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Original Article

Preparation and optimization of polymeric micelles as an oral drug delivery system for deferoxamine mesylate: *in vitro* and *ex vivo* studies

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Abstract

Deferoxamine mesylate (DFO) is administered as a slow subcutaneous or intravenous infusion due to its poor oral bioavailability and lack of dose proportionality. The aim of the present study was to prepare and optimize polymeric micelles containing DFO, as an oral drug delivery system for increasing permeability and oral bioavailability. Based on a full factorial design with three variables in two levels, eight polymeric micelle formulations were made using film hydration method. Two polymers including 0.1% of carbomer 934 and Poloxamer® P 407 and two blends of surfactant + co-surfactant including 1 and 2 fold of critical micelle concentration of Labrafil® + Labrasol® and Tween 80 + Span 20 were used to prepare polymeric micelles. The effect of variables on particle size (PS), entrapment efficiency (EE), drug release, thermal behavior, *in vitro* iron bonding and *ex vivo* rat intestinal permeability were evaluated. The PS of polymeric micelles was less than 83 nm that showed 80% EE with continuous drug release pattern. The change in type of polymer from carbomer to Ploxamer® significantly increased drug release. All polymeric micelles increased the iron-bonding ability of DFO compared to control. This could be due to surfactants that can play an important role in this ability. Polymeric micelles increased drug permeability through intestine more than 2.5 folds compared to control mainly affected by polymer type. Optimized polymeric micelle consists of Tween 80 and Span 20 with 1.35 folds of critical micelle concentration and Poloxamer® demonstrated 97.32% iron bonding and a 3-fold increase in permeation through the rat intestine compared with control.

Keywords: Deferoxamine mesylate; Iron chelators; Oral bioavailability; Polymeric micelle; Thalassemia.

INTRODUCTION

Although iron is vital for oxygen transport, DNA synthesis, and energy metabolism, it also catalyzes dangerous reactions that produce free radicals (1). Two species of iron including ferrous (Fe^{+2}) and ferric (Fe^{+3}) are produced; the former is highly toxic and the latter is insoluble in physiological pH (2). In plasma, iron is transported by transferrin, which prevents free radical production and ensures that iron is available for metabolic processes (3). In normal condition, only 25-30% of transferrin is saturated with iron while in iron-overload resulting from blood transfusion in thalassemic patients, it becomes completely saturated leading to high iron concentration in the body (4,5). The acute and chronic iron overload lead to toxicity and affect multiple organs such as heart and liver that causes metabolic acidosis, depression of myocardial contractility and reduction in cardiac output and hepatotoxicity (6). Iron chelators are currently being used for their benefits in limiting iron-induced oxidative damage. Deferoxamine mesylate (DFO) is the best iron chelator in clinical use that binds with unbounded iron and finally is excreted in the feces and urine (7).

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However, administration of this drug is limited to the parenteral rout due to its poor absorption and an oral bioavailability less than 2%. Since DFO has a very short plasma half-life, repetitive subcutaneous infusion is needed to maintain effective therapeutic levels (8). To achieve desired treatment, it is normally required to deliver high doses of DFO via slow subcutaneous infusion, over prolonged periods (8-12 h), several days a week. This is evidently not ideal and is associated with poor compliance of patients (9). Oral delivery can be considered as a viable alternative method for improving the pharmacokinetics and toxicity profiles of DFO. However, the main challenge is poor membrane permeability and oral bioavailability of DFO (10,11). Recently, an orally active iron-chelating agent, deferiprone, has been introduced showing effectiveness in decreasing body iron. However, some patients have shown a life-threatening decrease in their white blood cells during deferiprone treatment (12). The development of a safe and orally active iron-chelator still remains a major challenge and is given number one priority in recommendations for research by the National Institute of Health and National Heart, Lung and Blood Institute (13). A few formulations have been used in order to improve DFO biopharmaceutical properties such as conjugation to hydroxyethyl starch (14) and hyperbranched polyglycerol (15) for increasing plasma half-life. However, strategies for increasing DFO permeability through intestinal membrane has not been introduced. It is possible to improve drug absorption by using nano-carriers that indirectly improve drug absorption. In addition, protective nano-carriers avoid drug degradation in the gastrointestinal (GI) tract enhancing oral absorption and bioavailability.

To conquer this issue, a few solutions have so far been suggested. Among these methodologies, polymeric micelles, constituted of amphiphilic block copolymers, have recently enticed more interest (16,17). Polymeric micelles are self-assembled core-shell nanostructures formed in an aqueous solution consisting of amphiphilic block copolymers (18,19). The core of polymeric micelle can entrap hydrophilic and hydrophobic compounds and charged macromolecules through electrostatic, hydrophobic, hydrogen bonding interactions (20) and the stereo complex formation (21). Therefore, DFO with 7 and 12 hydrogen bond donors and acceptor counts can interact with this core and load into the polymeric micelle.

For free soluble drugs, such as DFO, poor membrane permeability results in poor bioavailability and low drug efficacy. Polymeric micelles with small size can positively influence the bioavailability of DFO by increasing the membrane permeability (22). Interaction between polymeric micelles and phospholipid facilitate hydrophilic component incorporation into the biological membrane (23). Therefore, polymeric micelle can facilitate DFO oral absorption.

On the other hand, hydrophilic drugs that are susceptible to degradation in the GI and blood can be protected by encapsulation in the polymeric micelles. After oral administration, micelles are exposed to pH variation, bile salts, and digestive enzymes and can be destroyed. But loading drug could improve the stability of polymeric micelles by decreasing free energy of micellar dispersions (24). *In vitro* stability of micelles under different pH was studied (16). The results of this study indicated that micelles made by chitosan are stable maintaining a narrow PS distribution for 3 days under pH 7.5, 6.8, and 5.9.

The small size of polymeric micelles also helps to reduce mononuclear phagocyte system in the liver and bypassing the filtration of inter-endothelial cells in the spleen leading to longer blood circulation (25). On the other hand, endocytosis of the polymeric micelles and drug release in the blood stream is another reason for using polymeric micelles in the oral delivery of poor membrane permeable drugs, such as DFO (26). Therefore, the main aim of this study was designing and optimizing a polymeric micelle formulation as oral delivery system for

increasing the oral DFO permeability. DFO-loaded polymeric micelles can finally be incorporated in soft gel capsules.

MATERIALS AND METHODS

Materials

DFO was purchased from Jaber Ebne Hayyan Pharmaceutical Company (Tehran, I.R. Iran). Cholesterol, lecithin, oleic acid, carbomer 934, Poloxamer (Pluronic® P 407), Tween 80, and Span 20 were purchased from Sigma-Aldrich (USA). Labrafil® M1944, and Labrasol® were gifted from Gattefosse Company (France). Dialysis bag was acquired from the Armaghane Kalaye Gavan Co (I.R. Iran). All chemicals and solvents were of analytical grade. Freshly double distilled water was employed in the experiments. Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared based on United States Pharmacopeia (USP 29). SGF was made by dissolving 2 g of sodium chloride and 3.2 g of purified pepsin (purchased from Sigma-Aldrich, USA) in 7 mL of hydrochloric acid and sufficient water to make 1000 mL at pH 1.2. SIF was also prepared by dissolving 6.8 g of monobasic potassium phosphate in 250 mL of water, mixed and 77 mL of 0.2 N sodium hydroxide and 500 mL of water were added. Then, 10 g of pancreatin (purchased from Sigma-Aldrich, USA) was added and mixed and the pH was adjusted to 6.8 and diluted with water to 1000 mL.

