J Nanostruct 12(1): 136-143, Winter 2022

RESEARCH PAPER

Carboxymethyl Chitosan Nano-Fibers for Controlled Releasing 5-Fluorouracil Anticancer Drug

Indah Raya [1], Supat Chupradit [2] *, Yasser Fakri Mustafa [3], Khulood H. Oudaha [4], Mustafa M. Kadhim [5], Abduladheem Turki Jalil [5,6], Abed J. Kadhim [7], Trias Mahmudiono [8], Lakshmi Thangavelu [9]

[1] *Departement of Chemistry, Faculty Mathematics and Natural Science, Hasanuddin University, Makassar,South Sulawesi, Indonesia*

[2] *Department of Occupational Therapy, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand*

[3] *Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul, Iraq*

[4] *Pharmaceutical Chemistry Department, College of Pharmacy, Al-Ayen University, Thi-Qar, Iraq*

[5] *College of technical engineering, The Islamic University, Najaf, Iraq*

[6] *Faculty of Biology and Ecology, Yanka Kupala State University of Grodno, Grodno, Belarus*

[7] *Department of Materials Engineering, Al-Nisour University, Baghdad, Iraq*

[8] *Departemen of Nutrition, Faculty of Public Health, Universitas Airlangga, Indonesia*

[9] *Department of Pharmacology, Saveetha university,Chennai, India*

ARTICLE INFO

Article History:
Received 02 September 2021
Accepted 26 December 2021
Published 01 January 2022

Keywords:
5-Fluorouracil
Anticancer Drug
Carboxymethyl Chitosan
Nano Fibers
pH responsive
Pharmacokinetic

ABSTRACT

In the present study, the pH responsive electrospun carboxymethyl chitosan nanofibers were prepared via electrospinning method and cross-linked with glutaraldehyde vapor for various times up to 48 h. The controlled release of 5-Fluorouracil (5-FU) from single layer and tri-layered nanofibers (5-FU in the middle layer) was compared to obtain a sustained delivery system of 5-FU anticancer drug. The release of 5-FU from nanofibers was investigated at 37 °C under acidic pH (pH 5.5) and physiological pH (pH 7.4). The release data were fitted by zero-order, Higuchi and Korsmeyer-Peppas pharmacokinetic equations to determine the 5-FU release mechanism from nanofibers. Tri-layered nanofibers exhibited the sustained delivery of 5-FU without initial burst release during 168 and 216 h at pH=5.5 and 7.4, respectively. The initial burst release followed by sustained release of 5-FU from single layer cross-linked carboxymethyl chitosan nanofibers occurred during 48 and 60 h. The "n" constant of Korsmeyer-Peppas equation indicated the non Fickian diffusion of 5-FU from single layer nanofibers at both pH values of 5.5, pH 7.4 and tri-layered nanofibers at pH 5.5. Whereas, the Fickian diffusion of 5-FU was occurred from tri-layered nanofibers at pH 7.4. The obtained results indicated the high capability of tri-layered nanofibers for controlled release of 5-FU compared to single layer nanofibers.

How to cite this article
Raya I, Chupradit S, Fakri Mustafa Y, Oudaha K H, Kadhim M M, Turki Jalil A, Kadhim A J, Mahmudiono T, Thangavelu L. Carboxymethyl Chitosan Nano-Fibers for Controlled Releasing 5-Fluorouracil Anticancer Drug. J Nanostruct, 2022; 12(1):136-143. DOI: 10.22052/JNS.2022.01.013

* *Corresponding Author Email: ch.researcher20@yahoo.com*

INTRODUCTION

The nanofibers prepared by electrospinning technique have been studied extensively for use as drug carrier [1-6]. The various forms of nanofibers such as core-sheath structure [7-9], multi-layered structure [10-13], nanoparticles-embedded nanofibers [14-17], and so on have been developed to decrease the adverse effects of drug-loaded single phase nanofibers with initial burst release. The electrospining of multi-layered nanofibers by the electrospinning method is easier than core-shell nanofibers and nanoparticles-embedded nanofibers. For instance, the multi-layered nanofibers of gelatin and cross-linked with glutaraldehyde (25% v/v aqueous solution) for controlled release of piperine were fabricated [18]. A zero-order release up to 48 h was achieved. The sustained release of oligomeric proanthocyanidin from multi-layered polycaprolactone nanofibers was achieved for 62 days against thrombosis [19]. Multi layered nanofibrous scaffold from polycaprolactone, alginate, and ZnO nanoparticles as a wound healing patch were synthesized [20].

