

Pharmacokinetic study of furosemide incorporated PLGA microspheres after oral administration to rat

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ABSTRACT

Objective(s): The purpose of the current study was to assess the feasibility of microspheres from biocompatible polymer for oral bioavailability (BA) enhancement of potent sulfonamide- type loop diuretic- Furosemide - which used in the treatment of congestive heart failure, caused edema, cirrhosis, renal disease and as an adjunct in acute pulmonary edema. The comparatively poor and inconstant BA of furosemide, which occurs site-specifically in the stomach and upper small intestine, has been ascribed to the poor dissolution of furosemide.

Materials and Methods: In attempt to enhance the drug BA, poly (dl-lactic-co-glycolic acid) (PLGA) microspheres of furosemide were obtained using solvent-evaporation method and the carrier characteristics were investigated subsequently.

Results: The *in vivo* performance of optimum formulation was assessed by pharmacokinetic evaluation of drug after orally administration of free and loaded in microspheres to rats (4 mg/Kg). For this reason, the concentration of drug in plasma was measured by a new developed and sensitive method of HPLC. Acceptable drug loading and encapsulation efficiency of microspheres were obtained to be 70.43 and 85.21 %, respectively. Microspheres provided improved pharmacokinetic parameters (C_{max} = 147.94 ng/ml, T_{max} = 1.92 hr) in rats as compared with pure drug (C_{max} = 75.69 ng/ml, T_{max} = 1.5 hr). The obtained AUC of drug in microsphere was 10 fold higher than of the free drug.

Conclusion: The results showed that the prepared microspheres successfully improved BA of the poorly water-soluble drug effectively.

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Introduction

Microspheres are belonging to the multiparticulate drug delivery systems (DDSs). These systems are prepared to obtain sustained or controlled drug delivery, to improve bioavailability (BA), stability and to target drug to specific organelles.

Microspheres can also offer several advantages including reducing of drug fluctuation in therapeutic range, side effects, and dosing occurrence and successful in access of patient compliance (1). Among modern drug delivery carriers, matrix-type microspheres containing uniformly incorporated drug, are a promising carrier for improvement of the oral BA of insoluble therapeutic drugs (2).

Several therapeutic agents have been developed to obtain an enhanced BA and a prolonged constant drug plasma concentration after administration of microspheres to rats (3-5). The biodegradability and as well as biocompatibility is a vital for the polymeric carrier that used in pharmacy science. Between the biodegradable polymers, poly (lactic-co-glycolic

acid) (PLGA) has been used for many years as a suitable material for DDS fabrication; this polymer can be degraded by the hydrolysis of their constituents, which are usual metabolic without any cytotoxicity (6-9).

PLGA is widely used for preparation of nanoparticles, microspheres, injectable depots, films, scaffolds and as a bulk implant for DDS designing due to low toxicity and tunable biodegradability (10). In the procedure of a new drug invention, animal data are traditionally used to estimate the first dose to human and to assess the efficacy and safety of a newly developed compound.

Predominantly, one of the purposes of animal studies is to expect pharmacokinetics (PK) and pharmacodynamics (PD) of the therapeutic agent in humans. The evaluation of PD and PK values are so important for both drug and the carrier (11-13). However, due to many differences in anatomy and physiology of animal species and humans, it is very difficult to completely predict the absorption kinetics in

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humans based on animal studies (12, 13). In the procedure of developing DDSs, various animal models have used to realizing of therapeutic dosage and to forecast their performances in humans (15, 16).

It is necessary to select the adequate animal model by consideration of the differences between selected animals and humans, which is appropriate for estimating the action of the DDSs (15). Furosemide is an anthranilic acid derivative commonly used in man as a potent tubule diuretic. It is frequently used in human edema treatment with CHF (congestive heart failure), hypertension and renal insufficiency.

