جهت مشاهده تمام تغییرات اعمال شده، ابتدا روی گزینه Review کلیک کنید و سپس گزینه Simple Markup را به All Markup تغییر دهید.

جهت مشاهده بدون نقص فایل، از نرم افزار ورد ورژن ۲۰۱۶ یا ۲۰۱۳ استفاده شود.

**Abstract**

**Objective:** Deferoxamine mesylate has poor permeability, and thus has poor oral bioavailability (less than 2%) and lacks dose proportionality. It is administered as a slow subcutaneous or intravenous infusion due to its poor bioavailability. Polymeric micelles are excellent potential drug delivery systems used to increase permeability and oral bioavailability of drugs. The aim of this study was to prepare and optimize polymeric micelles containing a hydrophilic drug, deferoxamine mesylate, as an oral drug delivery system.

**Methods:** Full factorial design with three variables including type of surfactant, surfactant concentration, and type of polymer in two levels was applied as an experimental study design. The effect of variables on formulation characteristics such as particle size, entrapment efficiency, drug release, thermal behavior, *in vitro* iron bonding, and *ex vivo* rat intestinal permeability was evaluated.

**Results:** Polymeric micelles showed 80% entrapment efficiency and particle size less than 83 nm, with a continuous drug release pattern. The change in the type of polymer from carbomer to ploxamer significantly increased drug release. All polymeric micelles increased the iron bonding ability of deferoxamine mesylate compared to the control, suggesting that surfactants play an important role in this ability. Polymeric micelles increased drug permeability more than 2.5-fold through the intestine compared to the control, which is mainly affected by polymer type.

**Conclusion:** The optimized polymeric micelle formula consists of Tween and Span with 1.35 critical micelle concentration and poloxamer that demonstrated 97.32% iron bonding and a 3-fold increase in deferoxamine mesylate permeation through the rat intestine compared with the control.

Key words: Deferoxamine mesylate, Polymeric micelle, Oral bioavailability, Optimization technique, Iron chelators

**Introduction**

Iron is essential for oxygen transport, DNA synthesis, and energy metabolism but it can also catalyze dangerous reactions that produce free radicals [1]. Two types of iron include ferrous (Fe+2)and ferric (Fe+3) forms. Ferrous iron is highly toxic and ferric iron is insoluble at physiological pH [2]. In the plasma, iron is transported by transferrin that prevents free radical production and ensures that iron is available for metabolic processes [3]. Transferrin is only 25-30% saturated under normal conditions, whereas transferrin can become completely saturated under conditions of iron overload resulting from blood transfusions required by thalassemia or other transfusion-dependent anemic patients that leads to dangerously high iron concentrations in the body [4,5]. Acute and chronic iron overload leads to toxicity and affects multiple organs such as mucous membranes of the gastrointestinal tract, heart, and liver causing metabolic acidosis, depression of myocardial contractility, reduction in cardiac output, and hepatotoxicity [6]. Iron chelation therapy is an effective treatment for iron overload and involves the use of molecules that bind with excess iron under physiological conditions to facilitate excretion via the feces and urine [7]. Deferoxamine mesylate (DFO) is a heavy metal antagonist that is used as an iron chelator for treatment of acute iron intoxication resulting from transfusion requiring anemic conditions like thalassemia and chronic iron overload. DFO acts by binding free iron in the bloodstream and enhancing its elimination in the [urine](http://en.wikipedia.org/wiki/Urine). DFO is rapidly absorbed after intramuscular bolus injection or slow subcutaneous infusion, but only poorly absorbed through the gastrointestinal tract in the presence of intact mucosa. The oral bioavailability of DFO is less than 2%; therefore, it is administered as a slow subcutaneous or intravenous infusion due to its poor bioavailability and short plasma half-life that causes neurotoxicity and swelling at the infusion site [8]. To achieve the optimal treatment, it is normally necessary to deliver high doses of DFO via slow subcutaneous infusion, over prolonged periods (8–12 h), several days a week. This is obviously not an ideal and compliant situation for the patients [9]. Oral delivery can be considered as a viable alternative for improving the pharmacokinetics and toxicity profile of DFO. The main challenge of DFO is poor membrane permeability and oral bioavailability, which is due to its low octanol/water partition coefficient (log p = -0.614) and high aqueous solubility [[10](#_ENREF_3)]. Typically, hydrophilic molecules such as DFO cannot freely diffuse through the intestinal membrane, due to their low affinity for lipid constituents [[11](#_ENREF_4)]. Recently, an orally active iron-chelating agent deferiprone has been introduced and shown to be effective at decreasing body iron. However, some patients experience a life-threatening drop in white blood cells during deferiprone treatment [12]. Development of a safe and orally active iron-chelator still remains a major problem and has been given high priority in recommendations for research by the National Institutes of Health - National Heart, Lung and Blood Institute [13]. It is possible to improve drug absorption by using permeation enhancers, but these agents are associated with a risk of toxicity by permitting the entry of unwanted pathogens through a leaky epithelium. As a consequence, the use of alternative approaches that indirectly improve drug absorption is generally preferred. Hence, protective carriers are able to avoid drug degradation in the gastrointestinal tract and can enhance oral absorption and bioavailability. In this context, polymeric micelles may play a very relevant role ([14-18](#_ENREF_5)). As previously mentioned, the main problem that hinders the utility of DFO is its poor oral bioavailability that requires it to be administered parenterally, thus increasing the cost of treatment and leading to poor patient compliance. [8]