Natural polymers are broadly used in drug delivery [21], gene therapy [22-25], and tissue engineering due to their high biocompatibility [26]. Chitosan and its derivates as pH responsive polymers have been used for anticancer drugs delivery systems [4, 27-30]. However, the use of chitosan due to its lower solubility is limited. Carboxymethyl chitosan (CMC) as a water-soluble polymer has a better biocompatibility compared to pure chitosan [31]. In recent studies, the electrospun CMC nanofibers have been used for biomedical applications such as drug delivery and tissue engineering [32, 33]. However, the electrospinning of pure CMC is difficult. The various polymers such as polyvinyl alcohol (PVA), polyethylene oxide (PEO) and so on were blended with CMC solution to facilitate its electrospinning. For instance, Ag nanoparticles were incorporated into the PVA/CMC nanofibers to increase its antimicrobial activity [34]. In another study, the antimicrobial capability of PEO/CMC/Ag composite nanofibers was investigated [35]. The PEO/CMC nanofibers were electrospun for fruit fresh keeping [36]. The osteogenic activity of PEO/CMC nanofibers was investigated [37]. Polycaprolactone/CMC nanofibers were used for bone tissue engineering [38]. UiO-66 metal organic framework nanoparticles were incorporated into the PEO/CMC/polyurethane core-shell nanofibers against MCF-7 breast cancer cells [39]. CMC/PCL/cobalt ferrite/Doxorubicin nanofibers were synthesized with core-shell structure for breast cancer treatment [40].

In this work, PEO/CMC nanofibers are prepared via electrospinning method. Then, nanofibers are cross-linked with glutaraldehyde to increase its stability in phosphate-buffered saline (PBS). The functional groups of CMC nanofibers before and after crosslinking are characterized using FTIR analysis. The degradation rate of nanofibers is evaluated for 10 days in water and PBS. The tri-layered nanofibers (5-FU in the middle layer) are prepared and 5-FU release behavior from both single layer and tri-layered nanofibers are studied under both acidic pH and physiological pH. The biocompatibility of synthesized nanofibers is also investigated for possible use in vivo studies. The aim of this study is to compare the controlled release of 5-Fluorouracil (5-FU) from single layer and tri-layered CMC nanofibers.

MATERIALS AND METHODS

Poly(ethylene oxide) (Mw:900 kDa, PEO) supplied from Sigma-Aldrich (USA) and N-Carboxymethyl chitosan (Mw:100–250 kDa, N-deacetylation≥95%, CMC) purchased from NAI Hangzhou Co. (Hangzhou, China) were used to fabricate PEO/CMC nanofibers. Glutaraldehyde solution (25 wt. % in H_2O, GTA) was utilized as crosslinking agent. 5-Fluorouracil (5-FU) anticancer drug was provided from Sigma-Aldrich (USA). Fourier transform Infrared (FTIR) spectroscopy was recorded by using of the Bruker-Vector spectrometer ranging from 500-4000 cm^{-1}. The morphology and fiber diameter of the surface of the nanofibers was implemented by using of a Scanning Electron Microscopy (SEM, VEGA / TESCAN-XMU model) after their coating with a thin layer of gold. UV-Vis spectroscopy (JAS. CO V-530, Japan) at a λ_{max} of 266 nm was used to determine the concentration of the 5-FU. The degradation rate of nanofibers was evaluated by their soaking in PBS at pH values of 5.5 and 7.4 for 10 days followed by measuring their weight before and after soaking.

Synthesis of PEO/CMC nanofibers and their crosslinking

CMC/PEO solution was prepared by mixing 5 wt.% CMC and 10 wt.% PEO solutions under stirring for 4 h (CMC to PEO ratio: 5:5 v/v). 5 wt.% CMC and

10 wt.% PEO solutions were previously obtained by adding predetermined amounts of CMC and PEO in distilled water under stirring for 4 h and 2 h, respectively. The electrospinning conditions for fabrication of single phase nanofibers were feeding rate, voltage, distance and electrospinning time of 0.5 mL/h, 25 kV, 15 cm, and 6 h, respectively. To load 5-FU anticancer drug into the nanofibers, the predetermined amounts of 5-FU (5 and 10 wt.% by weight of CMC/PEO solution w/w) were added into the CMC/PEO solution under stirring for further 5 h. Tri-layered nanofibers were prepared by sequential electrospinning of CMC/PEO, CMC/PEO/5-FU and CMC/PEO solutions on an aluminum foil placed on the collector for 2h, 2h and 2h, respectively. The crosslinking of nanofibrous samples was carried out by using GTA saturated vapor (25% v/v aqueous solution) for 15 and 30 min.

Drug encapsulation efficiency, loading content, release and pharmacokinetic studies

Drug encapsulation efficiency (DEE, %) and drug loading content (DLC, g drug/g nanofibers) were evaluated by its degradation in distilled water and measuring the final content of drug in nanofibers as follows:

$$DEE(\%) = \frac{Final\ \ content\ \ of\ \ drugs\ \ in\ fibers}{Initial\ \ content\ \ of\ \ drugs\ \ loaded - fibers} \times 100 \quad (1)$$

$$DLC(mg/g) = \frac{Final\ \ content\ \ of\ \ drugs\ \ in\ fibers}{weight\ \ of\ \ fibers} \quad (2)$$

To measure drug release behavior from nanofibers, drug-loaded nanofibers (2 cm × 3 cm of electropun nanofibers) were incubated in 50 mL of two PBS solutions under different pH values of 5.5 (acidic pH) and 7.4 (physiological pH) under stirring at 37 °C for 10 days to obtain the 5-FU release profiles from nanofibers. The release experiments were done three times and the average values were reported.