This drug belongs to the class of an IV of BCS system (Biopharmaceutics Classification System), which has poor and erratic absorption after orally administration and intersubject variation, in pharmacokinetic parameters (17, 18). The aim of the present study was to characterize the pharmacokinetic profile and the parameters of furosemide in rats after the oral administration of the furosemide incorporated microspheres which were prepared and characterized in our previous.

The new developed HPLC separation technique was optimized for furosemide in order to afford a simple and rapid methodology.

Materials and Methods

Materials

Furosemide was received as a gift sample from Atra Co (Tehran, Iran), Poly (DL, lactide-co-glycolid) (PLGA, 50:50 MW 12 000) was obtained from Boehringer Ingelheim Co (Ingelheim, Germany). Polyvinyl alcohol (PVA, MW 30 000 Da, 87% hydrolyzed) was a gift from Mowiol (Frankfurt, Germany). HPLC grade acetonitrile and the analytical grade dimethyl sulfoxide, chloroform, acetone, and perchloric acid (70%, w/v) were purchased from Merck (Darmstadt, Germany).

Microspheres loaded drug preparation

Furosemide loaded PLGA microspheres were prepared by o/w emulsion/solvent evaporation method. The procedure was as follows: the exact quantity of PLGA polymer and furosemide (10 mg) was accurately weighted and dissolved in dichloromethane. The organic phase was added drop-wise (0.5 ml/min) into the PVA aqueous solution and stirred magnetically at room temperature until the complete evaporation of the organic solvent. Subsequently, microspheres were separated by centrifugation at 10000 rpm for 15 min. The separated microspheres were dispersed and centrifuged two times in phosphate buffer (pH: 7.4) and distilled water to completely remove free drug and excess surfactants (19-21).

A Franz diffusion cell was used for the studies. In each case, 100 mg of optimized microsphere formulation was placed in the donor compartment of

the Franz diffusion cell separated from the receptor compartment by cellulose acetate membrane (pore size 0.22 μm). The receptor compartment was filled with phosphate buffer pH 7.4, simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.2) and maintained at a temperature of (37 °C) by means of a thermostatically controlled water bath, with agitation provided by a magnetic stirring bar at 50 rpm. Aliquot was removed and replaced by an equal volume of the receptor phase at different time interval and the samples collected analyzed spectrophotometrically.

Pharmacokinetics study

Laboratory animals

Pharmacokinetic studies were performed in twelve male Wistar rats (obtained from Institute Pasteur of Iran, Tehran) weighing about 250–300 g. All animal experimental protocols were approved by the animal welfare commission of the Kermanshah University of Medical Sciences.

The rats were fasted overnight before the experiment and had access to water easily. They were divided into two groups: (n=6, in each group) for the *in-vivo* study. All animals were housed in wire cages in a 12-hr light–dark cycle for a minimum of 5 days before the beginning of the experiment to allow them to adjust to the new environment.

Drug administration and sample collection

The animals were treated with optimized microspheres containing 4 mg/kg of furosemide. Microsphere dispersion was prepared freshly and diluted in physiological saline immediately before administration per animal through oral gavage. For comparison, a pharmacokinetic evaluation of free drug was also performed.

At predetermined time intervals, blood samples (0.5 ml) were collected via the inner canthus into heparinized tubes and separated immediately by centrifuging at 3500 rpm for 10 min and stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

Drug analysis

Extraction procedure of plasma sample

The following procedure was used in order to determine the furosemide in plasma samples. To 100 μl of plasma, 100 μl prazosin (50 $\mu\text{g}/\text{ml}$ as internal standard) and 50 μl HCl 0.2 M was added. Then 2 ml ether as the extraction solvent was added and stirred for 30 sec. After this time, the sample was centrifuged at 3000 rpm for 2 min and the supernatant was separated and dried (37 °C for 10 min) and was protected from light. The dried residue was reconstituted by 100 μl methanol and volume of 20 μl was injected to HPLC instrument.

The standard curve (n=3, $R^2=0.9988$) was used to calculate the unknown furosemide plasma concentrations.

