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Enhanced Intestinal Permeation of Doxorubicin Using Chitosan Nanoparticles

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Abstract

Purpose: Due to limited oral bioavailability of doxorubicin (Dox) many efforts during the last decades focused on the development of novel delivery systems to overcome these limitations. In the present study, Dox encapsulated chitosan nanoparticles were prepared to evaluate the intestinal permeation of Dox via oral administration.

Methods: Nanoparticles were fabricated based on ionic gelation method using tripolyphosphate. Some physicochemical properties, such as nanoparticle size and morphology, loading efficiency and in *vitro* drug release in 3 different pH values (5.0, 6.8 & 7.4) were evaluated. Intestinal permeations of free Dox and Dox loaded in nanoparticles were assessed using rat intestinal sac model.

Results: The nanoparticles were spherical shape with average size of 150 ± 10 nm. The entrapment and loading efficiency of Dox were up to 40% and 23%, respectively. According to the release profiles, up to 30% of loaded drug was released within 6hrs and the remaining amount of Dox was released more gradually, but this pattern was related to pH of the medium. The amount of drug released at acidic condition (pH 5.0) was greater than other pHs. The intestinal permeation of Dox increased nearly up to 90% by loading in chitosan nanoparticles.

Conclusion: Using chitosan nanoparticles presents a potential safe drug delivery system for oral administration of Dox. *In vivo* studies and the determined pharmacokinetic and pharmacodynamic of Dox loaded chitosan nanoparticles after oral administration are planned for future studies.

Introduction

Oral administration of drugs is one of the most common approaches. It has many advantages in comparison with intravenous rout, such as better safety, ease of administration, lower cost, convenience and patient compliance.[1-3] The common regimen for chemotherapy consists of the administration of anticancer drugs through i.v. injection or infusion. The poor physicochemical and biopharmaceutical properties of various anticancer drugs and biological barriers in the GI tract restrict the oral administration of these drugs.[4] Utilizing pharmaceutical approaches, such as nanocarriers can improve the oral absorption of such drugs.[3]

Polymeric nanoparticles, particularly chitosan nanoparticles have special features that can improve the oral absorption of drugs. Chitosan is the polycationic derivative of chitin with mucopolysaccharide structure similar to cellulose.[5-9] Chitosan nanoparticles have many advantages over natural polymers. The preparation methods of chitosan nanoparticles are simple and mild, and the resulting particles have good stability, and low toxicity (LD50>16g/kg). This nanoparticles can be used for different purposes. Hence, they are being considered

as a drug delivery carrier, especially in recent years. Chitosan has a linear structure with a number of free amine groups, and can easily form ionic cross-linkage with multivalent anions.[10] The mucoadhesive properties of chitosan increase the contact time of a drug with the absorption site, leading to increased absorption. This property is based on the interaction between the positive charge of the chitosan and the negative charge of the mucin in the intestine.[11] Furthermore, many studies have shown that chitosan can affect the tight junctions and induce their temporary opening, which increases the permeation of drugs through the intestinal epithelium.[3,10-13] The effect of chitosan and its derivatives on the oral absorption of some anticancer drugs has been studied.[10,12]

Dox is an anticancer drug with excellent properties, which is being used to treat several malignancies, such as lymphoma, osteosarcoma and other sarcomas, carcinomas, and melanoma.[14-17] Dox is an anthracycline glycoside antibiotic that inhibits the DNA synthesis and cell proliferation. Dox is one of the essential drugs in cytotoxic and adjuvant medicine of 20th WHO essential

medicine list (EML) for adult and 6th for children. It is administered through IV infusion. However, there is the possibility of unwanted side effects due to high initial concentration or its toxicity followed by fast decay if its administered below the minimum therapeutic level.[13,18] A relatively longer systemic exposure time with less fluctuation, can lead to lower toxicity and improved efficacy, which can be provided by oral delivery of this drug.[13,18]

During this project chitosan nanoparticles were prepared by ionic gelation method and the effect of some parameters, such as polymer concentration, poly anionic agent concentration, mixing speed, etc. on particle size were analyzed. Dox was loaded in the selected nanoparticles, and the intestinal permeability studies were carried out by intestinal sac model.

Materials and Methods

Low-molecular weight chitosan and pentasodium tripolyphosphate (TPP) were purchased from a domestic supplier of Sigma-Aldrich (USA) in Iran. Doxorubicin hydrocloride was obtained as a 2mg/ml solution in 0.9% (w/v) sodium chloride from EBEWE Pharma (Austria). All other chemicals, solvents and reagents used were of chemical or analytical grade as needed, which were used without further purification. All aqueous solutions were prepared with deionized water.

