Journal of Reports in Pharmaceutical Sciences 2014, 3(2), 169-178

Sustained Release of 5-fluorouracil from Chitosan Oligosaccharide-DeesterifiedTragacanth Core-Shell Nanoparticles

Ehsan Ahmadi[a], Behzad Shabazi[b,c], Komail Sadr javadi[b,c], Ali Fattahi*[c]

[a]Student Research Committee, Kermanshah University of Medical Sciences, Kermanshah, 6734667149, Iran
[b]School of pharmacy, Kermanshah University of Medical Sciences, Kermanshah, 6734667149, Iran
[c]Medical biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 2014-05-17
Revised: 2014-09-15
Accepted: 2014-10-25
ePublished:2014-11-09

***Keywords*:**
Chitosan oligosaccharide
De-estrifiedtragacanth
5-Fluorouracil
Nanoparticle

A B S T R A C T

A great challenge in using natural polysaccharide as drug delivery system is their fast drug release. The purpose of the present study was to prepare and evaluate chitosan oligosaccharide (CHO)-de-estrifiedtragacanth (DET) core-shell nanoparticles for controled release of water soluble drugs. CHO-DET nanoparticles were prepared by microemulsion method and characterized by FT-IR spectroscopy. Size, zeta potential, loading efficacy, and *in vitro* drug release were also investigated. Morphology of nanoparticles was evaluated by the scanning electron microscopy. The optimum particles with an average size of 400 nm were prepared. Loading efficacy of nanoparticles in drug/polymer ratios of 1/3.5 and 1/5 was 18.88 ± 3.95 and 17.55 ± 2.13. At the ratio of 1/5, release of 5-FU was sustained and less than 25% of drug was released at pH 7.4 and 11.14% of 5-FU was released at pH 1.5. At ratio of 1/3.5, release rate was faster than ratio of 1/5, and more than 30% of drug was released after 22 h at pH 7.4. drug release for this ratio was 29.14% at stomach pH. These phenomena make CHO-DET core-shell nanoparticles as a great candidate for controlled drug delivery system specially for colon targeted delivery where bacterial flora cleave polysaccharides and release their drug content in colon region.

*Corresponding Author: **Ali Fattahi**, **E-mail:** alifattahi@kums.ac.ir

Introduction

Polymeric nanoparticles (NPs) have shown interesting promise for drug and gene delivery. Nanoparticle drug delivery systems have outstanding advantages such as prolonged retention in blood stream, targeted delivery, controlled release, low toxicity and side effects etc[1].

Among polymeric NPs, natural polymer bases have attracted great interest. As natural biomaterials, polysaccharides are highly stable, safe, non-toxic, hydrophilic, and bioadhesive. They have abundant resources in nature and low cost in their processing. Particularly, most of natural polysaccharides have hydrophilic groups such as hydroxyl, carboxyl and amino groups, which are capable of easy modification of polysaccharides [1-3]. Tragacanth gum (TG) is a heterogeneous branched anionic polysaccharide that contains tragacantic acid (Fig.1) which is consisted of repeated galacturonic acid units in the backbone, and galactose, fucose and xylose in the side chains. We have recently produced a novel derivative of this gum which is completely de-esterified and soluble in water. De-esterified tragacanth (DET) can produce stable hydrogel with metallic cations and positively charged polymers [4, 5].

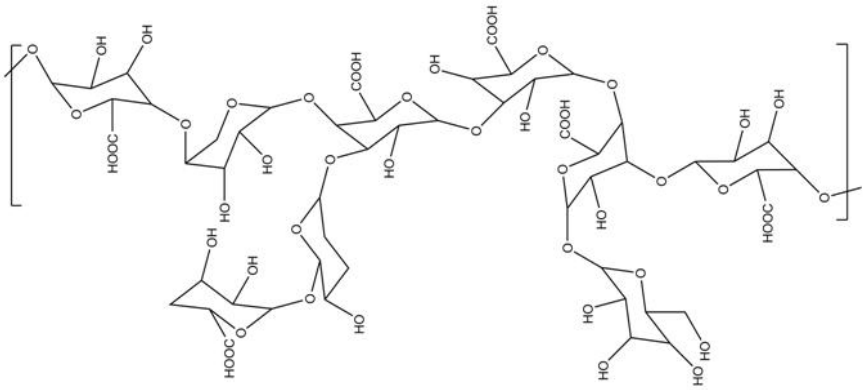

Fig. 1. Structure of tragacantic acid

Chitosan is a natural polysaccharide obtained by deacetylation of chitin, the major compound of exoskeletons in crustaceans. Its oligosaccharides have attracted much attention for drug and gene delivery. Not only CHO has higher solubility in physiological pH but also it inhibits tumor-induced angiogenesis which makes it a promising anticancer drug carrier [6].