Chromatographic condition

The separations were performed with Nucleodur C18 pyramid (5 μm) column using a mobile phase consisting of methanol and sodium phosphate buffer (0.05 M, pH 2, 50:50 V/V) containing 200 μl triethylamine. The flow rate was 1.0 ml/min and column temperature of 64 °C. The fluorescence detector excitation and the emission wavelengths were set at 360 and 413 nm, respectively.

Pharmacokinetic analysis

The pharmacokinetic analysis was performed by the compartmental analysis. The two-compartment model can be described by the following biexponential equation (Eq. 1):

$$C_t = A (e^{-Kt} - e^{-K_a t}) \quad \text{Eq. 1}$$

Where C_t is the drug concentration at time t. A is the Y-intercept, and K_a and K are the apparent first-order absorption and elimination rate constants. From equation 1 elimination rate constant was estimated by least squares regression of plasma concentration– time data points lying in the terminal log-linear region of the curve. Absorption half-life T_{1/2a} and elimination half-life T_{1/2e} calculated from the following equations respectively Eq. 2 and Eq. 3:

$$T_{1/2a} = 0.693 / K_a \quad \text{Eq. 2}$$

$$T_{1/2e} = 0.693 / K_e \quad \text{Eq. 3}$$

K_a, the absorption rate constant, obtained from residual method.

The area under the plasma concentration-versus time curve (AUC) was calculated using the trapezoidal rule with extrapolation to infinity (Eq. 4).

$$AUC_{0-t} = [(t_2-t_1)(C_2+C_1)/2] + \dots + [(t_n-t_{n-1})(C_n-1 + C_n)/2] \quad \text{Eq. 4}$$

Other pharmacokinetic factors include clearance (Cl) was calculated by dividing dose over AUC, volume of distribution at steady state (V_{ss}) and mean residence time (MRT) were calculated using following equations (Eq: 5-7 respectively):

$$Cl = \text{Dose} / AUC_{0-t} \quad \text{Eq. 5}$$

$$V_{ss} = \text{Dose} \cdot AUMC_{0-\infty} / AUC_{0-\infty}^2 \quad \text{Eq. 6}$$

$$MRT = AUMC_{0-\infty} / AUC_{0-\infty} \quad \text{Eq. 7}$$

Where AUMC (area under the first moment curve) is the area under the Ct plotted against t from time 0 to infinity (22, 23).

Statistical analysis

One-way analysis of variance was performed to compare the parameters between the loaded microspheres and free drug solution groups. The level of significance was P<0.05.

Results

Microspheres preparation and characterization

The obtained optimum formulation showed the morphological of spherical, nonporous, and uniform with smooth surface microspheres. The average diameter was 70±10 μm and the drug loading of more than 75.43%.

The release behavior of the furosemide from PLGA microspheres in various medium showed that approximately 8% of the furosemide was released in the SGF (pH 1.2) over a period of 24 hr and 60% in SIF (pH 7.4) and 50% in phosphate buffer (pH 7.4). The result indicated that in each three medium the release consisted of an initial rapid release phase, followed by a sustained release period for 24 hours, obeying Higuchi order kinetic (19).

Chromatographic assay results

The composition and pH of the mobile phase used in the present assay provided good separation of furosemide and prazosin and gave sharp peaks with no interferences from endogenous components in plasma. Figure 1 shows the HPLC chromatograms of blank plasma and a subject's plasma samples obtained by the above described method.

The chromatograms showed excellent resolution between furosemide and the internal standard, appearing at 5.08 and 3.08 min, respectively. No interference was found during the chromatographic run of the plasma in the area where furosemide or the internal standard peak appears (24, 25). Table 1 shows a summary of intraday and interday precision and accuracy of measurement of furosemide in plasma, respectively.

The LOD (limit of detection) and LOQ (limit of quantification) were determined to be 5 and 10 ng/ml, respectively.