Animals

Adult female Wistar rats (about 3 months of age) weighing 200 ± 9.8 g were obtained from the Laboratory Animal Care and Breeding Center of Ahvaz Jundishapur University of Medical Sciences (Ahvaz, I.R. Iran). Animals were kept for 5 days of acclimatization, given a standard diet and water, and treated according to the principles for the care and use of laboratory animals approved by the Ethical Committee of the Ahvaz Jundishapur University of Medical Sciences (Ahvaz, I.R. Iran). All rats sacrificed by intravenous ketamin injection and then animal intestines were removed completely and divided into three equal segments. All segments were then washed with cold ringer solution and their contents were removed. The guidelines followed were those laid down by the National Academy of Sciences published by the National Institutes of Health (Human services, Office of Laboratory Animal Welfare).

Deferoxamine mesylate assay

The quantitative determination of DFO was performed using a UV spectrophotometer Biochrom WPA BioWave II (England). The λ_{max} was set at 211 and 206 nm when the drug was dissolved in SGF and SIF, respectively. The assay was validated in terms of linearity, repeatability, accuracy, and limit of quantification (LOQ). Possible interferences of formulation's component on DFO assay were evaluated using blank samples.

Solubility studies

Solubility of DFO was determined in oleic acid, lecithin, castor oil and isopropyl myristate. An excess amount of DFO was added to 5 mL of the oil and stirred for 30 min at 45 °C, and for 24 h at room temperature (25 °C). The equilibrated samples were then was centrifuged at 8,000 rpm for 20 min to remove undissolved drug. The clear supernatant liquid then decanted and the concentration of DFO was determined using previously constructed calibration curve.

Determination of critical micelle concentration

Aqueous solutions of surfactant and co-surfactant with different concentrations but constant concentration of polymer were prepared. Surface tension of these solutions was measured at 25 °C with a Torsion balance (WHITE ELEC Model NO. 83944E). Then, the surface tension versus log concentration was plotted. There is a break in surface tension at a concentration associated with critical aggregation concentration (CAC), which corresponds to the onset of micelle formation on the polymer. With increasing surfactant concentration, a second break indicating critical micelle concentration (CMC) was observed.

Preparation of deferoxamine mesylate polymeric micelles

DFO-loaded polymeric micelles were prepared by film hydration method that previously reported by Zhang *et al.* (27). Lipophilic phase consisted of 1.5 g cholesterol, 1 g lecithin and 1.5 mL oleic acid were blended and dissolved in 10 mL chloroform and kept in a rotary evaporator at 120 rpm and 60 °C for 15 min to form a uniform lipid film. To remove residual amount of solvents, the films were placed in a vacuum oven at 40 °C, overnight. Then, dried lipid films were hydrated with aqueous solution containing DFO (5 mg/mL), surfactant, polymer and co-surfactant at 50 °C and 120 rpm and then sonicated in a bath sonicator (POWER SONIC 505, Korea) at 500 W at 25 °C for 5 min.

Optimization of polymeric micelles using full factorial design

A full-factorial design of three factors at two levels was used for optimization of the study. The correlation between independent variables and responses was evaluated using equation 1:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + \ldots\ldots \quad (1)$$

Where, Y is dependent variable (particle size (PS), entrapment efficiency (EE), drug release or drug permeability and X_1, X_2 and X_3 are surfactant type, surfactant concentration and polymer type respectively. The modeling was performed using Minitab 16 with stepwise linear regression technique with significant term $P < 0.05$. The Designed formulations of polymeric micelles are illustrated in Table 1.

Particle size

PS was measured at 25 °C by PS analyzer (SCATTER SCOPE 1 QUIDIX, South Korea) based on photon correlation spectroscopy with wide measurable size range (1-7000 nm). Each sample was measured in triplicate.

Percentage of entrapment efficiency

EE was determined by direct and indirect methods. In indirect method a polymeric micelle solution was added into centrifugal-ultrafiltration tubes (Microcon MWCO 3000, Millipore Co, USA) and centrifuged at 15,000 rpm for 25 min. Then, the amount of free DFO in the supernatant was measured by UV-V is at 206 nm. Then the amount of loaded DFO was calculated by subtracting the unloaded DFO from initial DFO added to the polymeric micelle. Percent of EE and loading capacity (LC) were then determined using equation 2 and 3. In direct method, the samples were centrifuged at 15,000 rpm for 25 min and nanoparticles were separated from the suspending medium and dissolved in methanol/water (70/30) to disrupt the polymeric micelle and amount of DFO was measured spectrophotometerically (28,29).

$$EE\ (\%) = \frac{\text{weight of DFO loaded}}{\text{weight of DFO initially added}} \times 100 \quad (2)$$

$$LC\ (\%) = \frac{\text{weight of DFO loaded}}{\text{weight of micelles}} \times 100 \quad (3)$$

In vitro release study

In vitro drug release studies were performed using the dialysis bag technique at the sufficiently sink condition as respected to DFO solubility. Polymeric micelle solutions including 10 mg DFO were placed in dialysis bags with acetate cellulose (Spectra/Por, molecular weight cut of 3000-4000 Da) membrane, tied and immersed into the 100 mL release medium stirred at 37 °C in basket dissolution apparatus. SGF (pH 1.2) and SIF (pH 6.8) were applied as release medium. At predetermined intervals 2 mL of sample was removed, filtered and DFO released amount determined by UV spectroscopy. Aqueous solution of DFO with the same concentration of the polymeric micelle solution was used as a control.

Scanning electron microscopy

Micelle morphology was studied by scanning electron microscopy (SEM, AIS-2100 SERON TECHNOLOGY, South Korea) and the samples were coated with gold prior to the measurements.

Thermal behavior of deferoxamine mesylate loaded polymeric micelles

The thermal behavior of polymeric micelles was performed using differential scanning calorimetry (DSC). Samples at first were heated to 50 °C and kept at this temperature for 5 min. Then, the temperature reduced to 0 °C at rate of 5 °C/min. The samples were kept at 0 °C for 5 min, and the temperature was then increased to 200 °C with the same rate. The possible incompatibility between drug and nanoparticles was also evaluated by measuring transition temperature and enthalpy.

Ex vivo permeation study through rat intestine

Male Wistar rats were sacrificed and their small intestines were excised and placed in the ice-cold bubbled ringer buffer (Carbogen, 95:5 O_2/CO_2). The jejunum 15-20 cm distal from the pyloric sphincter was removed and rinsed with ringer buffer. Two mL of polymeric micellar formulations containing defined amount of DFO was poured into the rat intestine and closed from both sides. Then tissue was kept in organ bath filled with 25 mL of phosphate buffer pH 7.4 with continuous aeration for 4 h at 37 °C. Two mL sample was taken at 0.5, 1, 2, 3, and 4 h from solution for spectrophotometric determination and replaced immediately with an equal volume of fresh solution. The same test was carried out for the solution of DFO in phosphate buffer pH 7.4 as a control. Percent of drug permeated after 4 h (P_4%) was calculated.