The 5-FU release data were analyzed by using of the zero-order, Higuchi [41], and Korsmeyer-Peppas [42] pharmacokinetic models to obtain the drug release mechanism from single and tri-layered nanofibers.

RESULTS AND DISCUSSION

Characterization

SEM images from CMC/PEO and 5-FU loaded

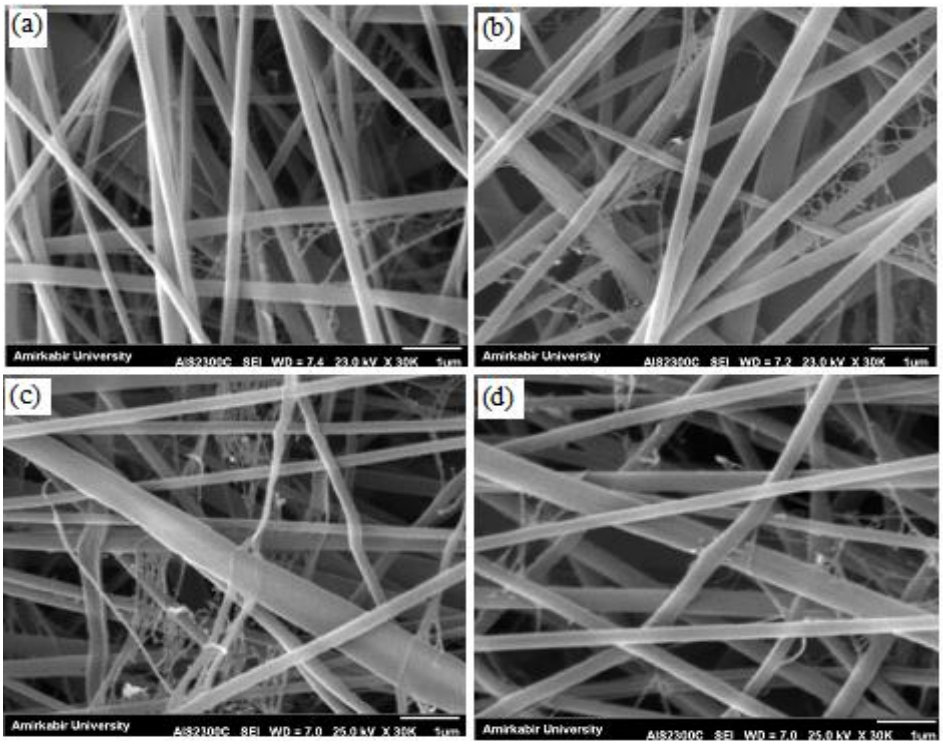

Fig. 1. SEM images from (a) PEO/CMC, (b) PEO/CMC/5-FU before crosslinking and (c) PEO/CMC, (d) PEO/CMC/5-FU after crosslinking with GTA for 30 min.

CMC/PEO nanofibers before and after crosslinking with GTA are illustrated in Fig. 1. As shown, the homogeneous nanofibers with an average diameter of 245 nm was obtained for CMC/PEO nanofibers. By loading 5-FU into the nanofibers, a gradual increase in the fiber diameter was obtained and the mean fiber diameter was increase to 270 nm. After crosslinking of nanofibers with GTA (30 min), the fiber diameters of both CMC/PEO and CMC/PEO/5-FU have been increased from 245 and 270 nm to 345 and 390 nm, respectively. The adhesion of some nanofibers together and linkage of some pores of nanofibers resulted in increasing nanofiber diameter after crosslinking.

The degradation rate of CMC/PEO/5-FU nanofibers before and after crosslinking with GTA under acidic and physiological pH is presented in Fig. 2. As shown, the mass loss percentage (%) of CMC/PEO/5-FU nanofibers before crosslinking was 100% after only 1 and 2 h under pH values of 5.5 and 7.4, respectively. By crosslinking of nanofbers with GTA for 15 min, the stability of nanofibers was significantly improved and lower than 40% and 50% of nanofibers were degraded after 10 days at physiological and acidic pH values. After crosslinking of nanofibers with GTA for 30 min, the mass loss percentage was found to be lower than 10 % and 18% under pH values of 7.4 and 5.5, respectively. Therefore, nanofibers cross-linked with GTA for 30 min was selected for further experiments.