In order to overcome this problem, several strategies such as micronization [[19](#_ENREF_11)], formation of solid solutions, micro emulsification, and novel drug delivery systems including nanoparticles and lipid-based vesicles have been proposed [20]. Among these approaches, polymeric micelles, consisting of amphiphilic block copolymers, have attracted much attention in past the decade [21].

Nanosized drug carriers have, as their name implies, nanoscale dimensions (typically 10-200 nm) and can be categorized into particulate systems and water-soluble macromolecular systems. The first category includes lipid based systems such as liposomes [22,23], systems based on surfactants such as emulsions [24,25], and systems based on synthetic polymers such as nanoparticles [26,27], polymeric micellar drug delivery systems (PMDDS) [28,29], and polymeric vesicles [30,31]. Polymeric micelles are self-assembled core–shell nanostructures formed in an aqueous solution consisting of amphiphilic block copolymers [32.33]. Typically, the hydrophobic blocks of the copolymers form the core of the micelles through hydrophobic interactions, although other interactions such as electrostatic interactions [34] and stereocomplex formation [35] can also be utilized as the driving force for core formation. The hydrophilic blocks of the copolymers form the shell of the micelles and stabilize the micellar structure.

For free soluble drugs, such as DFO, poor membrane permeability leads to poor bioavailability and low drug efficacy. Absorption through the oral route is also poor, resulting in failure to provide effective plasma drug concentrations through conventional oral administration. In this regard, polymeric micelles can positively impact bioavailability of DFO by increasing membrane permeability.

On the other hand, hydrophilic drugs that are susceptible to degradation in the gastrointestinal tract and blood can be protected by encapsulation in the polymeric micelles. In addition, drug-loaded polymeric micelles are small in size (~100 nm), favoring transport across the intestinal epithelium. [36] After oral administration, micelles are exposed to variations in pH, bile salts, and digestive enzymes that can destroy the micelles. Although, drug-loading could improve the stability of polymeric micelles by decreasing free energy of micellar dispersion [37]. Generally, critical micelle concentration (CMC) values less than 135 mg/ml denote resistance to dissociation by dilution in orally administered polymeric micelles [38]. However, CMC alone is not enough to estimate polymeric micellar stability within the gastrointestinal tract. Most *in vitro* studies involve investigating drug release from micelles in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) [39]. *In vitro* stability of micelles under different pH conditions was previously evaluated [40]. The results of this study indicated that micelles made with chitosan are stable and maintain a narrow particle size distribution for 3 days at pH 7.5, 6.8, and 5.9.

The small size of polymeric micelles also contributes to prolonged blood circulation through avoiding scavenging by the mononuclear phagocyte system in the liver and bypassing filtration of inter-endothelial cells in the spleen. In addition, encapsulation of drug inside the core of polymeric micelle may protect against rapid clearance from the circulation, which can lead to reduced amounts of drug available for absorption [41]. On the other hand, endocytosis of the polymeric micelles and drug release in the blood stream is another reason for using polymeric micelles for oral delivery of poor membrane permeable drugs such as DFO [36]. Therefore, the main aim of this study was to design and optimize a polymeric micelle formulation as an oral delivery system for increasing oral DFO permeability. The results of this study demonstrate that DFO-loaded polymeric micelles can finally be incorporated into soft gel capsules.