Dioctylsulfosuccinate sodium salt **(**AOT) is an anionic surfactant that is approved as oral, topical, and intramuscular excipient. Because of its double-tailed nature, the AOT molecule is hydrophilic and hydrophobic parts and is one of the few ionic surfactants that can form microemulsions without the addition of a co-surfactant [7, 8]. It has been used vastly for preparation of micro/nanoparticles by reverse microemulsion[9-11].

5-fluorourcil (5-FU), an analog of pyrimidine, is an anionic antineoplastic drug [12, 13] for the treatment of metastatic cancers of colon, breast, pancreas, head, neck and ovary. Its rapid clearance from systemic circulation and its enzymatic degradation in liver result in low level of drug near the site of action and increase the side effects. The controlled release of 5-FU can be achievedby the improvement of therapeutic efficacy and reducing incidence of side effects [14]. Our objective in this research was to develop a novel sustained release bacterially triggered core-shell nanoparticular system for colon drug delivery of 5-FU. Several studies have reported core-shell of chitosan with ionic polymers such as pectin, alginate, *β*-lactoglobulin and albumin [15-18]. But, to our knowledge, so far nanoparticles with CHO core and DET shell have not reported yet.

Materials and Methods

Materials

Chitosan oligosaccharide (90% deacetylated; verified by ^{1}H NMR, Mw = 8.6 kDa; verified by high performance liquid chromatography) was supplied by Yuhuan Marine Biochemistry Co., Ltd., Zhejiang, China. Ribbon type tragacanth gum (TG) from Astragalusgossypinus was purchased from local market of Isfahan, Iran.

Iso-octane was supplied by Merck, Germany. Dioctyl sodium sulfosuccinate (AOT), 5-fluorouacil (5-FU), as well as other compounds were purchased from Sigma-Aldrich, USA.

Preparation of de-esterified tragacanth

DET has been prepared by de-esterification of TG base on Fattahi et al. with minor modification [5]. Briefly, 2 g TG was dispersed in 1 L (0.1 M) sodium hydroxide and stirred for 4 h at 4°C, followed by precipitation of DET in 60% ethanol. Then, precipitated DET was dissolved in deionized water. In order to remove sodium traces, acetic acid was added to the DET solution and washed several times with increasing concentrations of ethanol (60, 70 and 94%) followed by dialysis against water for 24 hr.

Preparation of nanoparticles

Desired amount of dioctyl sodium sulfosuccinate (AOT) was dissolved in iso-octane to achieve 1-2.4% AOT in iso-octane. Different volumes of 1 wt% chitosan aqueous solution were dropped into the continuous phase while being spinned for 1 minute and then 1 mL of DET aqueous solution (0.5 wt%) was added into the continuous phase to achieve different CHO/DET ratios ranged from 1 to 10. To form solid nanoparticles, 0.5 mL of CaCl2 solution (0-0.3 wt%) was added into the solution and mixed for another 3 minutes to establish cross-linked tragacanth gel on surface of chitosan nano-droplets. Variables for each sample produced by above parameters are summarized in table 1. Once the two phases were clearly separated, the nanoparticles suspended in the aqueous phase were pelleted out by centrifugation at 15000 rmp for 30 minutes (Hetterich, Germany) [19]. NPs were washed by iso-octane and acetone for three times to strip off AOT surfactant remaining on the nanoparticle surface. Finally, NPs were freeze-dried for further studies. In the case of drug loading, desired amount of 5-Fu was added to CHO solution to achieve 5-FU/polymer weight ratios of 1/3.5 and 1/5.

Particle size and zeta potential measurements

The particle size and zeta potential of the RA–chitosan micelles were measured by a zetasizer(Zetasizer-ZEN3600 Malvern Instrument Ltd., Worchestershire, UK). All particle-size measurements were performed in distilled water using a He–Ne laser beam at 658 nm. The device calculated the zeta potential of the nanoparticles from their electrophoretic mobility using Smoluchowski's equation. All the experiments were performed in triplicate.

FT-IR analysis

FT-IR spectra were acquired in transmission mode from CHO and DET powders, dried CHO-DET particles and loaded nanoparticles. The spectra were recorded with an FT-IR spectrometer (IR prestige-21, Shimadzu, Japan), in the spectral range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}.