FTIR spectra of CMC/PEO before and after crosslinking, 5-FU and CMC/PEO/5-FU are presented in Fig. 3. For CMC/PEO, the detected peaks at 3430 cm^{-1}, 2921 cm^{-1}, 1735 cm^{-1}, 1580 cm^{-1}, 1410 and 1072 cm^{-1} were assigned to the NH_2 groups, C-H stretching vibration, COO^-, deforming NH_2 group, symmetric COO stretching vibrations and C-O absorption peak, respectively. After crosslinking of CMC with GTA, the C-O absorption peak was shifted to 1088 cm^{-1} and became stronger [43]. For 5-FU, the main peaks of NH, C=O, C=C, C-F, C-N and pyrimidine compound of 5-FU were detected at 3140 cm^{-1}, 1665 cm^{-1}, 1455 cm^{-1}, 1425 cm^{-1}, and 1340 cm^{-1}, respectively. The main peaks of both CMC/PEO and 5-FU were detected in the FTIR spectrum of CMC/PEO/5-FU nanofibers.

Drug loading efficiency, and drug loading content

The 5-FU drug loading content and 5-FU drug encapsulation efficiency for 5-FU-loaded single layer and tri-layered nanofibers with various initial amounts of 5-FU (5 and 10 wt.% by weight of polymer) are presented in Table 1. As shown, the maximum drug encapsulation efficiency (DEE%) was about 97.5±0.2% and 96.6±0.15% for tri-layered CMC/PEO nanofibers containing 5% and 10% 5-FU. The maximum drug content was found to be 96.6±1.5 mgg^{-1} for 10 wt.% 5-FU loaded-nanofibers. Whereas, the maximum DEE for 5 wt.% 5-FU-loaded CMC/PEO single layer nanofibers was about 88.2±0.5%. The lower DEE for single layer nanofibers was due to washing of unattached 5-FU molecules from nanofibers surface, Whereas, the incorporation of 5-FU drug in the middle layer of

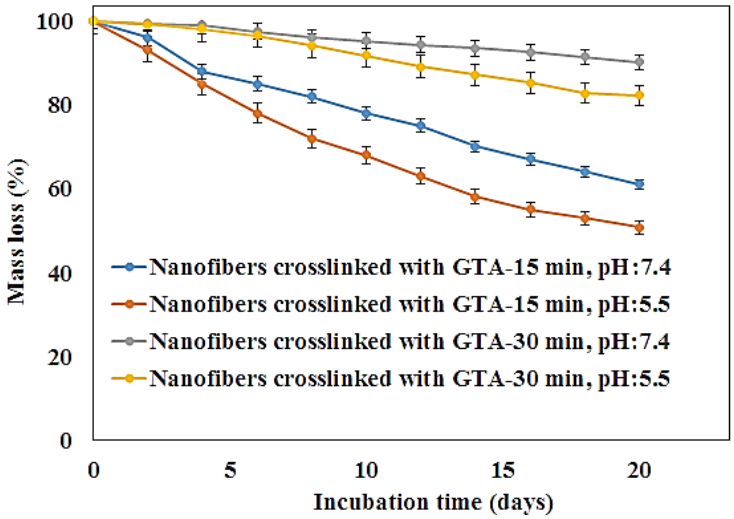

Fig. 2. Degradation rate of CMC/PEO nanofibers cross-linked with GTA.