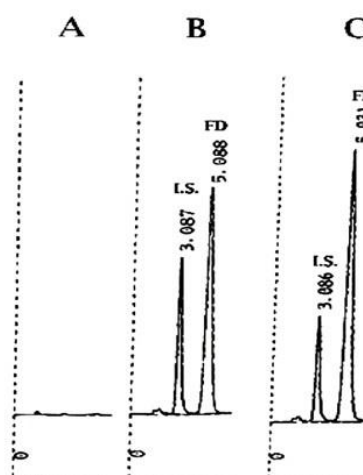


Figure 1. Representative HPLC chromatograms of furosemide and I.S. in rat plasma samples: (A) blank plasma; (B) blank plasma spiked with furosemide and the internal standard (100 ng/ml) and I.S. (50 μg/ml); (C) plasma after microspheres administration (4 mg/Kg)

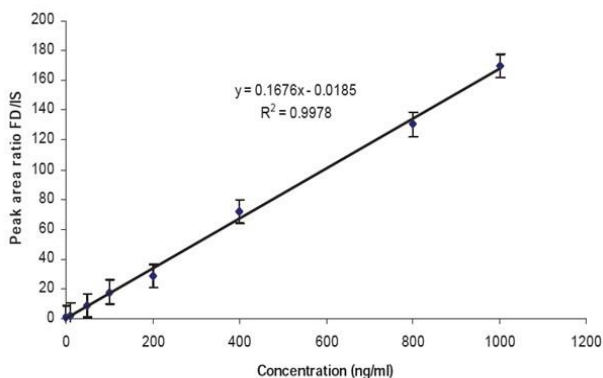


Figure 2. Calibration curve of furosemide in spike rat plasma (mean±SD, n=3)

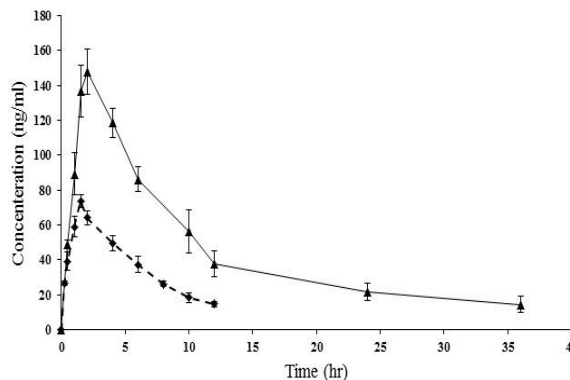


Figure 3. Plasma concentration– time curve of furosemide in free and loaded in microspheres following oral administration (4 mg/kg) to rats. (Each point represents the mean±SD, n=6)

The plasma concentration of drug in loaded and free forms

The calibration line was determined with the results gained from the standard plasma furosemide solutions, with the correlation coefficient of R²=0.9978 (Figure 2).

The mean plasma concentration-time profiles of furosemide following oral administration of both formulations (microspheres and free drug solution) in rats are shown in Figure 3. As shown after oral administration of free drug, plasma concentrations of furosemide declined at a relatively fast elimination rate, whereas plasma concentration- time profiles of furosemide in microsphere formulation showed a much slower elimination phase.

Pharmacokinetic analysis

The mean pharmacokinetic parameters of furosemide in forms of free furosemide and loaded in microspheres in rats and the comparisons of parameters for both cases are indicated in Table 1. It should be mentioned that according to the studies by Chen and coworkers, the route of administration seems to significantly impact the PK (26), as the elimination half-life and MRT of the drug following oral administration and in PLGA micro and nanoparticles of the same drugs such as anesthetics are significantly different (27).

Table 1. Intra- and inter-day precision and accuracy data

Concentration (ng/ml)	Mean± SD	CV (%)	Bias (%)
Intra-day (n=6)			
200	205.51± 2.59	1.26	2.75
100	95.26± 3.98	4.17	-4.74
50	52.35± 2.68	5.12	4.7
10	11.15± 1.21	10.85	11.5
Inter-day (n=6)			
200	210.04± 2.78	1.32	5.02
100	98.89± 2.38	2.40	-1.11
50	45.57± 3.34	7.33	-8.86
10	9.38± 0.58	6.18	-6.2

From the plasma concentration- time profile in the rats (Figure 3), it is clear that encapsulation of drug in a microcarrier markedly slowed down the elimination phase and resulted in about a 2.95-fold (P<0.0001) increase in the elimination half-life (T_{1/2e}) values of capsulated form compared with free drug. As shown after 8 hr, the drug was still detectable in plasma following oral administration of microspheres compared with 3 hr for free drug administration.