Preparation of Dox loaded chitosan nanoparticles

Chitosan nanoparticles loaded Dox (Dox-Cs-NPs) were prepared using an ionic gelation method. Nanoparticles were formed spontanously, by dropwise adding of TPP aqueous solution to the chitosan aqueous solution (in 1% v/v acetic acid) under stirring at room temperature. Probe sonicator or magnet stirrer were used to mix chitosan and TPP solutions. The effect of chitosan and TPP solutions concentration, and different mixing methods on the particle size and size distribution were analyzed. Selected formulations with the particle size less than 300 nm [3] and narrow size distribution were used for loading experiments. Dox was added into chitosan solution at different drug: polymer ratio (w:w) prior to adding TPP solution. The suspension was centrifuged at 15000 rpm for 30 min, and sedimented nanoparticles were washed with deionized water and lyophilized before storage.

Characterization of NPs
Particle size and particle shape analysis

Nanoparticle sizes were determined using a laser diffraction particle size analyzer (Microtrac, Nano-flex 180°, USA) at room temperature.

The shape and surface characteristics of the nanoparticles were observed by scanning electron microscopy (SEM) (TESCAN vega3,Czech).

Drug loading and encapsulation efficiency

The encapsulation efficiency of Dox-Cs-NPs was determined by indirect method. Dox-Cs-NPs were separated from the suspension by ultracentrifugation

(Hettich, Model Mikro220R, Germany) at 15000 rpm and 4°C for 30 min. The amount of free Dox in the supernatant was analyzed by HPLC.[19-22] The encapsulation efficiency (EE) was calculated by equation 1:

$$EE\% = ((T-F)/T)*100 \qquad (eq. 1)$$

And drug loading amounts (LA) were determined using equation 2:

$$LA\% = ((T-F)/W)*100 \qquad (eq. 2)$$

Where F is the free amount of drug in the supernatant, T is the total amount of drug added into the chitosan solution, and W is the weight of Dox-Cs-NPs. All formulations were carried out in triplicate.

The chromatographic system for Dox analysis consist of a Hichrom C18 column (250 × 4 mm, UK) and a Cecil instrument (Adept series and CE 4200 UV/Vis Detector, England). The mobile phase consisted of acetonitrile: distilled water (30:70 v/v) and the pH of the mobile phase adjusted to 3 using phosphoric acid. The flow rate was set to 1 mL/min and Dox was quantified at 233 nm. The HPLC method validation showed the linearity of method ($r^2 = 0.9993$) in the range of 1–50 µg/ mL. CV% was less than 9% and the accuracy was more than 88% within this range of concentrations (1–50 µg/ mL). The specificity was tested in the presence of the nanoparticles components.

In vitro drug release study

Profile of Dox release from the nanoparticles was analyzed in three different pH values of 5.0, 6.8 and 7.4 in phosphate buffer solution. Dox-Cs-NPs were suspended in 0.5 mL buffer solution in the microtubes. The tubes were put into shaker incubator (BIOER Mixing Block MB-102, China) and temperature was set to 37°C. At scheduled time points, the nanoparticles were separated by centrifugation at 10000 rpm for 10 min, and the released drug in the supernatant was measured by HPLC method as described earlier. Following supernatant decantation, the same volume of fresh pre-warmed buffer solution was replaced. All measurements were performed in triplicate. The release percentage at each time was calculated and cumulative released curves are depicted.

Analysis of release data

To describe the drug release mechanism, release data were analyzed using the Ritger and Peppas equation:

$$M_t / M_\infty = Kt^n \qquad (eq. 3)$$

Where M_t represents the amount of drug released in time t, M_∞ is the total amount of drug that should be released at infinite time, K is constant and "n" is the release exponent indicating the type of drug release mechanism.[23]

To assay the release kinetic, data were evaluated by zero order, first order, and Higuchi model. For zero order release kinetic, the cumulative percent of released drug versus time was assessed. In the cases of first order

kinetic and Higuchi model, the logarithm of the amounts of the remaining drug to be released versus time, and the cumulative percent of released drug versus square root of time were evaluated, respectively. The relevant correlation coefficients were used to determine the best model.