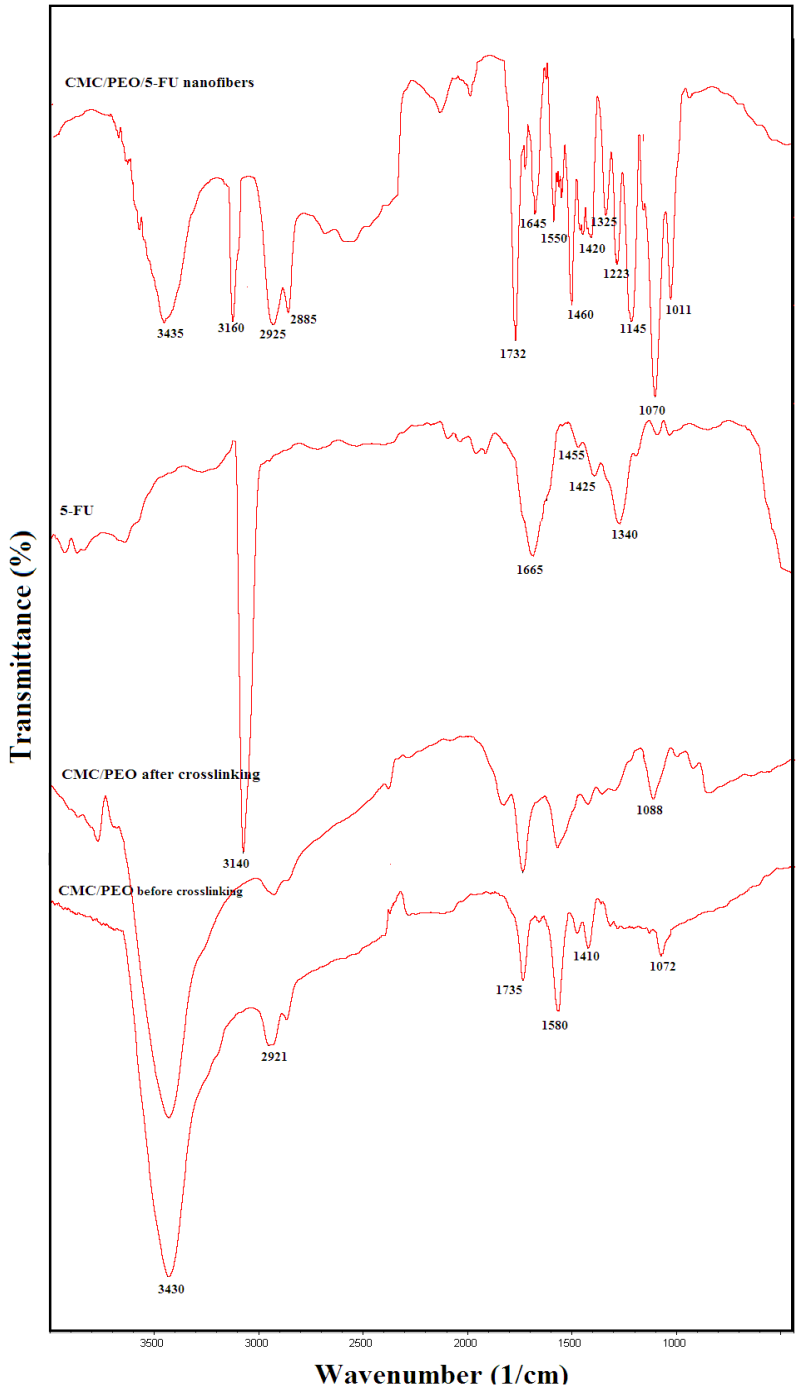

Fig. 3. FTIR spectra of CMC/PEO before and after crosslinking, 5-FU and CMC/PEO/5-FU nanofibers.

tri-layered nanofibers resulted in higher DLL and DLC for 5-FU loaded-tri-layered nanofibers. The obtained results demonstrated the high capability of nanofibers for loading of high amounts of 5-FU molecules.

Drug release and pharmacokinetic studies

The 5-FU release from single layer and tri-layered nanofibers containing 5% and 10% 5-FU under pH values of 5.5 and 7.4 is illustrated in Fig. 4. As can be seen, the increase in pH from 5.5 to

Table 1. Drug loading efficiency and drug loading content of synthesized CMC/PEO nanofibers (n=5)

Nanofibrous sample	DOX concentration (%)	DEE (%)	DLC (mg/g)
Single layer nanofibers	5	88.20±0.50	44.10±0.25
	10	85.40±0.60	85.40±6.00
Tri-layered nanofibers	5	97.50±0.20	48.75±0.10
	10	96.60±0.15	96.60±1.50

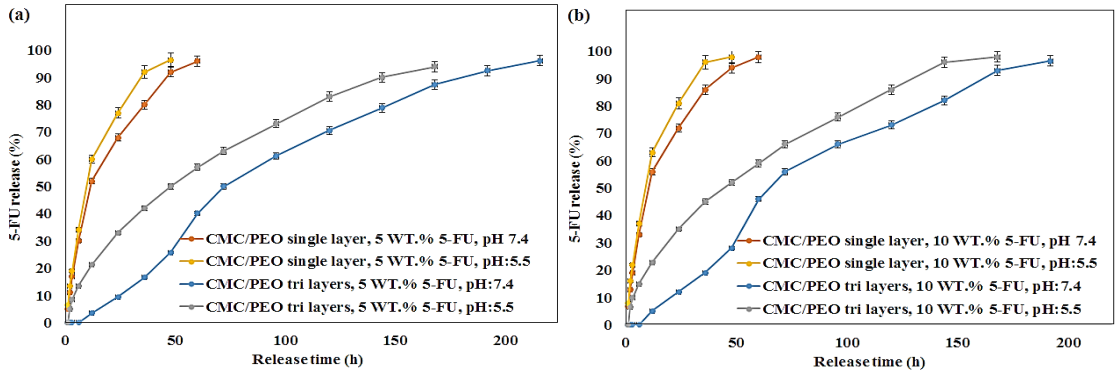

Fig. 4. Cumulative release of 5-FU from nanofibers containing (a) 5 wt.% 5-FU and (b) 10 wt.% 5-FU.

Table 2. Pharmacokinetic parameters of 5-FU release from nanofibers.