In the same time, the absorption half time (T_{1/2a}) for encapsulated drug increased in comparison with free drug. The amount of furosemide distribution also significantly decreased for microsphere formulation. V_{ss} was obviously lower for the carrier-loaded drug in comparison with similar values for the free drug (Table 1).

Therefore, it seems that the encapsulation of furosemide in microcarrier considerably delayed the kinetics of drug transfer from the plasma to the tissue compartment, which led to reduced drug distribution. Consequently, the drug release rate from carriers has a high effect on drug distribution. Further pharmacokinetic analysis indicated that after administration of microspheres, AUC and the MRT parameters were increased 3.5 and 2.34 fold (P<0.05) respectively, in comparison with the free drug (Table 1).

These results indicate that the prepared microspheres could reduce the clearance rate of drug and the carrier had obviously limited phagocytosis uptake by the reticuloendothelial system (as displayed in Table 1, the clearance of the encapsulated and free drug are 2.52±0.42 and 8.54±1.03, respectively) (28).

Discussion

The pharmacokinetics of furosemide in the form of free and loaded in PLGA microspheres was studied after orally administration to rat following a single dose of 4 mg/Kg. The plasma concentration of drug was determined using a simple, sensitive and

reproducible HPLC method, developed in this laboratory.

Recent studies showed that the encapsulation of these drugs in biodegradable nano and micro carriers was proposed as an alternative method to increase their oral bioavailability and lower variability in pharmacokinetic parameters (29-37).

In pharmacokinetic study of the camptothecin solid lipid nanoparticles (CA-SLN), it was shown that the AUC/dose and the mean residence times of CA-SLN were significantly higher than those of the drug solution. In addition, the incorporation of 7-ethyl-10-hydroxycamptothecin (SN-38), which has marked antitumor effects on many types of tumors, into NPs improved the stability of the lactone ring in serum-containing media (29).

Reddy and Murthy evaluated and compared the PK and the tissue distribution of doxorubicin (Dox) after intravenous and intraperitoneal injection of a free drug solution and drug-loaded NPs. For both routes of injection, the T_{1/2}, MRT, and AUC of the drug after the administration of Dox NPs were highly higher and the clearance (Cl) was lower than the expected values for the Dox-free drug solution.

The BA of Dox in NP form was greatly enhanced (about 2-fold) in comparison with the solution, which can improve the therapeutic efficacy of drug and reduce the Dox-associated systemic toxicity, especially cardiotoxicity (30). Song *et al* prepared copolymer of polymer PLGA-PEG-PLGA microspheres and studied the PK and the tissue distribution of Curcumin. In this study, the plasma AUC_{0-∞}, T_{1/2α}, T_{1/2β} and MRT of CUR micelles were increased by 1.31, 2.48, 4.54 and 2.67 fold in comparison with the CUR solution, respectively. Also the biodistribution study in mice indicated that the micelles decreased drug uptake by liver and spleen and enhanced drug distribution in lung and brain (5).

Narayanan and coworkers reported an engineered PLGA-casein, polymer-protein hybrid nanocarrier with a combination of chemically distinct hydrophobic and hydrophilic drug models. From this study, the *in vivo* pharmacokinetic studies on rats by HPLC method confirmed a sustained and sequential release of both the drugs in plasma, which indicated the prolonged circulation of the nanomedicine and enhanced the availability of the drugs in comparison with the bare drugs. From these works it was shown that PLGA-PEG-PLGA is a suitable carrier for *in vivo* drug delivery (31).