Morphological studies

Morphology of the particles was examined, using a scanning electron microscope (SEM, SERON technology, AIS2100, Korea). Prior to examination, the samples were lyophilised, fixed on a brass stub and coated with a gold–palladium layer under argon atmosphere, using a gold sputter module in a high vacuum evaporator.

Loading efficiency and In vitro drug release studies

To estimate the content of the loaded drug, indirect method was used and unloaded drug in supernatant was measured. The loading efficacy (LE) was calculated by the following equations:

$$\text{LE} = \frac{(Wo - W)}{Wo} * 100$$

Where **W** is the weight of the drug in supernatant and $\mathbf{W}_0$ is the weight of the initial feeding drug.

The *in vitro* drug release studies were carried out by dialysis method. For evaluation of stomach pH and colon pH effect on release rate of different formulations, release was investigated at pH 1.5 and pH 7.4 respectively. Nanoparticles equivalent to 10 mg of drug was placed in a cellulose dialysis bag (cut-off 12 kDa, Sigma, USA), and to this a little amount of release media was added. The dialysis bag was sealed at both ends after adding the release media. The dialysis bag was dipped into the glass bottle containing release medium. The

release media was stirred continuously at 100 rpm and maintained at temperature 37 ºC. In order to prevent evaporation of the release medium the bottle was closed. Samples were withdrawn at regular time intervals and the same volume was replaced with fresh release medium. The concentration of 5-FU was measured by UV Spectrophotometer at a wavelength of 266 nm.

Results and Discussion

Investigation of factors affecting the size of the CHO-DET particles

CHO-DET particles were synthesized successfully,the effect of different parameters such as CHO/DET weight ratio, percent of AOT and $CaCl_2$ concentration on particle size was investigated. At the constant concentration of AOT and $CaCl_2$, size of particles weresignificantly reduced by increasing polymers weight ratio from 1 to 2.5. A little increase was observed in particle size by increasing polymers weight ratio to 5 and at weight ratios higher than 5, particle size was increased into micron size. Therefore, weight ratios between 2.5 to 5 were considered as optimum CHO/DET weight ratios (Fig.2A). Whereas the concentration of $CaCl_2$ and CHO/DET weight ratio were constant, 1.7% AOT caused the lower particle size (fig.2B). As shown in fig.2C, variation in $CaCl_2$ had lower effect on particle size. Initially, particle size was reduced with increasing percent of calcium chloride from 0 to 0.1%, and then particle size was enhanced by increasing $CaCl_2$ concentration. This increase could be attributed to reduction of surface charge of particles by neutralization of galactronic acid of DET and increase of aggregation chance. Particle size and zeta of NPs produced at optimum condition were summarized in table 2. (see table 1 and 2)

Table1. Formulation codes and different parameters of production

Sample code	CHO:DET ratio	AOT%	$CaCl_2$
S1	1:1	0.25	0.1
S2	1:2.5	0.25	0.1
S3	1:5	0.25	0.1
S4	1:7.5	0.25	0.1
S5	1:10	0.25	0.1
S6	1:2.5	0.15	0.1
S7	1:2.5	0.2	0.1
S8	1:2.5	0.25	0.1
S9	1:2.5	0.3	0.1
S10	1:2.5	0.35	0.1
S11	1:2.5	0.25	0.0
S12	1:2.5	0.25	0.05
S13	1:2.5	0.25	0.1
S14	1:2.5	0.25	0.2
S15	1:2.5	0.25	0.3

Table 2. particle size and zeta potential of CHO-DET at the optimum condition.

Formulation	AOT (%)	CHO-DET ratio	$CaCl_2$ (%)	Particle size	Zeta potential
S1	0.1	1:1	0.25	399.57±20.59	-76.4±9.47