Intestinal permeation studies by intestinal sac model
Preparation of everted intestinal sac
Permeation of free Dox and Dox loaded chitosan nanoparticles across the intestinal wall was studied. Everted and normal intestinal sacs were prepared according to the standard procedure.[24-26]

Male Sprague Dawley rats (250-300g) were used for the intestinal permeation experiments. Animals were sacrificed and a longitudinal segment as long as 30 cm of the upper section of intestine was removed and rinsed with cold oxygenated physiological Ringer solution and cut into segments of 2 cm length. Each sac tied to a cotton thread and filled with 2 mL Ringer solution. For everting the intestine segment a glass rod was carefully used and the serosal side of the intestine was filled with 0.5 mL Ringer solution (acceptor part). Sacs were placed in a jacket glass tubes containing 40 mL physiologic Ringer solution that were continually bubbled with 95% O_2 and 5% CO_2 kept at 37°C (donor chamber).

Intestinal permeation
After equilibration at 37°C for 5 min, the exact volume of Dox solution or nanosuspension of Dox-Cs-NPs were added to the donor chamber. At scheduled time points after adding the drug to the donor chamber five intestinal sacs were removed. The liquid inside the sacs were centrifuged and analyzed with HPLC method. In the case of adding Dox-Cs-NPs to the donor chamber, the liquid inside the sacs at first was treated with acetic acid 1% and then ultrasonicated for 45 min to dissolve chitosan and to extract the entrapped drug within the nanoparticles. Finally, the solution was centrifuged and analyzed with HPLC.

Results and Discussion
Preparation of Dox-Cs-NPs
Preparation of chitosan nanoparticles was performed by using various concentrations of chitosan (0.2-0.5mg/mL) and TPP solutions (0.2-0.5mg/mL).[27] Chitosan nanoparticles were prepared through ionic gelation technique. This method is based on electrostatic interaction between the positive charge of amine groups of chitosan and negative charge groups of polyanion, such as tripolyphosphate. This technique is simple and performed in the aqueous medium. By adding TPP to the chitosan solution, nanoparticles were spontaneously formed during stirring at room temperature. For mixing the nanosuspension the effects of magnetic stirring and ultrasonic wave on the particle size and size distribution were analyzed. The results of particle size are shown in Table 1. Dispersion of the NPs with probe sonicator was much better than magnetic stirrer, and the homogenisity of the particle size improved when probe sonicator was used.

Table 1. Particle size of nanoparticles obtained by two methods of mixing. Data were reported as mean (n=3)

Formulation No	Chitosan Conc (W/V%)	TPP Conc (W/V%)	Nanoparticle Mean Size (nm)- Number size	Nanoparticle Mean Size (nm)- Volume size	Mixing Methods
F₁	0.2	0.2	52.4	418	Magnet Stirrer
F₂	0.3	0.2	49.3	572	
F₃	0.5	0.2	228.2	285.3	
F₄	0.2	0.3	2449	2521	
F₅	0.3	0.3	2582	2691	
F₆	0.5	0.3	39.7	503	
F₇	0.2	0.5	1765	1822	
F₈	0.3	0.5	6000	6000	
F₉	0.5	0.5	3470	3520	
F₁₀	0.5	0.1	440	738	Probe Sonicator
F₁₁	0.5	0.25	418	781	
F₁₂	**0.5**	**0.3**	**152.675**	**341.75**	
F₁₃	0.5	0.5	Unread	-	

The poly dispersity of obtained NPs with two methods is shown in Figure 1. The selected formulation had the minimum size (volume diameter 152.6 nm) and was prepared by adding 2 mL of TPP (0.3%w/v) to 3 mL of chitosan solution (0.5%w/v) and sonicated for 120 sec by 75% amplitude. This formulation was selected for Dox loading. Dox was added into chitosan solution at different drug: polymer ratio (w:w) prior to the addition of TPP solution.