**Materials and Methods**

DFO was purchased from Jabber Ebne Hayyan Pharmaceutical Company (TI). Cholesterol, lecithin, oleic acid, carbomer 934, poloxamer (pluronic P 407), Tween 80 and Span 20 were purchased from Sigma-Aldrich, labrafil M1944, and labrasol were gifts from Gattefosse Company (France). Dialysis bag was obtained from the Armaghane kalaye gavan Co (x). All chemicals and solvents were of analytical grade. Freshly double distilled water was used in the experiments. Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared based on United States Pharmacopeia (USP 29). SGF was prepared by dissolving 2 g of sodium chloride and 3.2 g of purified pepsin (Sigma-Aldrich) in 7 ml of hydrochloric acid diluted with water up to 1000 ml. The pH was adjusted to 1.2. SIF also was prepared by dissolving 6.8 g of monobasic potassium phosphate in 250 ml of water and adding 77 ml of 0.2N sodium hydroxide and 500 ml of water. Then 10 g of pancreatin (Sigma-Aldrich) were added to the solution and mixed. The pH was then adjusted to 6.8 and the solution was diluted with water up to 1000 ml. Minitab16 software was used for experimental design and evaluation of the effect of variables on responses.

***DFO assay method***

The quantitative determination of DFO was performed using a UV spectrophotometer Biochrom WPA BioWave II (England). The λmax was set to 211 nm when drug was dissolved in SGF and to 206 nm when drug was dissolved in SIF. The assay was validated in terms of linearity, repeatability, accuracy, and limit of quantification (LOQ). Interference of components within the formulation of the DFO assay was evaluated by placebo.

***Determination of critical micelle concentration (CMC)***

One way to evaluate the interaction between polymer and surfactant in the bulk solution is to measure the surface tension of the mixture, keeping the polymer concentration constant and varying the surfactant concentration. Surfactant and co-surfactant aqueous solutions with increasing concentrations and constant concentration of polymer were prepared. Surface tension of these solutions was measured at 25°C using a torsion balance (WHITE ELEC Model No. 83944E). Then surface tension versus log concentration was plotted. There is a break in surface tension at a concentration associated with the critical aggregation concentration (CAC), which corresponds to the onset of micelle formation of the polymer. With increasing surfactant concentrations, we observed a second break that correlated with the critical micelle concentration (CMC), which represents micelle formation by surfactant in the bulk solution. Therefore, the first and second breaks in the surface tension represent CAC and CMC, respectively.

***Preparation******of DFO polymeric micelles***

DFO-loaded polymeric micelles were prepared using the film hydration method [42]. The lipophilic phase consisted of 1.5 g cholesterol, 1 g lecithin, and 1.5 ml oleic acid mixed together and then 10 ml chloroform was added to this mixture and placed into a rotary evaporator at 120 RPM for 15 min at 60ºC to form a uniform lipid film. To remove residual amounts of solvent, the films were placed in a vacuum oven at 40oC overnight. Then the dried lipid films were hydrated with an aqueous solution containing DFO (5 mg/ml), surfactant, polymer, and co-surfactant and evaporated at 50oC and 120 RPM. Finally, the films were sonicated in a bath sonicator with a power of 500 w at 25ºC for 5 min.