Evaluation of interference between deferoxamine mesylate and iron

DFO is a chelating agent, which forms complex with ferric ion. The complex formation constant is 10^{31}. The affinity of DFO for complex formation with divalent ions such as Fe^{+2} is lower (complex formation constant is 10^{14} or less) than trivalent ions. Chelating occurs on a 1:1 molar ratio, so that 1 g of DFO forms complex with 85 mg of ferric ion (30). Various $Fe(No_3)_3$. 9 H_2O concentrations at 0.01, 0.02, 0.04, and 0.08 g/mL were prepared in SGF, pH 1.2 and SIF, pH 6.8 as stomach and intestinal mimetic media. Then, DFO-loaded polymeric micelles in ratio of 100:8.5 were added to iron solutions thermostatically maintained at 20 °C. After 3 h, the mixture was treated with 10% sodium acetate to provide a pH 2-3 and 2% sulfosalicylic acid was added as indicator to form the violet-red complex. Then, the mixture was heated to 40-50 °C. According to the following equation, remaining iron, which was not incorporated with micelles, was measured by complexometeric titration with 1% ethylenediaminetetraacetic acid solution (EDTA). (31, 32).

$$M_1V_1 = M_2V_2 \quad (4)$$

where, M_1 is the concentration of iron bonded to EDTA, M_2 is a concentration of EDTA, V_1 is the volume of iron solution and V_2 is the volume of EDTA. The free DFO solution was used as the control.

Micelle stability in refrigerated temperature

DFO-loaded polymeric micelles were stored at 4 °C for 3 months and time-dependent changes in micelle size and distribution was evaluated. The chemical stability of DFO was studied by measuring drug content.

Stability in media simulating physiological conditions and effect of dilution

Micelle formation may be influenced by dilution in GI system. Therefore, the stability of polymeric micelles was assessed by dilution of formulations in SGF, which mimics GI tract condition. The fluid volume in the GI depends on fasted or fed state. In the fasted state, the total volume in the stomach and small intestine is approximately 130 mL (33). For this purpose, 1 mL DFO-loaded polymeric micelles incubated in 1 and 10 mL buffer phosphate, pH 7.4; SGF, pH 1.2; and SIF, pH 6.8 with and without bile salts (5 Mm) for 12 h at room temperature. (34). Then at defined time intervals polymeric micellar solutions were filtered through 0.2 µm membrane filter and PS as a sign of physical stability and DFO content as a sign of chemical stability were analyzed. DFO-loaded polymeric micelles without any incubation were used as control.

Statistical analyses

Data are presented as mean ± standard deviation (SD) of at least three experiments. Statistical significance was tested by two-tailed student's *t*-test and one-way ANOVA. Analyses were performed using Minitab® software. $P < 0.05$ was considered significant.

RESULTS

Validity of drug measurement method

The calibration curve for DFO was linear in the range of 0.001-0.3 mg/mL with a correlation coefficient (R^2) of 0.988, signifying that 98.8% of the absorbance values are estimated by the concentration. Regression analysis revealed a significant relationship between concentration and light absorbance (P = 0.001). The lack-of-fit in the current study was not significant (P = 0.115), which appears in the estimated absorbance changes. LOQ was approximately 0.0009 mg/mL. Within-day and between-day relative errors were within 0.1 to 1.2% and 0.4 to 1.8% and the coefficients of variation (CV%) ranged from 0.2 to 1.07% and 0.35 to 1.39%, respectively. The results of the assay validation revealed desired repeatability and accuracy of the method.

Oil solubility of deferoxamine mesylate

To identify a suitable oil phase, studies of solubility were conducted in various types of oils. DFO solubility in lecithin, oleic acid, castor oil, and isopropyl myristate as oil phases was 4.16 ± 0.2, 5.43 ± 0.3, 2.31 ± 0.6, and 1.89 ± 0.5 mg/mL, respectively. Oleic acid and lecithin amongst tested oils demonstrated the desired solubility.

Critical micelle concentration and critical aggregation concentration

Data on CMC and CAC for a mixture of surfactants and polymers are shown in Table 2. The results demonstrated micelle formation occurred at lower concentration with mixture of Tween 80 and Span 20 compared to the mixture of Labrafil® and Labrasol® in the presence of polymers. Same as CMC, the Labrafil® and Labrasol® blend has higher CAC in comparison with Tween and Span when Poloxamer® is used as a polymer. In the presence of carbomer, Tween and Span blend did not show measurable CAC. The CMC/CAC ratios for Tween+ Span and Labrafil® + Labrafil® in the presence of Poloxamer® are 1.2 and 1.8, respectively. Concentration of mixed surfactants used in formulations, was above the CMC of that mixture. Thus, it can be concluded that micelles are formed.

Particle size distribution

PS and polydispersity index (PDI) of different polymeric micellar drug delivery systems (PMDDS) are presented in Table 3. Through this experiment, the different sizes of particles were between 14 and 82 nm with PDI of less than 0.5. Based on the regression analysis, following equation was obtained which show effect of each variable on PS.

$$Y_1 = 40.07 - 5.171\ X_1 + 3.80\ X_2 + 3.25\ X_3 - 13.70\ X_1X_2 + 12.20\ X_1X_3 - 12.35\ X_2X_3 \quad (5)$$

where, Y_1 is PS, X1, X_2 and X_3 are surfactant type, surfactant concentration and polymer type, respectively. A summary of the statistical analyses for PS and other responses is shown in Table 4.

Entrapment efficiency

EE was between 55 and 81% and LC was between 4.5-6.1% considered good value for hydrophilic compound such as DFO. Equation 6 demonstrates the effect of each variable on the EE.

$$Y_2 = 66.61 - 2.20\ X_1 + 2.71\ X_2 + 4.62\ X_3 - 3.19\ X_1X_2 + 6.37\ X_1X_3 - 3.14\ X_2X_3 \quad (6)$$

where,Y_2 is EE and X_1, X_2 and X_3 are defined previously.

Drug release in simulated gastric fluid solution

The cumulative DFO release profiles in SGF are presented in Fig. 1.

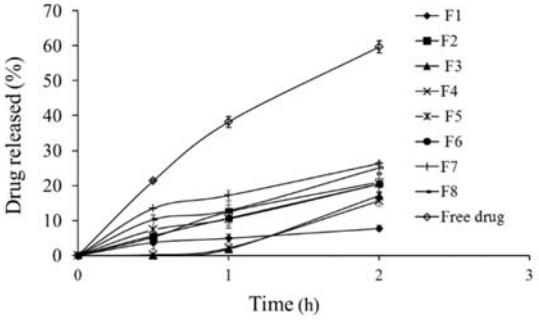

Fig. 1. Percent of drug released from all polymeric micelle formulations in simulated gastric fluid solution

Table 1. Compositions of different polymeric micellar drug delivery systems formulations using full factorial design.