Drug loading and encapsulation efficiency is shown in Figure 2. Dox were successfully entrapped into chitosan nanoparticles with the encapsulation efficiency up to 23% and loading amount of 40%.
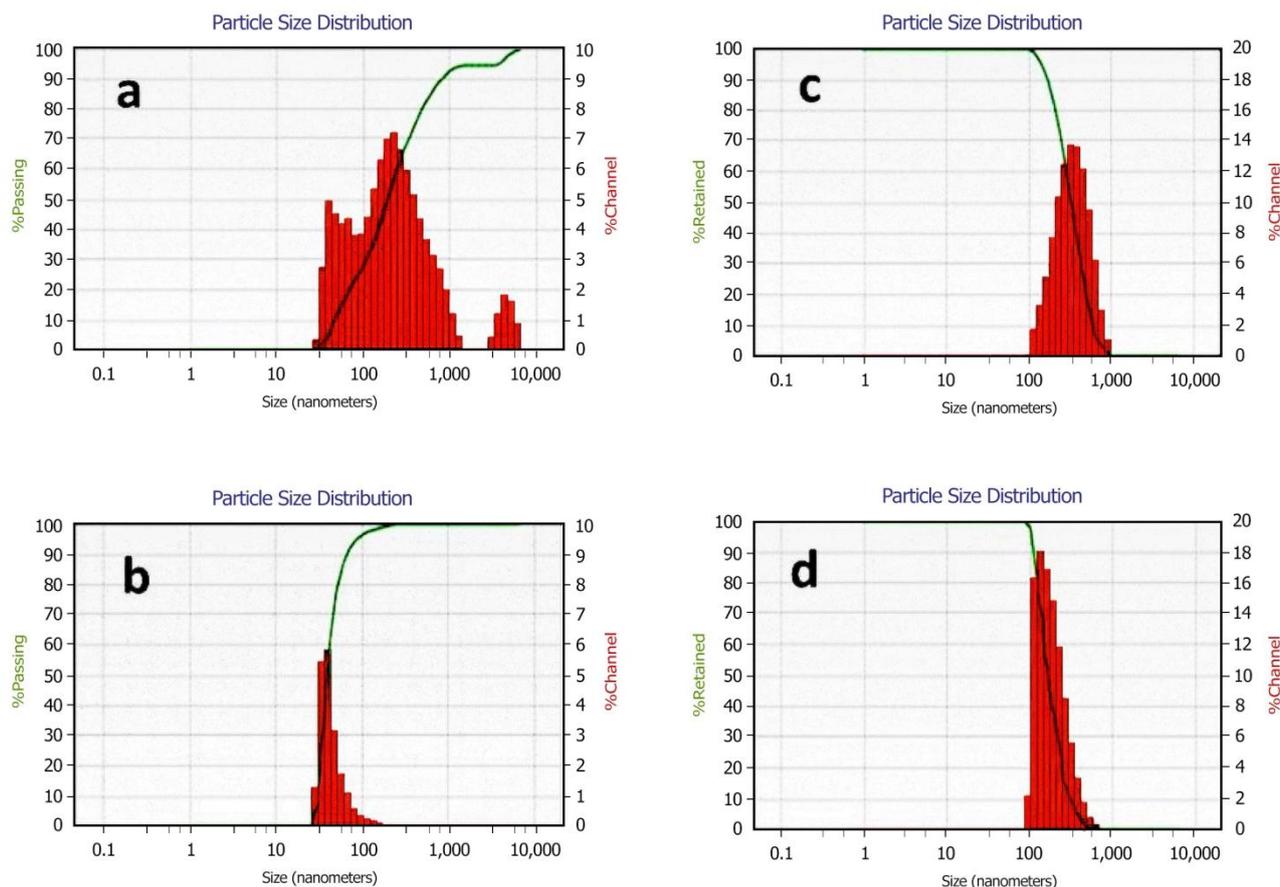
Figure 1. Volume (top) and number (bottom) size and size distribution of nanoparticle obtained by magnetic stirrer (a,b) and probe sonicator (c,d)
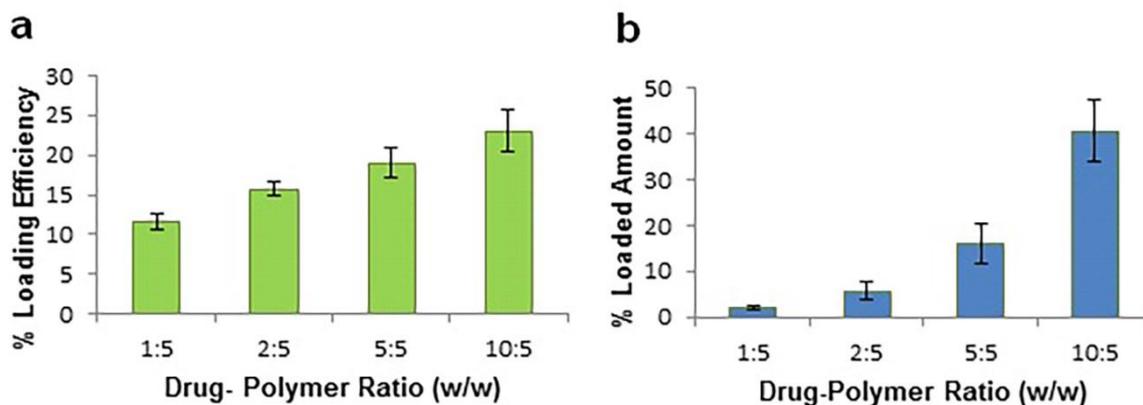