It has been previously reported that hydrophilic polymers could overcome the multidrug resistance phenotype mediated by P-glycoprotein (P-gp) leading to an increase in drug concentration inside the organs. Under these optimal conditions, the drug loaded within the carriers in the circulation is protected from metabolism and inactivation in the plasma. Also, owing to the size limitations in the transport of large molecules or carriers across healthy endothelium

membrane, the drug accumulation decreased in the healthy tissues (32, 33).

There are several hypotheses about effective oral delivery and intestinal transport of drug loaded in microcarrier, for example, the opening of the tight junctions shows a potential approach to increase the paracellular absorption of drugs (34).

Microparticles that possess bio-adhesiveness could be localized to the absorptive epithelium while larger particles with low bio-adhesiveness failed to penetrate enterocytes and were taken up preferentially in the Peyer's patches (35, 36). The literature shows that the modification and improvement of pharmacokinetic parameters, such as the tissue distribution profile, are achieved through the nano and microparticulate drug delivery.

Other advantages include improved BA and decreased toxicity due to high loading efficiency, which results in lower doses administered. These factors all lead to an increase in the patient compliance to treatment (37).

Taken together, on the basis of the obtained results, the increased efficacy compared with free drug in rats, prolonged half-life of the encapsulated form in plasma, and markedly improved pharmacokinetic parameters were achieved by the formulation of furosemide as polymeric microcarriers.

Therefore, because little is known about the PK of furosemide, it seems that further investigations are needed to clarify the effect of the route of administration on the PK of furosemide.

Conclusion

In this study, the micro formulation of furosemide was investigated in terms of *in-vivo* PK of the drug in the free and entrapped forms.

The prepared microspheres were characterized by efficient drug loading and prolonged circulation in rat plasma. The study of the biodistribution of the furosemide loaded microspheres in animal models could provide further support for the therapeutic advantage of the prepared formulation.

The statistical comparison of the PK parameters clearly indicated that the microsphere significantly improved these parameters compared to free drug. In this study, we indicated that the drug loaded in PLGA microspheres could be a promising carrier for new DDSs and have the potential to enhance the BA of drugs after the oral administration and are expected to allow a reduction in dosage and a decrease in systemic toxicity.

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Conflict of interest

The authors have no conflict of interest.