Formulations	Surfactant type	Surfactant concentration	Polymer type	Cholesterol (g)	Oleic acid (mL)	Lecithin (mL)	Propylene glycol (%)
1	Labrafil® + Labrasol®	2 CMC	Carbomer	1.5	1.5	1	0.1
2	Labrafil® + Labrasol®	2 CMC	Poloxamer®	1.5	1.5	1	0.1
3	Labrafil® + Labrasol®	1 CMC	Carbomer	1.5	1.5	1	0.1
4	Tween® 80 + Span® 20	2 CMC	Carbomer	1.5	1.5	1	0.1
5	Tween® 80 + Span® 20	1 CMC	Poloxamer®	1.5	1.5	1	0.1
6	Tween® 80 + Span® 20	1 CMC	Carbomer	1.5	1.5	1	0.1
7	Tween® 80 + Span® 20	2 CMC	Poloxamer®	1.5	1.5	1	0.1
8	Labrafil® + Labrasol®	1 CMC	Poloxamer®	1.5	1.5	1	0.1

CMC, critical micelle concentration.

Table 2. Amounts of CAC and CMC for mixture of surfactants and polymers (mean ± SD, n = 3)

Mixture of surfactant and polymer	CAC (mg/mL)	CMC (mg/mL)
Labrafil® : Labrasol® (1:1) + 0.1% Carbomer	0.056 ± 0.004	0.077 ± 0.003
Labrafil® : Labrasol® (1:1) + 0.1% Poloxamer®	0.049 ± 0.006	0.085 ± 0.005
Tween® 80 : Span® 20 (1:1) + 0.1% Carbomer	-	0.019 ± 0.002
Tween® 80 : Span® 20 (1:1) + 0.1% poloxamer®	0.014 ± 0.002	0.017 ± 0.003

CAC, Critical aggregation concentration; CMC, critical micelle concentration.

Table 3. Physicochemical properties of different polymeric micelle formulations (mean ± SD, n = 3).

Formulations	PS (nm)	PDI	EE (%)	D_2 SGF (%)	D_{24} SIF (%)	P_4 (%)
1	75.4 ±5.21	0.48 ± 0.02	78.14 ± 5.12	7.71 ± 1.15	48.40 ± 1.41	37.10 ± 3.10
2	12.28 ± 0.40	0.46 ± 0.27	70.49 ± 3.81	21.00 ± 3.23	52.95 ± 6.62	52.20 ± 3.80
3	37.7 ± 2.3	0.36 ± 0.14	66.19 ± 6.78	17.10 ± 0.86	36.95 ± 1.38	26.70 ± 2.80
4	81.23 ± 0.5	0.48 ± 0.11	80.48 ± 6.94	15.62 ± 1.31	51.18 ± 1.00	29.50 ± 1.90
5	15.32 ± 1.32	0.39 ± 0.19	54.19 ± 5.21	20.45 ± 2.60	84.60 ± 4.08	42.50 ± 3.80
6	44.25 ±3.48	0.42 ± 0.14	60.14 ± 4.58	20.46 ± 2.06	39.96 ± 0.14	28.10 ± 3.10
7	40.15 ± 0.42	0.35 ± 0.12	62.83 ± 4.61	26.31 ± 1.49	68.09 ± 8.27	39.20 ± 2.90
8	14.2 ± 1.19	0.49 ± 0.15	60.44 ± 3.32	25.09 ± 0.60	72.15 ± 4.16	40.90 ± 1.70
Free drug	-	-	-	59.68 ± 3.81	99.9 ± 5.32	13.10 ± 1.40

Ps, Particle size; PDI, polydispersity index; EE, entrapment efficiency; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; P4, percent of drug permeated after 4 h.

Table 4. Summary of the statistical analyses of the responses generated by full-factorial design.

	Y_1		Y_2		Y_3		Y_4		Y_5	
	Coefficient	***P* value**	**Coefficient**	***P* value**	**Coefficient**	***P* value**	**Coefficient**	***P* value**	**Coefficient**	***P* value**
Intercept	40.07	0.001	66.61	0.0001	19.22	0.01	56.8	0.0001	33.76	0.001
X_1	-5.17	0.28	2.20	0.16	-1.49	0.928	-4.17	0.34	-1.48	0.75
X_2	3.80	0.04	2.71	0.01	-1.56	0.414	-11.53	0.70	6.16	0.22
X_3	3.25	0.01	4.62	0.02	-4.00	0.0406	-0.31	0.03	-3.41	0.05
X_1X_2	-13.70	0.38	-3.19	0.40	-1.81	0.2270	3.86	0.73	6.21	0.36
X_1X_3	12.20	0.41	6.37	0.20	-1.32	0.3000	-1.63	0.88	3.04	0.58
X_2X_3	-12.35	0.41	-3.14	0.40	-2.00	0.2072	1.50	0.89	-3.21	0.57
Lack of fit	-	0.6353	-	0.1521	-	0.2283	-	0.1658	-	0.6096

Y_1, Particle size; Y_2, entrapment efficiency; Y_3, release after 2 h in simulated gastric fluid;Y_4, release after 24 h in simulated intestinal fluid; Y_5, drug permeation through rat intestine after 4h.

Table 5. Measured and predicted physicochemical characteristics of optimized formulation (mean ± SD)

Independent variables				
Surfactant type	Tween®80 + Span®20			
Surfactant concentration	1.35 critical micelle concentration			
Polymer type	Poloxamer®			
Dependent variables	**Measured**	**Predicted**	***P* value**	**Error (%)**
Particle size (nm)	27.33 ± 2.01	29.46	0.25	-7.23
Entrapment efficiency (%)	65.45 ± 1.48	62.33	0.15	5.00
Drug permeated after 4 h (%)	45.95 ± 2.61	44.78	0.32	2.61
Iron-bonding (%)	97.32 ± 0.89	-	-	-

Drug release study in SGF was evaluated within 2 h because maximum residence time in the stomach is 2 h. The percentage of drug release after 2 h (D_2%) is shown in Table 3. Equations 7 demonstrates the effect of each variable on the obtained D_2% in SGF.

$$Y_3 = +19.22 - 1.49X_1 - 1.56X_2 - 4.00X_3 - 1.81X_1X_2 - 1.32X_1X_3 - 2.00X_2X_3 \quad (7)$$

where, Y_3 is D_2% in SGF and X_1, X_2 and X_3 are as defined previously.

Drug release in simulated intestinal fluid solution

The percentage of drug release profiles in SIF is presented in Fig. 2. Also, the percentages of drug release after 24 h (D_{24}%) in SIF solution are shown in Table 3. Equation 8 demonstrates the effect of each variable on the obtained D_{24}% in SIF.

$$Y_4 = 56.8 - 4.17\ X_1 - 11.53X_2 - 0.31X_3 + 3.86\ X_1X_2 - 1.63\ X_1X_3 + 1.5\ X_2X_3 \quad (8)$$

where, Y_4 is D_{24}% in SIF and X_1, X_2 and X_3 are as defined already.

Drug release kinetics

In order to evaluate the drug release kinetics, the release profiles were fitted into zero-order kinetics, first-order kinetics, Higuchi and Hixon-crowell models. We found that first order kinetics (R^2 = 0.975) and Higuchi (R^2 = 0.986) were the best models to define the release kinetics for DFO.

Scanning electron microscopy

SEM picture of optimized DFO-loaded polymeric micelles is shown in Fig. 3 illustrating micelles with uniform shape and size.