Figure 2. Loading efficiency (a) and loaded amount (b) of Dox in chitosan nanoparticles. Data were reported as mean ± SD (n=3)

Characterization of Preparation of Dox
Morphology studies
The morphology of Dox-CS-NPs was assessed by SEM and the results are shown in Figure 3. As it can be seen, the particles are spherical with smooth surface. No aggregation was detected and the particle size in the micrographs confirms the results of laser diffraction particle size analysis.

In vitro drug release
The release profile of Dox from Dox-Cs-NPs at three different pH values is shown in Figure 4. A quick release was seen from Dox-Cs-NPs in the first 6 hrs (approximately 30% of the drug was released at pH 5.0) and a relatively slow and sustained release was observed in the following days. By decreasing the pH from 7.4 to 5.0, the percentage of the released drug increased, but by changing the pH of the release medium to 6.8 the amount

of drug released was reduced. The amount of drug released at acidic condition (pH 5.0) was greater than other pHs. This phenomenon is suitable for drug release at tumor region.[28] Another positive aspect of these nanoparticles is the release profile at pH 6.8. At this pH, the lowest amount of drug was released. Since in the *in vivo* condition the range of intestine pH is similar to this point, the Dox-Cs-NPs will have slight release before absorption. These differences in the release behavior at three pH media could be explained as the influence of protonation of amine groups of Dox, chitosan and changing the solubility of drug and polymer in these conditions.[28]
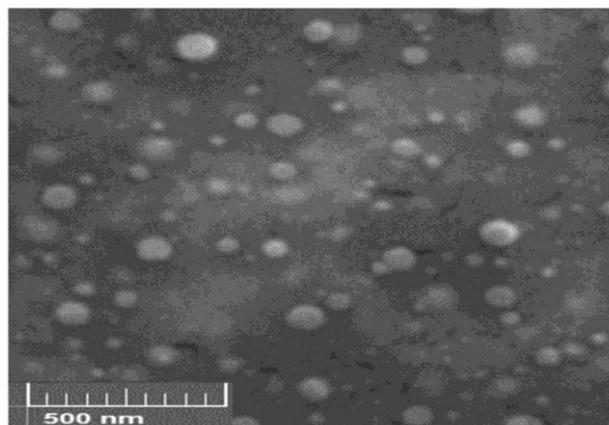

Figure 3. Scanning electron microscopic image of Dox-Cs-NPs
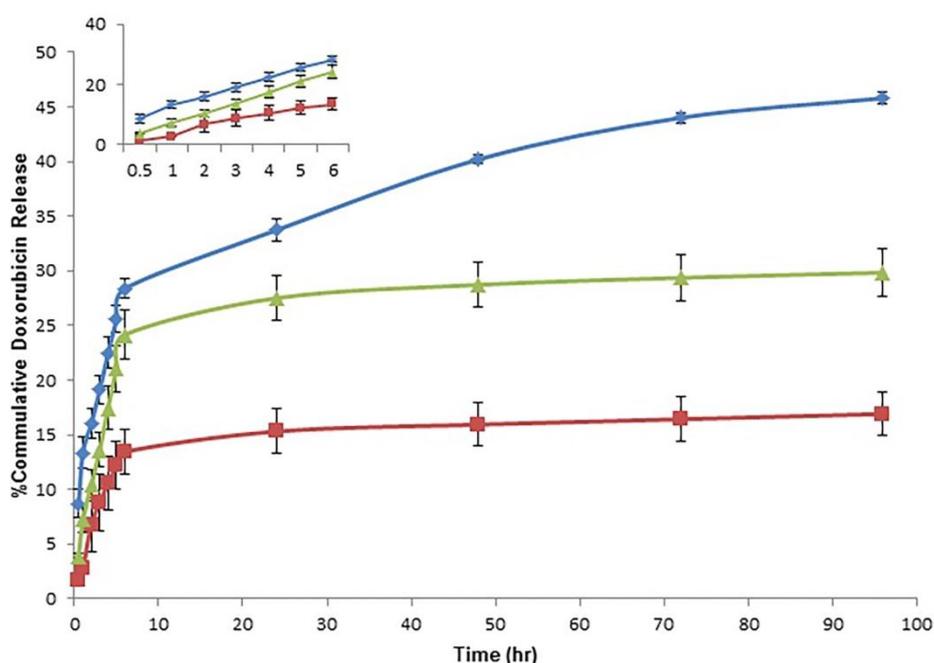