***Percent entrapment efficiency (%EE)***

The amount of DFO encapsulation efficiency was determined using the ultrafiltration method to separate the free DFO in micelle solution. A polymeric micelle solution with defined amounts of DFO was added to centrifugal-ultrafiltration tubes (Microcon MWCO 3000, Millipore Co, USA) and centrifuged at 15000 RPM for 25 min. Then, DFO content was determined using a UV-Vis spectrometer at 206 nm. Total DFO in the polymeric micelle solution was determined after dilution in methanol/water (70/30) to dissolve the polymeric micelle and completely release DFO. The amount of loaded DFO was calculated by subtracting the unloaded DFO from the initial DFO added to the polymeric micelle. Percent EE was then measured using equation 2 [43,44]:

× 100 (2)

***In vitro release studies***

*In vitro* drug release studies were performed using the dialysis bag technique at sufficiently sink condition with respect to DFO solubility. Polymeric micelle solutions containing 0.1 mg DFO were placed in dialysis bag with acetate cellulose membrane (Spectra/Por, molecular weight cut-off 3000-4000 Da), tied and immersed into 100 ml of release medium with stirring at 37°C in a basket dissolution apparatus. SGF (pH 1.2) and SIF (pH 6.8) were applied as release media. Dialysis bags including DFO-loaded polymeric micelle solution were incubated for 1 h in 100 ml SGF and then the sample bags were transferred into 100 ml of SIF and incubated for 96 h. At time intervals of 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 24 hours, 2 ml of sample was removed, filtrated, and released amount of DFO was determined by UV spectrometry [45]. In this experiment, an aqueous solution of DFO with the same concentration of polymeric micelle solution was used as a control.

***Micelle stability***

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

***Stability in media modeling physiological conditions and effects of dilution***

The stability of 1 ml DFO-loaded polymeric micelles was studied by incubation in 1, 10, and 50 ml buffer phosphate, pH 7.4; SGF, pH 1.2; and SIF, pH 6.8 with or without bile salts (5 mM) for 12 h at room temperature [46]. At defined time intervals, polymeric micellar solutions were filtered through 0.2 µm membrane filters and particle size as a sign of physical stability and DFO content as a sign of chemical stability were analyzed and compared with DFO solution as a control.

***Thermal behavior of DFO loaded in polymeric micelles***

The thermal behavior of polymeric micelles was analyzed using differential scanning calorimetry (DSC). Samples were first heated to 50oC and kept at this temperature for 5 minutes to remove their thermal history. Then the temperature was reduced to 0oC with at a rate of 5oC/min. Samples were kept at 0oC for 5 min and the temperature was then increased to 160oC using 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 the small intestine was excised and placed in ice-cold bubbled (carbogen, 95:5 O2/CO2) ringer buffer. The jejunum 15-20 cm distal from the pyloric sphincter was removed and rinsed with ringer buffer. Two milliliters of polymeric micellar formulation containing defined amounts of DFO was poured into the rat intestine and closed from both sides. Then the tissue was kept in an organ bath filled with 25 ml of phosphate buffer (pH 7.4) with continuous aeration for 4 hr at 37°C. Two milliliter samples at 0.5, 1, 2, 3, and 4 hr were obtained from the 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. Apparent permeability coefficient (Papp) Percentage permeated and permeability enhancement ratio were calculated using equations 3 and 4 [47].

 (3)

where dQ/dt is the steady state appearance rate on the acceptor side of the tissue. A is the area of the tissue (cm2) and C0 is the initial concentration of drug in the donor phase.

 (4)

***Evaluation of in vitro interference between DFO and iron***

DFO is a chelating agent that forms complexes with ferric ion. The complex formation constant is equal to 1031. The affinity of DFO for complex formation with divalent ions such as Fe +2 is much lower (complex formation constant equal to 1014 or less) than trivalent ions. Chelating occurs on a 1:1 molar basis, so that 1 gram of DFO forms complexes with 85 mg of ferric ion [48]. Increasing concentrations of Fe (NO3)3·9 H2O (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 and blank polymeric micelles at a ratio of 100:8.5 were added to iron solutions thermostatically maintained at 20ₒC. After 3 hr, the mixture was treated with 10% sodium acetate to provide a pH of 2-3 and 2% sulfosalicylic acid was added as an indicator to form the violet-red complex. Then the mixture was heated to 40-50°C. Afterwards, the concentration of Fe+3 ions was determined by titration with 0.1 M EDTA. The amounts of adsorbed ions were calculated by subtracting the amount of Fe+3 ions from the initial amount [49]. According to the following equation, remaining iron that was not incorporated into micelles was measured by complexometery titration with 1% EDTA as shown in equation 5 [50].

**M1 V1 = M2 V2  (5)**

where M1 is the concentration of iron that is bound to EDTA, M2 is the concentration of EDTA, V1 is the volume of iron solution, and V2 is the volume of EDTA. Free DFO solution was used as a control.