Differential scanning calorimetry

Thermograms of DFO pure powder, DFO-loaded polymeric micelles and blank polymeric micelles are shown in Fig. 4. DFO powder indicated one endothermic transition around 190 °C that disappeared in DFO-loaded polymer micelle thermogram. This implies that DFO is dissolved in formulation and thermotropic state of polymeric micelle was not changed.

Permeability study

The percentage of DFO permeated through rat intestine within 4 h (P_4%) from different formulations is given in Table 3. The effect of each variable on the obtained P_4% is demonstrated by the following equation:

$$Y_5 = 33.76 - 1.48\ X_1 + 6.16\ X_2 - 3.41\ X_3 + 6.21\ X_1X_2 + 3.04\ X_1X_3 - 3.21\ X_2X_3 \quad (9)$$

where, Y_5 is P_4 % and other Symbols are as defined previously.

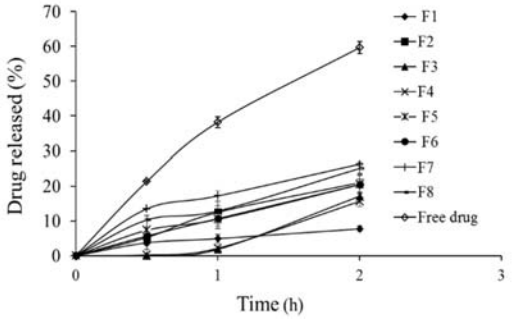

Fig. 1. Percent of drug released from all polymeric micelle formulations in simulated gastric fluid solution

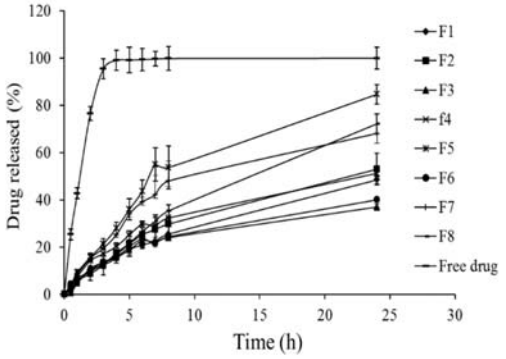

Fig. 2. Percent of drug released from all polymeric micelle formulations in simulated intestinal fluid solution

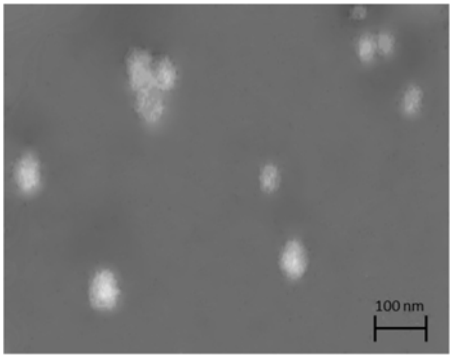

Fig. 3. Scanning electron microscopy of optimized polymeric micelles

Iron-bonding ability

The percentage of iron bonded to DFO-loaded polymeric micelles and DFO aqueous solution, is shown in Fig. 5. All polymeric micelles and controls showed DFO-iron bonding between 94.75 and 97.88%.

Micelle stability in refrigerated temperature

DFO-loaded polymeric micelles that were stored at 4 °C for 3 months indicated less than 5% increasing in PS that was not significant. Therefore, it seems that micelles have desirable stability.

Micelle stability in media mimicking physiological conditions

Results indicated that in SGF and SIF without bile salt, micelle size and drug content did not change significantly. But incubation in SGF/Bile salt and SIF/bile salt decreased micelle size by 12.58 % ± 0.95 and 9.89 % ± 0.55, respectively. Under these circumstances drug content did not change significantly indicating DFO-loaded polymeric micelles was stable.

Optimization was performed with Minitab 16 software to find the level of independent variables that would obtain a minimum value of PS and maximum value of EE. Optimized formulation with desirability factor 0.974 was prepared and the PS, EE%, P_4 % and its iron bonding ability were measured. The differences between actual and predicted values of dependent variables were evaluated by *t*-test and *P*-values are reported in Table 5. Based on the results, at 5% significance level, there was no significant difference between the actual and predicted values ($P > 0.05$).

DISCUSSION

The present research designed to prepare DFO-loaded polymeric micelles for increasing oral absorption of DFO. When a surfactant is added to a dilute polymer solution, a micelle-like aggregation is formed at CAC that depends on the nature of the surfactant and polymer. The CAC is always lower than CMC by a factor between 3 and 10 for simple system and 10-1000 for a complex long chain polymer. In the presence of a polymer, the CMC of the surfactant mixture may be affected by the interaction between surfactant and polymer.

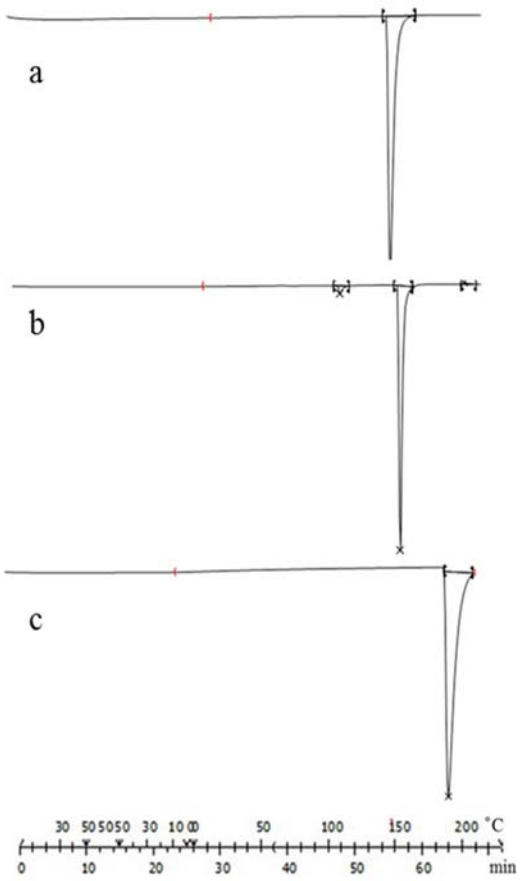

Fig. 4. Differential scanning calorimetry thermograms of (a) blank micelle, (b) deferoxamine-loaded polymeric micelle, and (c) deferoxamine powder.

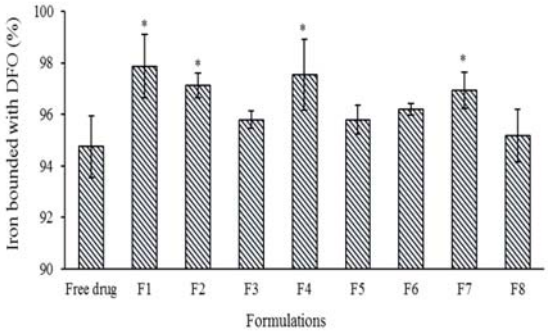

Fig. 5. Percent of iron bounded with DFO-loaded polymeric micelles and free drug solution. *$P < 0.05$ compared with free drug. DFO, Deferoxamine.