Figure 4. In vitro release profile of Dox from Dox-Cs-NPs in three different pH values. Blue line is related to pH 5, green to pH 7.4 and red to pH 6.8

According to the Figure 4 the release of Dox from chitosan nanoparicles occurred in 2 different manners. At first, a fast release profile was observable showing that some part of Dox was absorbed on the surface of the nanoparticles and this part was released at the beginning up to the first 6 hrs showing a burst release, and the second part of the drug could be released up to 96 hrs from the beginning. In this regards release kinetics were assessed in two different time intervals. Release kinetic was evaluated for 0-6 hrs and 6-96 hrs, separately. Release kinetic was calculated by data fitting to zero order, first order, Higuchi model, and Ritger and Peppas equation. The relevant correlation coefficients were used to determine the best model (Table 2). According to the results, the drug release followed the Higuchi model. The mechanism of drug release is highly related to the pH. It seems that Fickian diffusion at pH 5.0 and anomalous mechanism (Fickian diffusion and polymer relaxation) at

pH 7.4 and 6.8 are the major drug release mechanisms. In addition, erosion of the chitosan nanoparticles at pH 6.8 is also involved.

Intestinal permeation
To evaluate the role of chitosan nanoparticles in the intestinal permeation of Dox, everted rat gut sac technique was employed. This is a simple and accurate model.[24,26,27] Permeation of Dox from simple solution and Dox-Cs-NPs across the everted and not- everted gut is shown in Figure 5. The transport of Dox encapsulated in the nanoparticles was significantly higher than the free Dox (approximately12.7 folds). When Dox solution was placed at the mucosal surface of intestine, the very low amount of drug (7%) transported across the intestinal wall (Figure 5a). In spite of the proper log P of Dox (log P=1.3) for diffusion across the intestinal wall, presence of P-glycoprotein pumps (P-gp pumps) as an efflux pumps in

the luminal part of the enterocytes could show this discrepancy. In contrast, Dox can diffuse from the serosal site to mucosal site of intestine. Assessment of the Dox loaded Cs-NPs permeation via everted gut and bypassing the P-gp pump showed that permeation of the drug will be increased profoundly up to 89% (Figure 5b). The presence of chitosan nanoparticles increased the intestinal permeation of Dox approximately by 12.7 folds. This observation also showed that when Dox is entrapped into

the chitosan nanoparticles, it is not available for P-gp pumps and can be transported across the intestinal wall. In addition, this significant increase in the intestinal permeation could be explained by the effect of chitosan on the opening of tight junctions and facilitation of the paracellular transport of Dox. However, in the not everted gut experiment, simple diffusion of drug was inhibited by entrapping the drug into the nanoparticles. Therefore, no Dox could be transport from serosal to mucosal site.

Table 2. Results of release kinetics according to various equations

R² Correlation of Doxorubicin Chitosan Nanoparticles Release Data								
Release Model	Zero Order		First Order		Higuchi		n*	
pH	0-6 (hr)	6-96 (hr)	0-6 (hr)	6-96 (hr)	0-6 (hr)	6-96 (hr)	0-6 (hr)	6-96 (hr)
5	0.9804	0.9476	0.9846	0.9374	0.9882	0.9885	0.4393	0.1793
6.8	0.935	0.7891	0.9641	0.7893	0.9806	0.9051	0.9127	0.07
7.4	0.9957	0.7492	0.9971	0.7563	0.9846	0.88	0.7074	0.0707