References

- Haznedar S, Dortunç B. Preparation and *in vitro* evaluation of Eudragit microspheres containing acetazolamide. *Int J Pharm* 2004; 269:131-140.
- Wang Z, Chen B, Quan G, Li F, Wu Q, Dian L. Increasing the oral bioavailability of poorly water-soluble carbamazepine using immediate-release pellets supported on SBA-15 mesoporous silica. *Int J Nanomedicine* 2012; 7: 5807-5818.
- Huang JJ, Wigent RJ, Bentzley CM, Schwartz JB. Nifedipine solid dispersion in microparticles of aminomethacrylate copolymer and ethylcellulose binary blend for controlled drug delivery: effect of drugloading on release kinetics. *Int J Pharm* 2006; 319: 44-45.
- Wan Sh, Sun Y, Sun L, Tan F. Chitosan microparticles for oral bioavailability improvement of the hydrophobic drug curcumin. *Pharmazie* 2012; 67: 525-528.
- Song Z, Feng R, Sun M, Guo C, Gao Y, Li L, Zhai G. Curcumin-loaded PLGA-PEG-PLGA triblockcopolymeric micelles: Preparation, pharmacokinetics and distribution *in vivo*. *J Colloid Interface Sci* 2011; 354: 116-123.
- Dawes GJS, Fratila-Apachitei LE, Mulia K, Apachitei I, Witkamp G-J, Duszczak J. Size effect of PLGA spheres on drug loading efficiency and release profiles. *J Mater Sci Mater Med* 2009; 20: 1089-1094.
- Afshari M, Derakhshandeh K, Hosseinzadeh L. Characterisation, cytotoxicity and apoptosis studies of methotrexate-loaded PLGA and PLGA-PEG nanoparticles. *J Microencapsul* 2014; 31: 239-245.
- Derakhshandeh, K., Fathi, S. Role of chitosan nanoparticles in the oral absorption of Gemcitabine. *Int J Pharm* 2012; 437: 172-177.
- Gentile P, Chiono V, Carmagnola I, Hatton PV. An Overview of Poly (lactic-co-glycolic) Acid (PLGA)-Based Biomaterials for Bone Tissue Engineering. *Int J Mol Sci* 2014; 15: 3640-3659.
- Ramesh V, Sairam M, Kallappa M, Aminabhavi M. Preparation of sodium alginate-methylcellulose blend microspheres for controlled release of nifedipine. *Carbohydrate Polymers*. 2007; 69: 241-250.
- Cooper DL, Wood RC, Wyatt JE, Harirforoosh S. Pharmacokinetic interactions between rebamipide and selected nonsteroidal anti-inflammatory drugs in rats. *Eur J Pharm Sci* 2014; 53: 28-34.
- Ezan E. Pharmacokinetic studies of protein drugs: Past, present and future. *Adv Drug Deliv Rev*. 2013; 65:1065-1073.
- Kirchhofer C, Vargas M, Huwyler J, Keiser J. Pharmacokinetics of the fasciocidal drug candidates MT04 and OZ78 in uninfected rats and *in vitro* pharmacodynamics studies. *Int J Parasitol Drugs Drug Resist*. 2012; 2: 121-125.
- Sun F, Sui C, Teng L, Liu X, Teng L, Meng Q, Li Y. Studies on the preparation, characterization and pharmacological evaluation of tolterodine PLGA microspheres. *Int J Pharm* 2010; 397: 44-49.
- Nagahara N, Akiyama Y, Higaki K, Kimura T. Animal models for predicting potency of oral sustained-release adhesive microspheres in humans. *Int J Pharm* 2007; 331: 46-53.
- He Z, Wan X, Schulz A, Bludau H, Dobrovolskaia MA, Stern ST, Montgomery SA, Yuan H, Li Z, Alakhova D, Sokolsky M, Darr DB. A high capacity polymeric micelle of paclitaxel: Implication of high dose drug therapy to safety and *in vivo* anti-cancer activity. *Biomaterials* 2016; 101: 296-309.
- Lindenberg M, Kopp S, Dressman B. Classification of orally administered drugs on the World Health Organization Model list of Essential Medicines according to the biopharmaceutics classification system. *Eur J Pharm Biopharm* 2004; 58:265-278.
- Derakhshandeh K, Hosseinalizadeh A, Nikmohammadi M. The effects of PLGA microparticles on intestinal absorption of P-glycoprotein substrate using the everted rat intestinal sac model. *Arch Pharm Res* 2011; 34:1989-1997.
- Derakhshandeh, K., Erfan, M., Dadashzadeh, S. Encapsulation of 9-nitrocamptothecin, a novel anticancer drug, in biodegradable nanoparticles: factorial design, characterization and release kinetics. *Eur J Pharm Biopharm*. 2007; 66:34-41.
- Derakhshandeh K, Soheili M, Dadashzadeh S, Saghiri R. Preparation and *in vitro* characterization of 9-nitrocamptothecin-loaded long circulating nanoparticles for delivery in cancer patients. *Int J Nanomed* 2010; 5:463-471.
- Derakhshandeh K, Hochhaus G, Dadashzadeh, S. *In vitro* cellular uptake and transport study of 9-nitrocamptothecin PLGA nanoparticles across Caco-2 cell monolayer model. *Iran J Pharm Res* 2011; 10: 425-434.
- Shargel L. *Applied Biopharmaceutics & Pharmacokinetics*. 5th ed. USA: Appleton and longe; 2004.p. 146-175.
- López-Arnau R, Martínez-Clemente J, Carbó M, Pubill D, Escubedo E, Camarasa J. An integrated pharmacokinetic and pharmacodynamic study of a new drug of abuse, methylone, a synthetic cathinone sold as "bath salts". *Prog Neuro-psychopharmacol & Biol Psychiatry* 2013; 45:64-72.
- Lin ET, Smith DE, Benet L, Hoener BA. High-performance liquid chromatographic assays for furosemide in plasma and urine. *J Chromatography* 1979; 163:315-321.
- Carolina gomez G, Carlos von Plessing R, C. Gloria Godoy M, Rolando Reinbach H, Ricardo Godoy R. Method validation for the determination of furosemide in plasma by liquid-liquid extraction and high-performance liquid chromatography with fluorescence detection. *J Chil Chem Soc* 2005; 50: 479-482.
- Chen J, Ping Q, Guo J, Chu X, Song M. Pharmacokinetics of lactone, carboxylate and total 9-nitrocamptothecin with different doses and administration routes in rats. *Biopharm Drug Dispos* 2006; 27:53-59.
- Ratajczak-Enselme M, Estebe JP, Dollo G, Chevanne F, Bec D, Malinovsky JM. Epidural, intrathecal and plasma pharmacokinetic study of