Table 5. Measured and predicted physicochemical characteristics of optimized formulation (mean ± SD)

Independent variables				
Surfactant type	Tween®80 + Span®20			
Surfactant concentration	1.35 critical micelle concentration			
Polymer type	Poloxamer®			
Dependent variables	**Measured**	**Predicted**	***P* value**	**Error (%)**
Particle size (nm)	27.33 ± 2.01	29.46	0.25	-7.23
Entrapment efficiency (%)	65.45 ± 1.48	62.33	0.15	5.00
Drug permeated after 4 h (%)	45.95 ± 2.61	44.78	0.32	2.61
Iron-bonding (%)	97.32 ± 0.89	-	-	-

At CAC, polymer chains become saturated with surfactants and more surfactant-polymer interaction tendencies occur at higher CAC. According to the findings of the present study, Labrafil® and Labrasol® blend showed higher CAC and CMC compared with Tween 80 and Span 20 due to higher tendencies of Labrafil® and Labrasol® for interaction with polymers leading to micellar formation at higher concentrations (35). In the current study, lecithin and cholesterol were used to serve as lipid core for polymeric micelle. Addition of cholesterol and lecithin decreased CMC value and particle size while promoted stability of the micelles. In a previous study, docetaxel-loaded lecithin-stabilized micellar drug delivery system exhibited good stability with particle size of below 200 nm (36). Emami *et al* also reported that the absence of lecithin in the oil phase of nanostructured lipid carriers caused an increase in particle size probably due to the increase of micelle core viscosity (37). Transition from micelles to vesicles may occur in the mixture of surfactant and phospholipid in a region where micelles and vesicles coexist. However, vesicles do not appear when the concentration of surfactant is higher than CMC, which is the case in our study. Incorporation of a small amount of cholesterol does not affect this transition (38).

PS is one of the most effective characteristics on drug release and permeation. In this study, polymeric micelles with PS less than 81 nm provide sufficient areas for efficient oral absorption. In the previous studies DFO-loaded PLGA (39) and poly (lactide-co-glycolide)-poly (ethylene glycol)-poly (lactide-co-glycolide) (40) nanoparticles showed mean PS of 220 and 116 nm, respectively. According to the results, the most effective factors on the PS relate to polymer type and surfactant concentration. Larger PS was resulted when higher concentrations of surfactant were used in the aqueous phase resulting in higher viscosity. This finding is in accordance with some earlier studies (41,37). Furthermore, the micelles prepared using carbomer showed larger particle size which could be attributed to the ionization of acrylate group of carbomer in aqueous medium, polymer swelling and increasing viscosity which in turn led to larger particles (42). On the other hand, the incorporation of poloxamer in the micelles significantly reduced PS. Hydrophobic propylene oxide domain of poloxamer could be adsorbed to the lipid core of the micelle and hydrophilic ethylene oxide domain produce protective hydrophilic shield preventing particle agglomeration. Similar results have been reported in previous studies (43,37).

As shown in table 4, EE was affected significantly by surfactant concentration and polymer type. Higher EE was observed when higher concentrations of surfactant and carbomer were used. PS increasing leading to more drug accommodation in the micelles. Carbomer exhibited high viscosity at low concentration in aqueous medium due to the hydration of its acrylate group which caused faster micelle formation preventing drug escape from the micelle leading to more EE (44).

Sustained drug release is another feature that is very important for DFO delivery systems. This increases DFO residence time and reduces dosing frequency. DFO-loaded micelles prepared in this study significantly decreased release rate of DFO in comparison with free drug which is more favorable than some DFO-loaded nanoparticles such as poly(ε-caprolactone)-

bloack-poly(propylene adipate) that showed 100% DFO release during 12 h (45). Based on regression analysis, in SGF, the correlation between polymer type and D_2% was significant and polymeric micellar drug delivery systems containing poloxamer® showed higher D_2%. Similarly, in SIF, significant correlations between D_{24}% and polymer type was observed ($P < 0.05$). In this manner, formulations containing Poloxamer® indicated higher drug release rate, which might be due to smaller size of the poloxamer-containing micelles leading to increased specific surface area and drug release rate. These results are in accordance with the results reported by Varshosaz *et al*. (46) which showed Pluronic® accelerated the drug release from liquid crystal formulations and Emami *et al* who reported that the presence of poloxamer in solid lipid nanoparticles caused an increase in drug release rate due to smaller particle size (47). In addition, as mentioned before, carbomer-containing micelles demonstrated higher viscosity compared to poloxamer-incorporating micelles which could decrease drug diffusion from the micelle to dissolution medium. Comparison between both media showed that percentage of drug release was not affected by pH of the medium.

The release kinetics of the micellar formulations were best fitted to the Higuchi model. Release exponents calculated using Pepas equation was equal or less than 0.5 in all formulations, which indicate DFO release is mainly controlled by drug diffusion.

All polymeric micelles exhibited an increase in P_4% and even some formulations demonstrated more than 3 folds in permeability compared to the control. The maximum value for permeability was observed for formulation 8, which corresponds to 3.32 folds higher than free DFO. According to the results, there was a significant relationship between polymer type and drug permeation, such that polymeric micelles containing Poloxamer® demonstrated higher DFO permeability. Previous studies have shown that poloxamer enhanced oral absorption and bioavailability of hydrophilic and hydrophobic drugs (48). Ying *et al.* reported that poloxamer was effectively promoted intranasal absorption of the drug possibly due to permeation of poloxamer into lipid membranes changing the lateral packing density of membranes leading to enhanced drug absorption (49). Conversely, Fischer *et al* reported no apparent increasing effect of poloxamer on ketoprofen intestinal absorption (50). However, PS may affect permeability. It is generally assumed that the membrane absorption is inversely proportional to particle size, and most published data support this hypothesis (51). The micelle containing poloxamer exhibited smaller size than those of containing carbomer which could be a reason for increased permeability.

Effective iron chelation is achieved if iron chelators can remove equal or greater amounts of iron accumulated in the body due to transfusion. This needs chelators to be able to reach the target site at appropriate concentration (52). DFO-loaded polymeric micelles demonstrated good iron-binding capacity. The highest iron-bonding ability demonstrated by formulation 1, which was about 97.9%. Not all formulation with surfactant concentration equal to CMC showed significant differences in iron bonding ability compared to free drug. A slight increase in surfactant concentration could lead to more micelle formation causing higher DFO loading and iron-binding capacity. On the other hand, increasing in surfactant concentration increased DFO entrapment, which leads to improvement of iron bonding ability. However, surfactant concentrations greater than 1.5 fold of CMC led to micelle aggregation with lower DFO loading or iron-binding capacity. DFO-loaded polymeric micelles demonstrated good stability leading to DFO protection against degradation in the SGF and SIF. CMC less than 0.135 mg/mL is resistant to rapid dissociation upon dilution in GI tract following oral administration of polymeric micelles (16). Therefore, Polymeric micelles prepared in the present study with CMC around 0.017 mg/mL demonstrate resistance to dissociation in GI tract. However, CMC per se is not adequate to ensure polymeric micelle stability in GI tract and other factors might be involved.