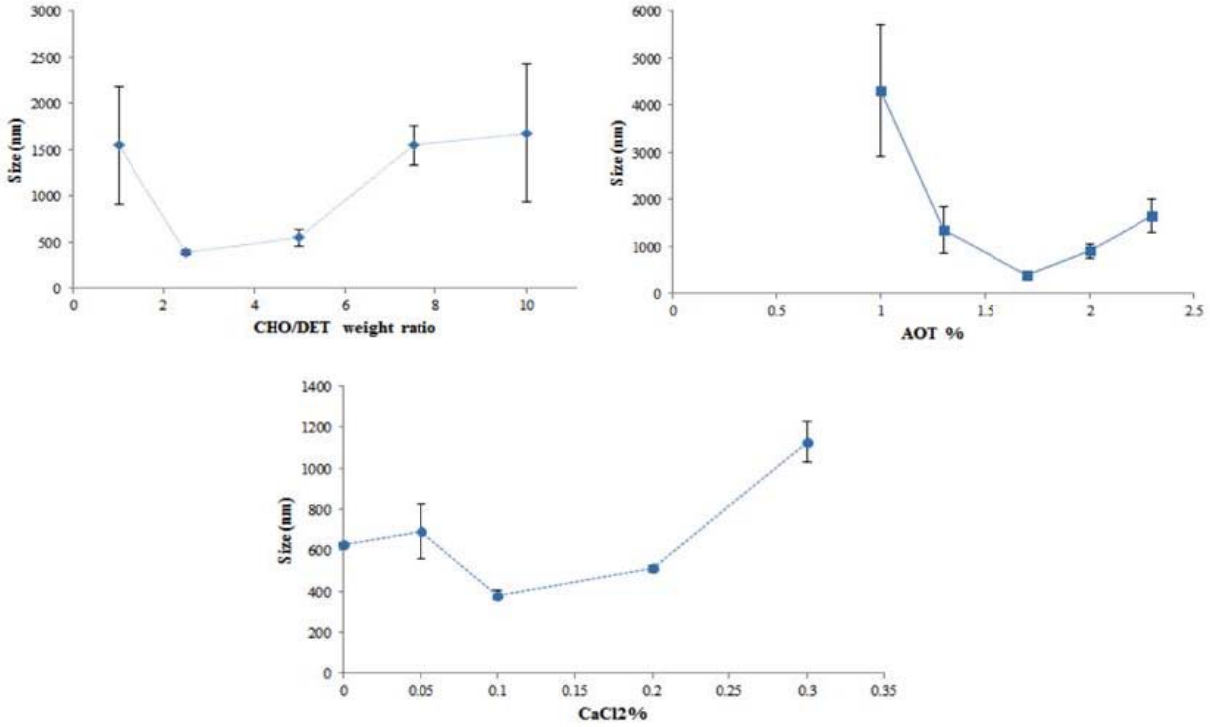

Fig. 2. Effect of CHO/DET weight ratio (A), AOT percentage (B) and concentration of calcium chloride (C) on size of CHO-DET core-sell particles.

The SEM microphoto (Fig. 3) shows spherical particles with smooth surface. Some non-spherical particles were also observed in micrograph which are probably aggregated particles. This is in agreement with results of zetasizer where a small fraction of aggregated in the micros size was observed (Fig. 4)

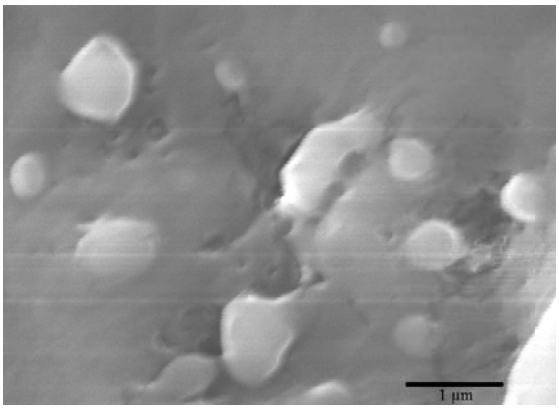

Fig. 3. Microphoto of CHO-DET NPs at optimum condition.