- epidural ropivacaine in PLGA-microspheres in sheep model. *Eur J Pharm Biopharm* 2009; 72:54-61.
28. Dadashzadeh S, Derakhshandeh K, HoseiniShirazi F. 9-Nitrocamptothecin polymeric nanoparticles: cytotoxicity and pharmacokinetic studies of lactone and total forms of drug in rats. *Anti-Cancer Drugs* 2008; 19:805-811.
29. Onishi H, Machida Y, Machida Y. Antitumor properties of irinotecan containing nanoparticles prepared using poly (DL-lactic acid) and poly (ethylene glycol)-block-poly (propylene glycol) - block poly (ethylene glycol). *Biol Pharm Bull* 2003; 26:116-119.
30. Reddy HL, Murthy RSR. Pharmacokinetics and biodistribution studies of doxorubicin loaded poly (butyl cyanoacrylate) nanoparticles synthesized by two different techniques. *Biomed Pap* 2004; 148:161-166.
31. Narayanan S, Pavithran M, Viswanath A, Narayanan D, Mohan CC, Manzoor K. Sequentially releasing dual- drug-loaded PLGA-casein core/shell nanomedicine: Design, synthesis, biocompatibility and pharmacokinetics. *Acta Biomaterialia* 2014; 10:2112-2124.
32. Krishna R, Mayer LD. Multidrug resistance (MDR) in cancer-mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. *Eur J Cancer Sci* 2000; 11:265-283.
33. Derakhshandeh K, Nikmohammadi M, HosseinalizadehA. The effects of PLGA microparticles on intestinal absorption of P-glycoprotein substrate by the everted rat intestinal sac model. *Arch Pharm Res* 2011; 34:1989-1997.
34. Rassa G, Soddu E, Cossu M, Brundu A, Cerri G, Marchetti N, Ferraro L, Regan RF, Giunchedi P, Gavini E, Dalpiaz A. Solid microparticles based on chitosan or methyl- β -cyclodextrin: A first formulative approach to increase the nose-to-brain transport of deferroxamine mesylate. *J Control Release* 2015; 201: 68-77.
35. Marais E, amman J, du Plessis L, Lemmer R, Steenekamp J. Eudragit L100/N-Trimethyl chitosan Chloride microspheres for oral insulin delivery. *Molecules* 2013; 18:6734-6747.
36. Fasano A. Novel approaches for oral delivery of macromolecules. *J Pharm Sci* 1998; 87:1351-1356.
37. Booyesen LL, Kalombo L, Brooks E, Hansen R, Gilliland J, Gruppo V. *In vivo/in vitro* pharmacokinetic and pharmacodynamic study of spray-dried poly-(dl-lactic-co-glycolic) acid nanoparticles encapsulating rifampicin and isoniazid. *Int J Pharm* 2013; 444:10-17.