Nanofibrous carrier	pH	Zero-order		Higuchi		Korsmeyer-Peppas		
		K_0 (hr^{-1})	R^2	K_H ($hr^{-0.5}$)	R^2	n	K_{KP}	R^2
CMCPEO single layer, 5 wt.% 5-FU	7.4	0.2546	0.968	3.215	0.955	0.652	2.66	0.992
	5.5	0.2895	0.965	3.652	0.952	0.721	2.85	0.991
CMCPEO single layer, 10 wt.% 5-FU	7.4	0.2651	0.975	3.512	0.960	0.699	2.72	0.990
	5.5	0.2987	0.971	4.012	0.959	0.755	2.92	0.994
CMCPEO tri layers, 5 wt.% 5-FU	7.4	0.2015	0.955	2.952	0.958	0.378	2.12	0.993
	5.5	0.2145	0.954	3.111	0.960	0.541	2.35	0.992
CMCPEO tri layers, 10 wt.% 5-FU	7.4	0.2085	0.951	3.015	0.958	0.395	2.23	0.992
	5.5	0.2201	0.949	3.245	0.961	0.568	2.40	0.993

7.4 resulted in a slower release of 5-FU from both single layer and tri-layered nanofibers. On the other hand, the initial burst release of 5-FU from single layer nanofibers was obtained. Whereas, the sustained release of 5-FU without initial burst release was achieved for 5-FU- loaded tri-layered nanofibers and release was begun after 12 h. Thus, the fastest release was achieved at pH 5.5 from single layer nanofibers. The 5-FU release from single layer and tri-layered nanofibers was occurred after 48 h, 60 h, and 168 h, 216 h at pH of 5.5, and 7.4, respectively. The increase in the 5-FU

content in nanofibers resulted in the faster release of 5-FU from nanofibers due to the lower distance between the 5-FU molecules in the nanofibers matrix by increasing 5-FU concentration. The faster release of 5-FU from single layer nanofibers compared to tri-layered nanofibers could be attributed to the easier diffusion of 5-FU molecules from single layer nanofibers. The weakness of some functional groups of CMC/PEO nanofibers (carboxyl groups) resulted in the faster release of 5-FU from nanofibers at pH 5.5 compared to the 5-FU release at pH 7.4.

The comparison of correlation coefficients of pharmacokinetic models indicated that the Korsmeyer-Peppas model (R^2 > 0.99) was best described the 5-FU release data (Table 2). The "n" constant of Korsmeyer-Peppas equation indicated the non Fickian diffusion of 5-FU from single layer nanofibers at both pH values of 5.5, pH 7.4 and tri-layered nanofibers at pH 5.5. Whereas, the Fickian diffusion of 5-FU was occurred from tri-layered nanofibers at pH 7.4.

CONCLUSION

CMC/PEO single layer and tri-layered nanofibers were successfully fabricated via electrospinning method and cross-linked with GTA. The crosslink of nanofibers with GTA for 30 min resulted in fabricating of stable nanofibers with lower than 10 wt.% mass loss after 10 days. Whereas, the pure CMC/PEO nanofibers without crosslinking, degraded after only 2 h. After crosslinking of nanofibers with GTA (30 min), the fiber diameters of both CMC/PEO and CMC/PEO/5-FU have been increased from 245 and 270 nm to 345 and 390 nm, respectively. FTIR spectra of nanofibrous samples demonstrated the physical loading of 5-FU anticancer drug into the nanofibers. The maximum DEE% was about 97.5±0.2% for tri-layered CMC/PEO nanofibers containing 5 wt.% 5-FU. Whereas, the maximum DEE for 5 wt.% 5-FU-loaded CMC/PEO single layer nanofibers was about 88.2±0.5%. The 5-FU release from single layer and tri-layered nanofibers was occurred after 48 h, 60 h, and 168 h, 216 h at pH of 5.5, and 7.4, respectively. Korsmeyer-Peppas model best described the 5-FU release data from both single layer and tri-layered nanofibers.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Pant B, Park M, Park S-J. Drug Delivery Applications of Core-Sheath Nanofibers Prepared by Coaxial Electrospinning: A Review. Pharmaceutics. 2019;11(7):305.