**Discussion**

The present research was designed to prepare polymeric micelles for DFO oral delivery system to increase DFO oral absorption. Here, we showed that PMDDS with particle size less than 81 nm provided 80% EE and led to DFO protection against degradation in the gastrointestinal tract and supplied sufficient area for oral absorption. PMDDS increased DFO permeability through rat intestine more than 3-fold compared to aqueous solutions. The correlation between PMDDS and physicochemical properties such as particle size, %EE, D1%, and D24% with Papp was evaluated. Our results demonstrated a significant and direct correlation between %EE and Papp. Therefore, Papp was controlled by %EE such that increased Papp resulted from increased %EE%. It appears that PMDDS increased DFO permeability without any effect on intestinal structure. Similarly, increased %EE resulted from increased PMDDS particle size, increased surfactant concentration, or using carbomer as the polymer. Micelles were formed by carbomer with higher concentration showing increased PMDDs particle size and %EE compared to poloxamer. Finally, polymer type affected Papp by altering PMDDS characterstics such as particle size and %EE. As a result, Papp is controlled directly and indirectly by EE% and particle size, respectively. The effect of particle size on drug permeation through different membranes such as intestine and skin has been reported in several studies. Researchers reported that as the size of gold nanoparticles increased, the permeability coefficient and diffusion coefficient through rat skin and intestine membrane decreased [53]. In another study, vitamin B12 transport across Caco-2 cell membranes was increased to 2-3-fold after nanocapsulation that was directly dependent on particle size [54]. All PMMDS tested demonstrated good iron-binding capacity and did affect DFO iron binding ability but actually increased this capacity. Therefore, PMMDS showed two concordant effects, increasing DFO permeations through intestine membranes and improving iron-binding capacity. Both of these processes were affected by components that were used in the PMMDS. It means that DFO permeability and iron-binding capacity were mainly touched by polymeric micelles components. Based on check point analysis, we found that CMC play a critical role in PMMDS behavior. PMMDS with an intermediate CMC was introduced as the optimized formulation. It appears that after micelle formation, a small increase in concentration above the CMC led to formation of larger micelles that increased DFO loading and iron binding capacity. At a concentration above 1.5 CMC, increased amounts of micelles led to formation of micelle aggregation that did not increase DFO loading or iron-binding capacity. We can conclude that polymeric micelles did not interact covalently with iron and the main mechanism for improving iron binding may be due to iron and DFO loading into the micelles. Effective iron chelation is obtained if iron chelators can remove equal or greater amounts of iron accumulated due to transfusion. This requires that chelators be able to reach the target site at relevant concentrations [55]. Based on this concept, DFO loaded in polymeric micelles prepared in this study provided high iron-binding capacity and presented a novel vehicle that increased DFO delivery to the blood by improving its intestinal absorption. It seems that polymeric micelles present a good opportunity for DFO target delivery. Another criterion that is very important for DFO delivery systems is release of DFO in a sustained pattern. This criterion increases DFO half-life and decreases the need to repeat the dose. We found that diffusion or dissolution controlled the release rate of DFO from polymeric micelles, and geometrical shape was kept constant. This indicates that optimized polymeric micelles that were developed in the present study demonstrated a sustained release pattern. Different approaches have been previously used for improving DFO bioavailability by conjugation of DFO to different polymer backbones such as PEG methacrylate [56], hyperbranched polyglyceroles [57], hydroxamic acid [58], 3-hydroxypyridine [59], and dextran [60]. In 2005, Polomoscanik et al. produced non-toxic DFO hydroxamic acid based iron chelating hydrogels and evaluated their ability to prevent iron absorption in the gut [61]. These gels were effective at preventing gastric iron absorption, but Zn and Cu did compete with iron moderately. In the present study, Zn and Cu binding ability of DFO-loaded micelles was not evaluated and should be investigated in future studies. Previously published studies were performed based on DFO conjugation to polymeric backbones but there have been no previously reported systematic studies using polymeric micelles. The present study identified a novel DFO-loaded polymeric micelle that not only is stable and forms immediately but also increases DFO permeability through intestine membranes.