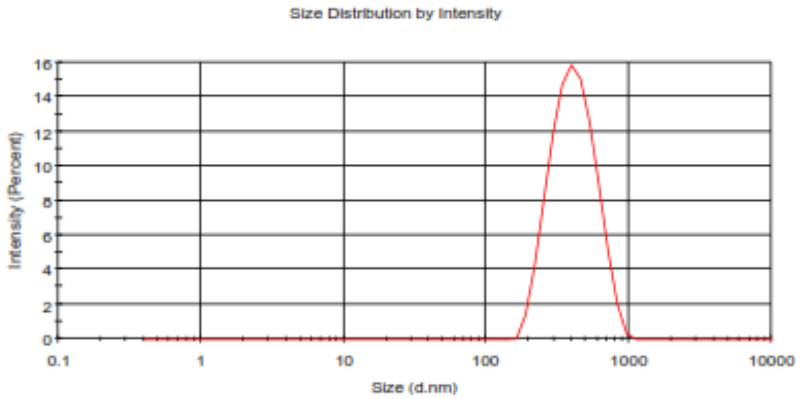

Fig. 4. Particle size distribution of CHO-DET nanoparticles produced by

FT-IR

The characteristic peaks of CHO, DET, CHO-DET NPs and 5-FU loaded NPs were shown in Fig.5. Characteristic infrared absorption bands of water soluble chitosan (Fig. 5A) appeared at 1631.78 cm^{-1} (amide I), 1516 cm^{-1} (NH bend) and 1381.1 cm^{-1} (CH bending). The characteristic peaks of DET consist of asymmetric stretching of COO (attributed to sodium salt of carboxylic acid) at 1616.35 cm^{-1} and symmetric stretching of COO at 1415.75 cm^{-1} (Fig. 5B). In CHO–DET nanoparticle spectrum (Fig. 5C), intensity of NH bend of chitosan at 1519.91 cm^{-1} is reduced. The peak at 1631.78 is related to amide of chitosan and the amine salt of carboxylic acid. A sharp peak at 1732 cm^{-1} is related to ester bonds of AOT. As shown in Fig. 5D, presence of characteristic peak at 821.68 cm^{-1} (vibration of CF=CH group) and the peak at 1275 cm–1atribiubited to C-F stretching band related to 5-FU in drug loaded nanoparticles showed successful encapsulation of 5-FU.

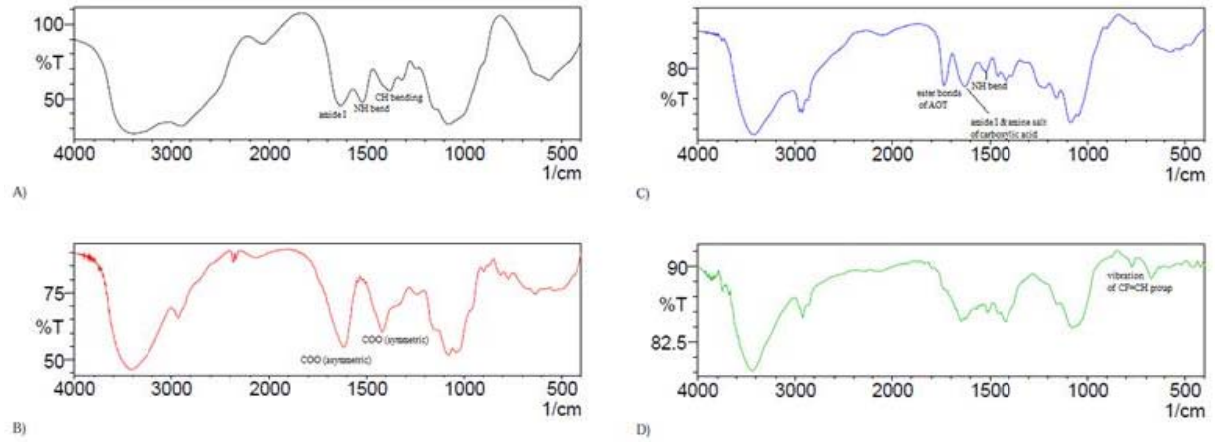

Fig. 5. FT-IR spectra of (A) CHO, (B) DET, (C) CHO–DET nanoparticles, (D) 5-Fu loaded NPs.

Preparation and characterization of 5-FU loaded CHO-DET NPs

Previous studies have shown that nanoparticles can significantly enhanced therapeutic effect of nanoparticle-encapsulated drug by sustained release of drug and improving cell uptake. However, the use of polysaccharide based nanoparticles for cellular delivery of small molecular weight, water-soluble drugs have been limited by poor drug encapsulation efficiency and rapid release of the encapsulated drug [20]. So many studies have been done on 5-FU loaded polysaccharide based NPs such as alginate, chitosan and pectin NPs which suffered by low loading efficacy. Most of the formulations had a loading efficacy lower than 10% and maximum loading efficacy was around 20% [20-

[22]. Loading efficacy of NPS in drug/polymer ratios of 1/3.5 and 1/5 was 18.88 ± 3.95 and 17.55 ± 2.13, respectively which are high loading efficiency for such a system. In these two ratios that we have used, no significant difference was observed in loading efficacy. We have added 5-FU to the CHO solution. 5-FU is a negatively charged drug [13, 23] which can interact with positively charged CHO and increases loading efficacy as aconsequence.