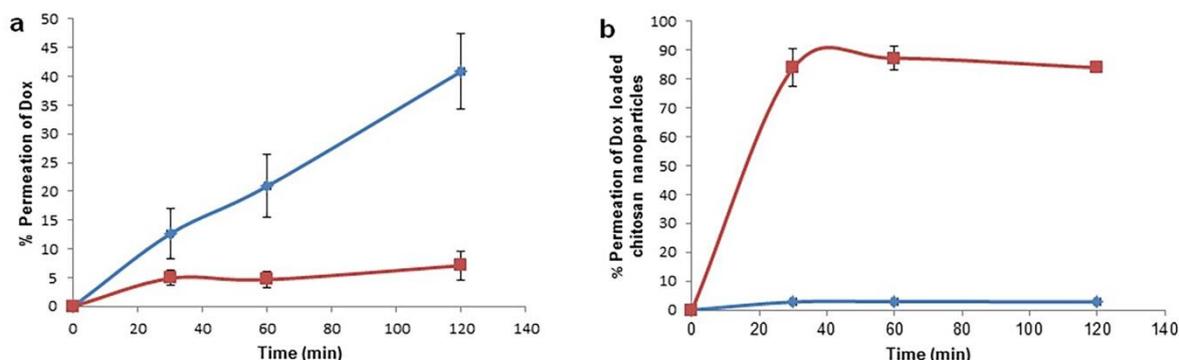n*: Pepas mechanism slope equation line

Figure 5. Intestinal permeation of Dox solution (a) and Dox loaded in chitosan nanoparticles (b) across the everted (red line) and not-everted (blue line) rat gut

Conclusion
By using the chitosan nanoparticles, the intestinal permeation of Dox approximately reached to 90%. Considering this effect, chitosan nanoparticles can present a promising platform for the development of oral delivery of poorly absorbable drugs. *In vivo* studies and determination the pharmacokinetic and pharmacodynamic of Dox loaded chitosan nanoparticles after oral administration are planned for future studies.

Ethical Issues
The protocol of the animal study was approved by the local Ethics Committee of Shiraz University of Medical Sciences (approval number 11114).

Conflict of Interest
Authors state that there is no conflict of interest.

References
1. Aulton ME, Taylor K. Aulton's pharmaceutics: The design and manufacture of medicines. Churchill Livingstone/Elsevier; 2013.
2. Schoener CA, Peppas NA. Oral delivery of chemotherapeutic agents: Background and potential of drug delivery systems for colon delivery. *J Drug Deliv Sci Technol* 2012;22(6):459-68. doi: 10.1016/S1773-2247(12)50081-X
3. Thanki K, Gangwal RP, Sangamwar AT, Jain S. Oral delivery of anticancer drugs: Challenges and opportunities. *J Control Release* 2013;170(1):15-40. doi: 10.1016/j.jconrel.2013.04.020
4. Montaseri H, Mohammadi-Samani S, Zarea B, Sobhani Z, Ahmadi F. Enhanced oral bioavailability of paclitaxel by concomitant use of absorption enhancers and P-glycoprotein inhibitors in rats. *J Chemother* 2013;25(6):355-61. doi: 10.1179/1973947813Y.0000000088

5. Chen MC, Mi FL, Liao ZX, Hsiao CW, Sonaje K, Chung MF, et al. Recent advances in chitosan-based nanoparticles for oral delivery of macromolecules. *Adv Drug Deliv Rev* 2013;65(6):865-79. doi: 10.1016/j.addr.2012.10.010

6. Ensign LM, Cone R, Hanes J. Oral drug delivery with polymeric nanoparticles: The gastrointestinal mucus barriers. *Adv Drug Deliv Rev* 2012;64(6):557-70. doi: 10.1016/j.addr.2011.12.009

7. Kurita K. Chitin and chitosan: Functional biopolymers from marine crustaceans. *Mar Biotechnol (NY)* 2006;8(3):203-26. doi: 10.1007/s10126-005-0097-5

8. Mourya V, Inamdar NN. Chitosan-modifications and applications: Opportunities galore. *React Funct Polym* 2008;68(6):1013-51. doi: 10.1016/j.reactfunctpolym.2008.03.002

9. Dodane V, Vilivalam VD. Pharmaceutical applications of chitosan. *Pharm Sci Technolo Today* 1998;1(6):246-53. doi: 10.1016/S1461-5347(98)00059-5

10. Derakhshandeh K, Fathi S. Role of chitosan nanoparticles in the oral absorption of gemcitabine. *Int J Pharm* 2012;437(1-2):172-7.doi: 10.1016/j.ijpharm.2012.08.008

11. Sailaja AK, Amareshwar P, Chakravarty P. Chitosan nanoparticles as a drug delivery system. *Res J Pharm Biol Chem Sci* 2010;1(3):474-84.

