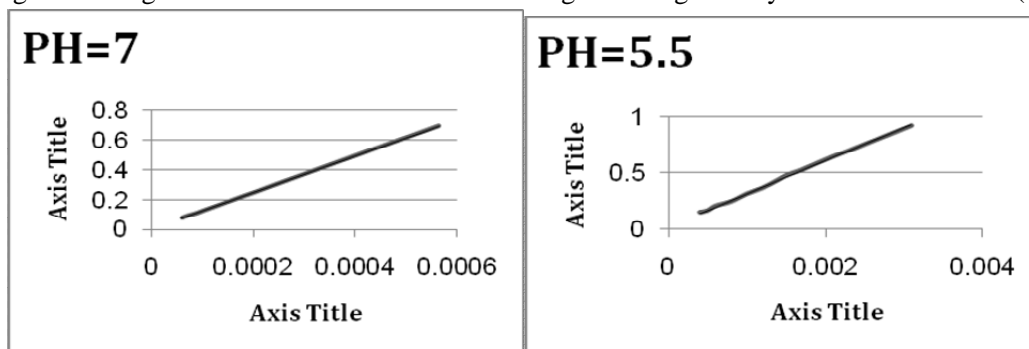


Fig (6). The standard graph drug.

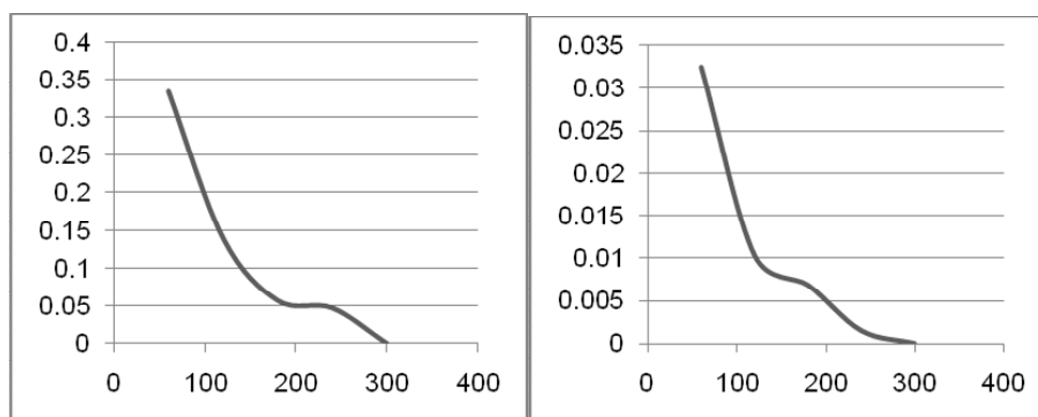


Fig (7). The graph of drug release from nanofibers made of PCL-Chitosan ratios 70-30 A- PH=5.5 and B- PH=7.

According to numbers adsorption obtained from absorption spectrophotometer and based on standard charts And charts drawn from all three modes S_{12} , S_4 , S_1 can be seen The release rate in S_4 more than two other and the highest amount of release in both simulated skin PH = 5.5 and simulated body environment with PH = 7 is shown. The test results of drug release indicate a total release rate was high (Fig 7). Because link of chitosan and tamoxifen with together , in a state in S_1 S_{12} too weak and too strong, so in that case S_4 a good fit Fast and convenient delivery and high in total.

4. DISCUSSION

In this study, a new method of delivery of the drug tamoxifen has been introduced according to the materials in drug delivery and in two different concentrations the PH of the skin and the body was carried out. This drug delivery using nanofibers containing medicinematerials such as PCL-chitosan -tamoxifen-acetic acid is formed. Put the nanofibers containing medication on the skin, the drug can be absorbed through the skin. Unlike oral and injectable drug delivery methods in the closest location cancer cells finds release, The non-cancerous and healthy cells less in the direction of drug release And at risk of serious injury from fall through oral and injectable. Given that the drug release from the scaffolds with different concentrations and ratios were examined, scaffold nanofiber made of PCL-chitosan 70-30 as optimal sample the carrier of tamoxifen drug was introduced. Based on the results of tests it was

found that nano-fiber PCL-chitosan-containing drug with a ratio of 70-30 and a concentration of 1 gr and 0.3 gr, speed And release better itself show compared to concentrations and the other ratios. The test results of drug release shown a total release rate was high.

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Conflicts of interest: Authors declared No conflict of interest

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