In vitro drug release

Fig.6 shows *in vitro* release of NPs at pH 7.4 and 1.5. At the ratio of 1/5, release of 5-FU was sustained and less than 25% of drug was released after 80 h at pH 7.4 (fig. 6A), and 11.14% at pH 1.5 after 3.5 h. With increasing of drug content at ratio of 1/3.5, release rate was faster than ratio of 1/5, and more than 30% of drug was released at pH 7.4 and 29.14% of drug was released at pH 1.5. Core-shell structure of NPs together with Van der Waals interaction of 5-FU and polymers probably caused this sustained release. Previous studies on AOT-chitosan and AOT-alginate NPs was also verified this sustained release. Outer layer of AOT was the main reason of sustained release of water soluble drugs from these surfactant-polymeric NPs [7, 20, 24]. Such a sustained release profile makes the CHO-DET nanoparticles a good candidate for colon targeting drug delivery and long active drug eluting stents. Another limitation of polysaccharide based NPs is burst and fast release of loaded drug. Nagarwal et al. investigated 5-FU release from chitosan-alginate NPs and chitosan coated chitosan-alginate NPs[22]. The release of 5-FU was sustained by NPs comparing to free drug, and chitosan coated NPs had lowest release rate [22]. Other studied on core-shell nanoparticles were also indicated that layer by layer coating reduced release rate [25, 26]. Both of CHO and DET are the good candidates for colon drug delivery because they are un-degradable in stomach and small intestine while they are degraded by colon flora. Most of polysaccharide based colon drug delivery systems suffered by fast release of drug content and release of huge portion of their content in upper parts of GI as a consequence. In the CHO-DET core-shell NPs, maximum drug which was released at pH 7.4 was less than 25% for feeding ratio of 1/5, and 11.14% of drug was released at pH 1.5 for this ratio. It can be assumed that more than 75% of 5-FU can be released in colon by enzymatic degradation of CHO and DET. Recent study on drug eluting stent for treatment and prevention of restenosis in gastrointestinal cancers, colon diseases and malignant airway had also shown importance of long active and sustained released delivery system for anticancer drugs [27-30]. 5-FU as anticancer drug also attracted much attention in the field of drug eluting stent [14, 31, 32]. CHO-DET NPs can be also used for encapsulation into stent with purpose of long active drug delivery system because of their sustained release property and nano-particle size.