2. Ghafoor B, Aleem A, Najabat Ali M, Mir M. Review of the fabrication techniques and applications of polymeric electrospun nanofibers for drug delivery systems. J Drug Deliv Sci Technol. 2018;48:82-87.
3. Wsoo MA, Shahir S, Mohd Bohari SP, Nayan NHM, Razak SIA. A review on the properties of electrospun cellulose acetate and its application in drug delivery systems: A new perspective. Carbohydr Res. 2020;491:107978.
4. Shikhi-Abadi PG, Irani M. A review on the applications of electrospun chitosan nanofibers for the cancer treatment. Int J Biol Macromol. 2021;183:790-810.
5. Khodadadi M, Alijani S, Montazeri M, Esmaeilizadeh N, Sadeghi-Soureh S, Pilehvar-Soltanahmadi Y. Recent advances in electrospun nanofiber-<scp>mediated drug</scp>delivery strategies for localized cancer chemotherapy. Journal of Biomedical Materials Research Part A. 2020;108(7):1444-1458.
6. Zarghami A, Irani M, Mostafazadeh A, Golpour M, Heidarinasab A, Haririan I. Fabrication of PEO/chitosan/PCL/olive oil nanofibrous scaffolds for wound dressing applications. Fibers and Polymers. 2015;16(6):1201-1212.
7. Irani M, Mir Mohamad Sadeghi G, Haririan I. Electrospun biocompatible poly (ε-caprolactonediol)-based polyurethane core/shell nanofibrous scaffold for controlled release of temozolomide. International Journal of Polymeric Materials and Polymeric Biomaterials. 2017;67(6):361-366.
8. Farboudi A, Nouri A, Shirinzad S, Sojoudi P, Davaran S, Akrami M, et al. Synthesis of magnetic gold coated poly (ε-caprolactonediol) based polyurethane/poly(N-isopropylacrylamide)-grafted-chitosan core-shell nanofibers for controlled release of paclitaxel and 5-FU. Int J Biol Macromol. 2020;150:1130-1140.
9. Faraji Dizaji B, Hasani Azerbaijan M, Sheisi N, Goleij P, Mirmajidi T, Chogan F, et al. Synthesis of PLGA/chitosan/zeolites and PLGA/chitosan/metal organic frameworks nanofibers for targeted delivery of Paclitaxel toward prostate cancer cells death. Int J Biol Macromol. 2020;164:1461-1474.
10. Abdul Q, Attiq-Ur-Rehman K, Naqeebullah K, Samiullah, Abdul H, Rehana K, et al. The antioxidant, antimicrobial, and clinical effects with elemental contents of pomegranate (Punica granatum) peel extracts: A review. Baghdad Journal of Biochemistry and Applied Biological Sciences. 2021;2(01):21-28.
11. Shalaby MN, Abdo Sakoury MM. THE ROLE OF PHYSICAL ACTIVITY ON THE SUPPORT AND ENHANCE THE NATURAL BEHAVIOR OF STEM CELLS AND CHOSEN PHYSIOLOGICAL VARIABLES FOR PLAYERS ATHLETICS. DRASSA Journal of Development and Research for Sport Science Activities. 2019;4(1):74-92.
12. Afrashi M, Semnani D, Talebi Z, Dehghan P, Maheronnaghsh M. Novel multi-layer silica aerogel/PVA composite for controlled drug delivery. Materials Research Express. 2019;6(9):095408.
13. Jeckson TA, Neo YP, Sisinthy SP, Gorain B. Delivery of Therapeutics from Layer-by-Layer Electrospun Nanofiber