12. Feng C, Sun G, Wang Z, Cheng X, Park H, Cha D, et al. Transport mechanism of doxorubicin loaded chitosan based nanogels across intestinal epithelium. *Eur J Pharm Biopharm* 2014;87(1):197-207. doi: 10.1016/j.ejpb.2013.11.007

13. Kalaria DR, Sharma G, Beniwal V, Ravi Kumar MN. Design of biodegradable nanoparticles for oral delivery of doxorubicin: In vivo pharmacokinetics and toxicity studies in rats. *Pharm Res* 2009;26(3):492-501. doi: 10.1007/s11095-008-9763-4

14. Jain AK, Swarnakar NK, Das M, Godugu C, Singh RP, Rao PR, et al. Augmented anticancer efficacy of doxorubicin-loaded polymeric nanoparticles after oral administration in a breast cancer induced animal model. *Mol Pharm* 2011;8(4):1140-51. doi: 10.1021/mp200011f

15. Lacy CF, Armstrong LL, Lance LL, Goldman MP. Drug information handbook: A comprehensive resource for all clinicians and healthcare professionals. Lexi-Comp; 2012.

16. Di Marco A, Gaetani M, Scarpinato B. Adriamycin (NSC-123,127): A new antibiotic with antitumor activity. *Cancer Chemother Rep* 1969;53(1):33-7.

17. Alldredge BK, Corelli RL, Ernst ME, Koda-Kimble MA, Guglielmo BJ, Jacobson PA, et al. Koda-kimble and young's applied therapeutics: The clinical use of drugs. Lippincott Williams & Wilkins; 2012.

18. Kim JE, Yoon IS, Cho HJ, Kim DH, Choi YH, Kim DD. Emulsion-based colloidal nanosystems for oral delivery of doxorubicin: Improved intestinal paracellular absorption and alleviated cardiotoxicity. *Int J Pharm* 2014;464(1-2):117-26. doi: 10.1016/j.ijpharm.2014.01.016

19. Han J, Zhang J, Zhao H, Li Y, Chen Z. Simultaneous determination of doxorubicin and its dipeptide prodrug in mice plasma by hplc with fluorescence detection. *J Pharm Anal* 2016;6(3):199-202. doi: 10.1016/j.jpha.2015.12.005

20. Reddy LH, Meda N, Murthy RR. Rapid and sensitive hplc method for the estimation of doxorubicin in dog blood--the silver nitrate artifact. *Acta Pharm* 2005;55(1):81-91.

21. Ricciarello R, Pichini S, Pacifici R, Altieri I, Pellegrini M, Fattorossi A, et al. Simultaneous determination of epirubicin, doxorubicin and their principal metabolites in human plasma by high-performance liquid chromatography and electrochemical detection. *J Chromatogr B Biomed Sci Appl* 1998;707(1-2):219-25. doi: 10.1016/S0378-4347(97)00610-5

22. Farvadi F, Tamaddon AM, Abolmaali SS, Sobhani Z, Yousefi GH. Micellar stabilized single-walled carbon nanotubes for a ph-sensitive delivery of doxorubicin. *Res Pharm Sci* 2014;9(1):1-10.

23. Samani SM, Montaseri H, Kazemi A. The effect of polymer blends on release profiles of diclofenac sodium from matrices. *Eur J Pharm Biopharm* 2003;55(3):351-5. doi: 10.1016/S0939-6411(03)00030-4

24. Ritschel WA, Kearns GL. Handbook of basic pharmacokinetics- including clinical applications. American Pharmaceutical Association; 1999.

25. Borchardt RT, Smith PL, Wilson G. Models for assessing drug absorption and metabolism. Springer; 1996.

26. Gamboa JM, Leong KW. In vitro and in vivo models for the study of oral delivery of nanoparticles. *Adv Drug Deliv Rev* 2013;65(6):800-10. doi: 10.1016/j.addr.2013.01.003

27. Sobhani Z, Mohammadi Samani S, Montaseri H, Khezri E. Nanoparticles of chitosan loaded ciprofloxacin: Fabrication and antimicrobial activity. *Adv Pharm Bull* 2017;7(3):427-32. doi: 10.15171/apb.2017.051

28. Unsoy G, Khodadust R, Yalcin S, Mutlu P, Gunduz U. Synthesis of doxorubicin loaded magnetic chitosan nanoparticles for ph responsive targeted drug delivery. *Eur J Pharm Sci* 2014;62:243-50. doi: 10.1016/j.ejps.2014.05.021