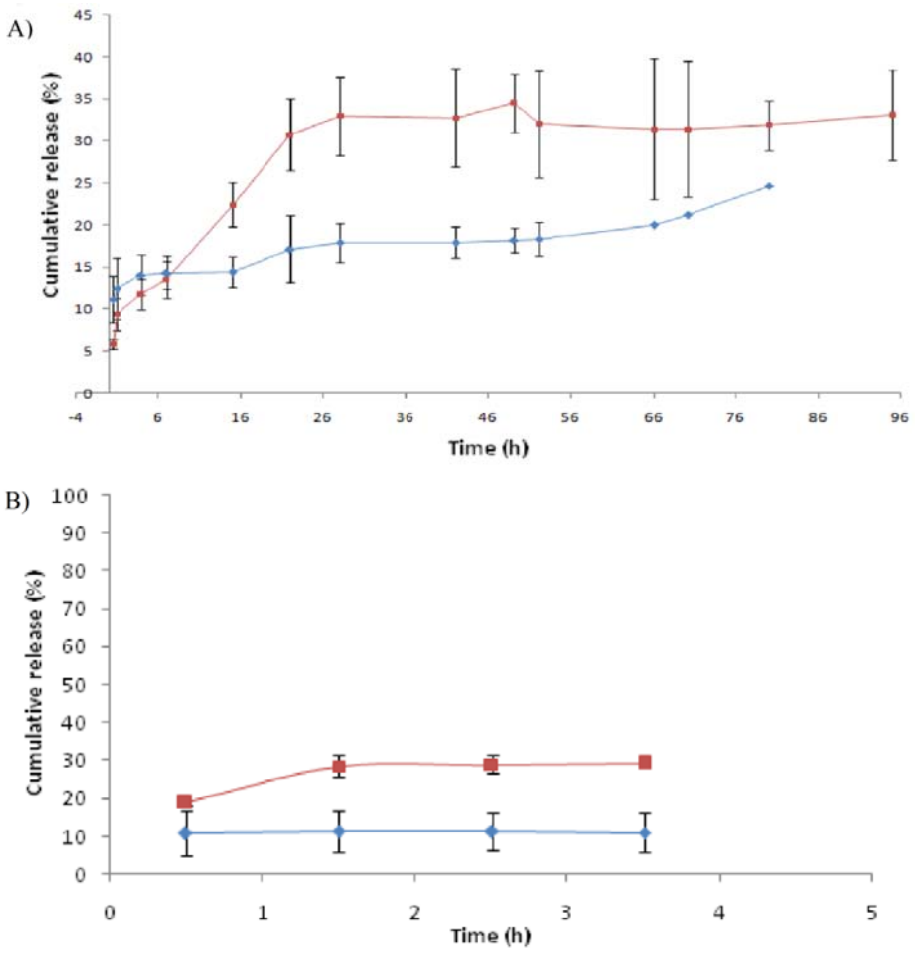

Fig. 6. Cumulative release of 5-FU from CHO-DET NPs at feeding ratios of 1/3.5 (■) and 1/5 (◆) at pH 7.4 (A) and pH 1.5 (B)

Conclusion

In this study and for the first time, CHO-DET NPs were prepared and 5-FU as an anticancer drug model was successfully loaded in NPs. Small particle size, good loading efficacy and slow release of NPs all together, with considering of bacterially trigger ability of polysaccharides indicated that CHO-DET NPs are great candidates for colon targeted drug delivery. As sustained anticancer drug delivery system, these core-shell nanoparticles potentially can also be used in the drug eluting stent.

Acknowledgement

This work is supported by the research grant No. 91424 from Vice–Chancellery of Research &Technology Affairs of the Kermanshah University of Medical Sciences.

Conflict of Interests

I certify that no actual or potential conflict of interest in relation to this article exists.

References

[1] Liu Z, Jiao Y, Wang Y, Zhou C, Zhang Z. Polysaccharides-based nanoparticles as drug delivery systems. Adv Drug Deliver Rev. 2008;60:1650-62.

[2] Rana V, Rai P, Tiwary AK, Singh RS, Kennedy JF, Knill CJ. Modified gums: Approaches and applications in drug delivery. CarbPol. 2011;83:1031-47.

[3] Sinha V, Kumria R. Polysaccharides in colon-specific drug delivery. Inter J pharm. 2001;224:19-38.