Francis *et al.* examined cyclosporine release from micelles in SGF and SIF and found that in both fluids, the drug release reached a plateau within 4 h with less than 12% of drug release (51). They concluded that this phenomenon was due to good micelle integrity under this condition. In our study, similar experiment was performed and results showed that D_4% in SIF and SGF was less than 20%; this may indicate polymeric micelles are able to resist dissociation in GI tract. Different approaches have been previously used for improving DFO bioavailability by conjugation of DFO to different polymer backbone such as PEG methacrylate (53), hyperbranched polyglyceroles (15), hydroxamic acid (54), 3-hydroxypyridine (55), and dextran (14). Polomoscanik *et al*. produced a non-toxic DFO hydroxamic acid-based iron-chelating hydrogels and evaluated its ability to prevent iron absorption in the GI tract (56). These gels were effective in preventing gastric iron absorption where Zn and Cu did compete with iron moderately. In the present study, the ability of DFO-loaded for Zn and Cu binding was not evaluated and it should be performed in future studies. Present research demonstrated a novel DFO-loaded polymeric micelle that is not only stable and easily forming but increases DFO permeability through intestine membrane.

CONCLUSIONS

DFO as an iron chelator demonstrates poor membrane permeability and low oral bioavailability and for this reason, it is administered as a slow subcutaneous or intravenous infusion. The main aim of this study was to design and optimize a polymeric micelle formulation for increasing oral DFO absorption. The results revealed that all polymeric micelles increased permeability through the rat intestine compared to control. Polymeric micelles with low PS provided sufficient area for oral absorption and iron binding. Majority of polymeric micelles did not decrease iron-binding ability compared to DFO aqueous solution. Polymeric micelles established perfect carrier for DFO encapsulation and protection against degradation that was proved by stability study in acidic and alkaline conditions. Polymeric micelles showed a sustained release pattern that is a good feature for reducing the frequency of DFO dosing. In conclusion, optimized polymeric micelle including Tween and Span with 1.35 CMC and Poloxamer® indicated perfect intestine permeation and iron-binding ability, which might be a suitable system for oral DFO administration.

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REFERENCES

1. Wang J, Pantopoulos K. Regulation of cellular iron metabolism. Biochem J. 2011;434(3):365-381.
2. Aisen P, Enns C, Wessling-Resnick M. Chemistry and biology of eukaryotic iron metabolism. Int J Biochem Cell Biol. 2001;33(10):940-959.
3. Franzer DM, Anderson GJ. The regulation of iron transport. Biofactors. 2014;40(2):206-214.
4. Hoffbrand AV, Taher A, Capellini MD. How I treat transfusional iron overload. Blood. 2012;120(18):3657-3669.
5. Tygi P, Kumar Y, Gupta D, Singh H, Kumar A. Therapeutic advancements in management of iron overload-A review. Int J Pharm Sci. 2015;7(8): 35-44.
6. Porter JB, Garbowski M. The pathophysiology of transfutional iron overload. Hematol Oncol Clin North Am. 2014;28(4):683-701.
7. Brittenham GM. Iron-Chelating therapy for transfutional iron overload. N Engl J Med. 2011;364(2):146-156.
8. Delea TE, Edelsberg J, Sofrygin O, Thomas SK, Baladi JF, Phatak PD, *et al*. Consequences and costs of noncompliance with iron chelation therapy in patients with transfusion-dependent thalessemia: a literature review. Transfusion. 2007;47(10): 1919-1929.
9. Schnebli HP, Hassan I, Hamilton KO, Lynch S, Jin Y, Nick HP, *et al*. Toward Better Chelation Therapy: Current Concepts and Research Strategy.

In: Bergeron RJ, Brittenham GM, editors. The Development of Iron Chelators for Clinical Use. Boca Raton: CRC Press; 1994. pp. 131-149.
10. Ihnat PML, Vennerstrom JL, Robinson DH. Synthesis and solution properties of deferoxamine amides. J Pharm Sci. 2000;89(12):1525-1536.
11. Daugherty AL, Mrsny RJ. Regulation of the intestinal epithelial paracellular barrier. Pharm Sci Technolo Today. 1999;2(7):281-287.
12. Ensign LM, Cone R, Hanes J. Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers. Adv Drug Deliv Rev. 2012;64(6):557-570.
13. Neufeld EJ. Oral chelators defevasirox and deferiprone fpr transfusional iron overload in the thalassemia major: new data, new questions. Blood. 2006; 107(9): 3436-3441.
14. Hallaway PE, Eaton JW, Panter SS, Hedlund BE. Modulation of deferoxamine toxicity and clearance by covalent attachment to biocompatible polymers. Proc Natl Acad Sci U S A. 1989;86(24): 10108-10112.
15. Imran ul-hag M, Hamilton JL, Lai BF, Shenoi RA, Horte S, Constantinescu I, *et al*. Design of long circulating nontoxic dendritic polymers for the removal of iron *in vivo*. ACS Nano. 2013;7(12):10704-10716.
16. Lu Y, Park K. Polymeric micelles and alternative nanonized delivery vehicles for poorly soluble drugs. Int J Pharm. 2013;453(1):198-214.
17. Wu C, Ying A, Ren S. Fabrication of polymeric micelles with core-shell-corona structure for application in controlled drug release. Colloid Polym Sci. 2013;291(4);827-834.
18. Bagheri M, Bigdeli E, Pourmoazzen Z. Self-assembled micellar nanoparticles of a novel amphiphilic cholestryl-poly(L-lactic acid)-b-poly (poly(ethylene glycol)methacrylate) block-brush copolymer. Iran Polym J. 2013;22(4):293-302.
19. Vlassi E, Papagiannopoulos A, Pispas S. Amphiphilic poly(2-oxazoline) copolymers as self-assembled carriers for drug delivery applications. Eur Polym J. 2017;88:516-523.
20. Zhang J, Ma PX. Polymeric core-shell assemblies mediated by host-guest interactions: versatile nanocarriers for drug delivery. Angew Chem Int Ed Engl. 2009;48(5):964-968.
21. Smeets NMB. Amphiphilic hyperbranched polymers from the copolymerization of a vinyl and divinyl monomer: The potential of catalytic chain transfer polymerization. Eur Polym J. 2013;49(9): 2528-2544.
22. Kang N, Perron ME, Prud'homme RE, Zhang Y, Gaucher G, Leroux JC. Stereocomplex block copolymer micelles: core-shell nanostructures with enhanced stability. Nano Lett. 2005;5(2):315-319.
23. Srinivas G, Mohan RV, Kelkar AD. Polymer micelle assisted transport and delivery of model hydrophilic components inside a biological lipid vesicle: a coarse-grain simulation study. J Phys Chem B. 2013;117(40):12095-12104.
24. Torchilin VP. Structure and design of polymeric surfactant based drug delivery systems. J Control Release. 2001;73(2-3):137-172.
25. Simões SM, Figueiras AR, Veiga F, Concheiro A, Alvarez-Lorenzo C. Polymeric micelles for oral drug administration enabling locoregional and systemic treatments. Expert Opin Drug Deliv. 2015;12(2):297-318.
26. Moazeni E, Gilani K, Rouholamini Najafabadi A, Rouini MR, Mohajel N, Amini M, *et al*. Preparation and evaluation of inhalable itraconazole chitosan based polymeric micelles. Daru. 2012;20(1):85-94.
27. Zhang X, Jackson JK, Burt HM. Development of amphiphilic diblock copolymers as micellar carriers of taxol. Int J Pharm. 1996;132(1-2):195-206.
28. Cao XT, Kim YH, Park JM, Lim KT. One-pot syntheses of dual-responsive core cross-linked polymeric micelles and coavalently entrapped drug by click chemistry. Eur Polym J. 2016;78:264-273.
29. Shahin M, Safaei-Nikouei N, Lavasanifar A. Polymeric micelles for pH-responsive delivery of cisplatin. J Drug Target. 2014;22(7):629-637.
30. Davis BA, Porter JB. Results of long term iron chelation treatment with deferoxamine. Adv Exp Med Biol. 2002;509:91-125.
31. Burke A, Yilmaz E, Hasirci N. Evaluation of chitosan as a potential medical iron (III) ion adsorbent. Turk J Med Sci. 2000;30(4):341-348.
32. Bolton S. Chelatometric determination of ferrous iron with 2-pyridinealdoxime as an indicator. J Pharm Sci. 1963;52(9):858-860.
33. Schiller C, Frohlich CP, Giessmann T, Siegmund W, Monnikes H, Hostern N, *et al*. Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging. Aliment Pharmacol Ther. 2005;22(10):971-977.
34. Dabholkar RD, Sawant RM, Mongayt DA, Devarajan PV, Torchilin VP. Polyethylene glycol-phosphatidylethanolamine conjugate (PEG-PE)-based mixed micelles: some properties, loading with paclitaxel, and modulation of P-glycoprotein-mediated efflux. Int J Pharm. 2006;315(1-2): 148-157.
35. dos Santos S, Medronho B, dos Santos T, Antunes FE. Amphiphilic Molecules in Drug Delivery Systems. In: Coelho J, editor. Drug Delivery Systems: Advanced Technologies Potentially Applicable in Personalised Treatment. Dordrechet: Springer; 2013. pp. 35-87.
36. Su CY, Liu JJ, Ho YS, Huang YY, Chang VH, Liu DZ, *et al*. Development and characterization of docetaxel-loaded lecithin-stabilized micellar drug delivery system (LsbMDDs) for improving the therapeutic efficacy and reducing systemic toxicity. Eur J Pharm Biopharm. 2018;123:9-19.
37. Emami J, Rezazadeh M, Sadeghi H, Khadivar K. Development and optimization of transferrin-conjugated nanostructured lipid carriers for brain