Matrix for Wound Healing: An Update. J Pharm Sci. 2021;110(2):635-653.
14. Irani M, Mir Mohamad Sadeghi G, Haririan I. A novel biocompatible drug delivery system of chitosan/temozolomide nanoparticles loaded PCL-PU nanofibers for sustained delivery of temozolomide. Int J Biol Macromol. 2017;97:744-751.
15. Bergia RE, Hudson JL, Campbell WW. Effect of whey protein supplementation on body composition changes in women: a systematic review and meta-analysis. Nutr Rev. 2018;76(7):539-551.
16. Al-Bedairy I, Shamsa M, Salim Sa, Mahdi M, Dawood K, Al Faisal AH. FOXA1 expression in Iraqi women with ER+ breast cancer. Baghdad Journal of Biochemistry and Applied Biological Sciences. 2020;2(02):106-119.
17. Alwan S, Al-Saeed M, Abid H. Safety assessment and biochemical evaluation of the effect of biogenic silver nanoparticles (using bark extract of C. zeylanicum) on Rattus norvegicus rats. Baghdad Journal of Biochemistry and Applied Biological Sciences. 2021;2(03):133-145.
18. Laha A, Sharma CS, Majumdar S. Sustained drug release from multi-layered sequentially crosslinked electrospun gelatin nanofiber mesh. Materials Science and Engineering: C. 2017;76:782-786.
19. Ma K, Rozet S, Tamada Y, Yao J, Ni Q-Q. Multi-layer nanofibrous tubes with dual drug-release profiles for vascular graft engineering. J Drug Deliv Sci Technol. 2019;53:100900.
20. Dodero A, Alloisio M, Castellano M, Vicini S. Multilayer Alginate–Polycaprolactone Electrospun Membranes as Skin Wound Patches with Drug Delivery Abilities. ACS Applied Materials & Interfaces. 2020;12(28):31162-31171.
21. George A, Shah PA, Shrivastav PS. Natural biodegradable polymers based nano-formulations for drug delivery: A review. Int J Pharm. 2019;561:244-264.
22. Rai R, Alwani S, Badea I. Polymeric Nanoparticles in Gene Therapy: New Avenues of Design and Optimization for Delivery Applications. Polymers. 2019;11(4):745.
23. Radhakrishnan R, Shetty S, Sharma M, Kabekkodu S, Anil Kumar NV, Satyamoorthy K. Understanding the molecular mechanism associated with reversal of oral submucous fibrosis targeting hydroxylysine aldehyde-derived collagen cross-links. J Carcinog. 2021;20(1):9.
24. Salman MM, Al-Obaidi Z, Kitchen P, Loreto A, Bill RM, Wade-Martins R. Advances in Applying Computer-Aided Drug Design for Neurodegenerative Diseases. Int J Mol Sci. 2021;22(9):4688.
25. Sowmya SV, Rao R, Prasad K. Prediction of metastasis in oral squamous cell carcinoma through phenotypic evaluation and gene expression of E-cadherin, β-catenin, matrix metalloproteinase-2, and matrix metalloproteinase-9 biomarkers with clinical correlation. J Carcinog. 2020;19(1):8.
26. Rai K, Sun Y, Shaliutina-Kolesova A, Nian R, Xian M. Proteins: Natural Polymers for Tissue Engineering. Journal of Biomaterials and Tissue Engineering. 2018;8(3):295-308.
27. Rostami E. Progresses in targeted drug delivery systems using chitosan nanoparticles in cancer therapy: A mini-review. J Drug Deliv Sci Technol. 2020;58:101813.
28. Shafabakhsh R, Yousefi B, Asemi Z, Nikfar B, Mansournia MA, Hallajzadeh J. Chitosan: A compound for drug delivery system in gastric cancer-a review. Carbohydr Polym. 2020;242:116403.
29. Shanmuganathan R, Edison TNJI, LewisOscar F, Kumar P, Shanmugam S, Pugazhendhi A. Chitosan nanopolymers: An overview of drug delivery against cancer. Int J Biol Macromol. 2019;130:727-736.
30. Garg U, Chauhan S, Nagaich U, Jain N. Current Advances in Chitosan Nanoparticles Based Drug Delivery and Targeting. Advanced Pharmaceutical Bulletin. 2019;9(2):195-204.
31. Lv J, Yin X, Zeng Q, Dong W, Liu H, Zhu L. Preparation of carboxymethyl chitosan nanofibers through electrospinning the ball-milled nanopowders with poly (lactic acid) and the blood compatibility of the electrospun NCMC/PLA mats. Journal of Polymer Research. 2017;24(4).
32. Ibrahim HM, Reda MM, Klingner A. Preparation and characterization of green carboxymethylchitosan (CMCS) – Polyvinyl alcohol (PVA) electrospun nanofibers containing gold nanoparticles (AuNPs) and its potential use as biomaterials. Int J Biol Macromol. 2020;151:821-829.
33. Tao F, Cheng Y, Tao H, Jin L, Wan Z, Dai F, et al. Carboxymethyl chitosan/sodium alginate-based micron-fibers fabricated by emulsion electrospinning for periosteal tissue engineering. Materials & Design. 2020;194:108849.
34. Zhao Y, Zhou Y, Wu X, Wang L, Xu L, Wei S. A facile method for electrospinning of Ag nanoparticles/poly (vinyl alcohol)/carboxymethyl-chitosan nanofibers. Appl Surf Sci. 2012;258(22):8867-8873.
35. Fouda MMG, El-Aassar MR, Al-Deyab SS. Antimicrobial activity of carboxymethyl chitosan/polyethylene oxide nanofibers embedded silver nanoparticles. Carbohydr Polym. 2013;92(2):1012-1017.
36. Yue T-T, Li X, Wang X-X, Yan X, Yu M, Ma J-W, et al. Electrospinning of Carboxymethyl Chitosan/Polyoxyethylene Oxide Nanofibers for Fruit Fresh-Keeping. Nanoscale Research Letters. 2018;13(1).
37. Zhao X, Zhou L, Li Q, Zou Q, Du C. Biomimetic mineralization of carboxymethyl chitosan nanofibers with improved osteogenic activity in vitro and in vivo. Carbohydr Polym. 2018;195:225-234.
38. Sharifi F, Atyabi SM, Norouzian D, Zandi M, Irani S, Bakhshi H. Polycaprolactone/carboxymethyl chitosan nanofibrous scaffolds for bone tissue engineering application. Int J Biol Macromol. 2018;115:243-248.
39. Farboudi A, Mahboobnia K, Chogan F, Karimi M, Askari A, Banihashem S, et al. UiO-66 metal organic framework nanoparticles loaded carboxymethyl chitosan/poly ethylene oxide/polyurethane core-shell nanofibers for controlled release of doxorubicin and folic acid. Int J Biol Macromol. 2020;150:178-188.
40. Abasalta M, Asefnejad A, Khorasani MT, Saadatabadi AR. Fabrication of carboxymethyl chitosan/poly(ε-caprolactone)/doxorubicin/nickel ferrite core-shell fibers for controlled release of doxorubicin against breast cancer. Carbohydr Polym. 2021;257:117631.
41. Higuchi T. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci. 1963;52(12):1145-1149.
42. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. Int J Pharm. 1983;15(1):25-35.
43. Farag R, Mohamed R. Synthesis and Characterization of Carboxymethyl Chitosan Nanogels for Swelling Studies and Antimicrobial Activity. Molecules. 2012;18(1):190-203.