[4] Fattahi A, Petrini P, Munarin F, Shokoohinia Y, Golozar MA, Varshosaz J, et al. Polysaccharides derived from tragacanth as biocompatible polymers and Gels. J Appl Polym Sci. 2013;129:2092-102.
[5] Fattahi A, Sadrjavadi K, Golozar MA, Varshosaz J, Fathi MH, Mir-mohammad-sadeghi H. Preparation and characterization of oligochitosan–tragacanth nanoparticles as a novel gene carrier. CarbPol. 2013;97:277-83.
[6] Fattahi A, Golozar M-A, Varshosaz J, Sadeghi HM, Fathi M. Preparation and characterization of micelles of oligomeric chitosan linked to all-trans retinoic acid. CarbPol. 2012;87:1176-84.
[7] Chavanpatil MD, Khdair A, Patil Y, Handa H, Mao G, Panyam J. Polymer-surfactant nanoparticles for sustained release of water-soluble drugs. J pharm sci. 2007;96:3379-89.
[8] You JO, Peng CA, editors. Calcium-Alginate Nanoparticles Formed by Reverse Microemulsion as Gene Carriers. Macromol Symp. 2005;219:147-53.
[9] Chakraborty S, Sahoo B, Teraoka I, Miller LM, Gross RA. Enzyme-catalyzed regioselective modification of starch nanoparticles. Macromolecules. 2005;38:61-8.
[10] Kim H, Cho M, Sah H. Development of new reverse micellar microencapsulation technique to load water-soluble drug into PLGA microspheres. Arch pharmacal res. 2005;28:370-5.
[11] Grillo R, de Melo NF, de Araújo DR, de Paula E, Rosa AH, Fraceto LF. Polymeric alginate nanoparticles containing the local anesthetic bupivacaine. J drug targ. 2010;18:688-99.
[12] Kim T-H, Lee GJ, Kang J-H, Kim H-J, Kim T-i, Oh J-M. Anticancer Drug-Incorporated Layered Double Hydroxide Nanohybrids and Their Enhanced Anticancer Therapeutic Efficacy in Combination Cancer Treatment. BioMed res inter. 2014;DOI: 10.1155/2014/193401.
[13] Aydin R, Pulat M. 5-Fluorouracil encapsulated chitosan nanoparticles for pH-stimulated drug delivery: evaluation of controlled release kinetics. J Nanomat. 2012; DOI: 10.1155/2012/313961.
[14] Lei L, Liu X, Guo S, Tang M, Cheng L, Tian L. 5-Fluorouracil-loaded multilayered films for drug controlled releasing stent application: Drug release, microstructure, andex vivo permeation behaviors. J Control Release. 2010;146:45-53.
[15] Taqieddin E, Amiji M. Enzyme immobilization in novel alginate–chitosan core-shell microcapsules. Biomaterials. 2004,25,10,1937-45.
[16] Chen L, Subirade M. Chitosan/β-lactoglobulin core–shell nanoparticles as nutraceutical carriers. Biomaterials. 2005;26:6041-53.
[17] Karimi M, Avci P, Mobasseri R, Hamblin MR, Naderi-Manesh H. The novel albumin–chitosan core–shell nanoparticles for gene delivery: preparation, optimization and cell uptake investigation. J nanopart res. 2013;15:1-14.
[18] Puga AM, Lima AC, Mano JF, Concheiro A, Alvarez-Lorenzo C. Pectin-coated chitosan microgels crosslinked on superhydrophobic surfaces for 5-fluorouracil encapsulation. Carbpol. 2013;98:331-40.
[19] You J-o, Liu Y-c, Peng C-A. Efficient gene transfection using chitosan–alginate core-shell nanoparticles. Inter J nanomed. 2006;1:173-80.
[20] Chavanpatil MD, Khdair A, Panyam J. Surfactant-polymer nanoparticles: a novel platform for sustained and enhanced cellular delivery of water-soluble molecules. Pharm res. 2007;24:803-10.
[21] Dutta RK, Sahu S. Development of a novel probe sonication assisted enhanced loading of 5-FU in SPION encapsulated pectin nanocarriers for magnetic targeted drug delivery system. Europ J Pharm Biopharm. 2012;82:58-65.
[22] Nagarwal RC, Kumar R, Pandit J. Chitosan coated sodium alginate-chitosan nanoparticles loaded with 5-FU for ocular delivery: *in vitro* characterization and *in vivo* study in rabbit eye. Europ J Pharm Sci. 2012;47:678-85.
[23] Anirudhan T, Mohan AM. Novel pH switchable gelatin based hydrogel for the controlled delivery of the anti cancer drug 5-fluorouracil. RSC Adv. 2014;4:12109-18.
[24] Chavanpatil MD, Khdair A, Gerard B, Bachmeier C, Miller DW, Shekhar MP, et al. Surfactant–polymer nanoparticles overcome P-glycoprotein-mediated drug efflux. Mol pharm. 2007;4:730-8.
[25] 25. Haidar ZS, Hamdy RC, Tabrizian M. Protein release kinetics for core–shell hybrid nanoparticles based on the layer-by-layer assembly of alginate and chitosan on liposomes. Biomaterials. 2008;29:1207-15.
[26] Ye S, Wang C, Liu X, Tong Z. Deposition temperature effect on release rate of indomethacin microcrystals from microcapsules of layer-by-layer assembled chitosan and alginate multilayer films. J control release. 2005;106:319-28.
[27] Chao Y-K, Liu K-S, Wang Y-C, Huang Y-L, Liu S-J. Biodegradable cisplatin-eluting Tracheal Stent for malignant airway obstruction: *In vivo* and *in vitro* studies. CHEST Journal. 2013;144:193-9.
[28] Park S-J, Shim WH, Ho DS, Raizner AE, Park S-W, Hong M-K, et al. A paclitaxel-eluting stent for the prevention of coronary restenosis. New Eng J Med. 2003;348:1537-45.
[29] Moon S, Yang S-G, Na K. An acetylated polysaccharide-PTFE membrane-covered stent for

the delivery of gemcitabine for treatment of gastrointestinal cancer and related stenosis. Biomaterials. 2011;32:3603-10.
[30] Loras C, Pérez Roldan F, Gornals J, Barrio J, Igea F, González-Huix F, et al. Letter: the role of stent in the treatment of Crohn's disease strictures–authors' reply. Aliment pharmacol ther. 2013;37:503-4.
[31] Lei L, Liu X, Shen Y-Y, Liu J-Y, Tang M-F, Wang Z-M, et al. Zero-order release of 5-fluorouracil from PCL-based films featuring trilayered structures for stent application. Europ J Pharm Biopharm. 2011;78:49-57.
[32] Lu F, Lei L, Shen Y-Y, Hou J-W, Chen W-L, Li Y-G, et al. Effects of amphiphilic PCL–PEG–PCL copolymer addition on 5-fluorouracil release from biodegradable PCL films for stent application. Inter J pharm. 2011;419:77-84.