delivery of paclitaxel using Box-Behnken design. Pharm Dev Technol. 2017;22(3):370-382.
38. Long MA, Kaler EW, Lee SP. Structural characterization of the micelle-vesicle transition in lecithin- bile salt solutions. Biophys J. 1994;67(4):1733-1742.
39. Vignesh S, Annapoorna M, R J, Subramania I, Shantikumar VN, *et al*. Injectable deferoxamine nanoparticles loaded chitosan-hyaluronic acid coacervate hydrogel for therapeutic angiogenesis. Colloids Surf B Biointerfaces. 2018;161:129-138.
40. Qiu M, Wang C, Chen D, Shen C, Zhao H, He Y. Angiogenic and osteogenic coupling effects of DFO-loaded poly(lactide-c0-glycolide)-poly(lactide-co-glycolide) nanoparticles. Appl Sci. 2016;6(10):290-304.
41. Zweers ML, Grijpma DW, Engbers GH, Feijen J. The preparation of monodisperse biodegradable polyester nanoparticles with a controlled size. J Biomed Mater Res B Appl Biomater. 2003;66(2):559-566.
42. Carlos Bregni, Diego Chiappetta, Natalia Faiden, Adriana Carlucci, Roberto García And Ricardoc Pasquali. Release study of diclofenac from new carbomer gels. Pak. J. Pharm. Sci., Vo.21, No.1, January 2008, pp.12-16.
43. Muller RH, Radtke M, Wissing SA. Solid lipid nanoparticles and nanostructured lipid carriers in cosmetic and dermatological preparations. Adv Drug Deliv Rev. 2002;54:S131-S155.
44. Taş C, Ozkan Y, Savaşer A, Baykara T. *In vitro* and *ex vivo* permeation studies of chlorpheniramine maleate gels prepared by carbomer derivatives. Drug Dev Ind Pharm. 2004;30(6):637-647.
45. Nanki SG, Pantopoulos K, Bikiaris DN. Synthesis of biocompatible poly(ε-caprolactone)-block-poly(propylene adipate) copolymers appropriate for drug nanoencapsulation in the form of core-shell nanoparticles. Int J Nanomedicine. 2011;6:2981-2995.
46. Kazemi M, Varshosaz J, Tabbakhian M. Preparation and evaluation of lipid-based liquid crystalline formulation of fenofibrate. Adv Biomed Res. 2018;7:126.
47. Emami J, Mohiti H, Hamishehkar H, Varshosaz J. Formulation and optimization of solid lipid nanoparticle formulation for pulmonary delivery of budesonide using Taguchi and Box-Behnken design. Res Pharm Sci. 2015;10(1):17-33.
48. Lai J, Lu Y, Yin Z, Hu F, Wu W. Pharmacokinetics and enhanced oral bioavailability in beagle dogs of cyclosporine A encapsulated in glyceryl monooleate/poloxamer 407 cubic nanoparticles. Int J Nanomedicine. 2010;2;5:13-23.
49. Li Y, Li J, Zhang X, Ding J, Mao S. Non-ionic surfactants as novel intranasal absorption enhancers: *in vitro* and *in vivo* characterization. Drug Deliv. 2016;23(7):2272-2279.
50. Fischer SM, Parmentier J, Buckley ST, *Reimold I, Brandl M, Fricker G*. Oral bioavailability of ketoprofen in suspension and solution formulations in rats: the influence of poloxamer 188. J Pharm Pharmacol. 2012;64(11):1631-1637.
51. Francis MF, Cristea M, Yang Y, Winnik FM. Engineering polysaccharide-based polymeric micelles to enhance permeability of cyclosporin A across Caco-2 cells. Pharm Res. 2005;22(2):209-219.
52. Hamilton JL, Kizhakkedathu JN. Polymeric nanocarriers for the treatment of systemic iron overload. Mol Cell Ther. 2015;3:3-17.
53. Rossi NA, Mustafa I, Jackson JK, Burt HM, Horte SA, Scott MD, *et al*. *In vitro* chelating, cytotoxicity, and blood compatibility of degradable poly(ethylene glycol)-based macromolecular iron chelators. Biomaterials. 2009;30(4):638-648.
54. Winston A, Varaprasad DV, Metterville JJ, Rosenkratz H. Evaluation of polymeric hydroxamic acid iron chelators for treatment of iron overload. J Pharmacol Exp Ther. 1985;232(3):644-649.
55. Zhou T, Kang XL, Liu ZD, Liu DY, Hider RC. Synthesis and iron (III)-chelating properties of novel 3-hydroxypyridin-4-one hexadentate ligand-containing copolymers. Biomacromolecules. 2008;9(5):1372-1380.
56. Polomoscanik SC, Cannon CP, Neenan TX, Holmes-Farley SR, Manderille WH, Dhal PK. Hydroxamic acid-containing hydrogels for nanoabsorbed iron chelation therapy; synthesis, characterization and biological evaluation. Biomacromolecules. 2005;6(6):2946-2953.