Deferoxamine-Based Materials and Sensors for Fe(III) Detection

Subjects: Chemistry, Analytical

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Deferoxamine (DFO) is a siderophore widely studied for its ability to bind iron(III) strongly. Thanks to its versatility, it is suitable for several clinical and analytical applications, from the recognized iron(III) chelation therapy to the most recent applications in sensing. The presence of three hydroxamic functional groups enables Deferoxamine to form stable complexes with iron(III) and other divalent and trivalent metal ions. Moreover, the terminal amino group in the DFO molecule, not involved in metal ion complexation, allows modification or functionalization of solid phases, nanoobjects, biopolymers, electrodes and optical devices.

Keywords: deferoxamine ; DFO ; iron(III) ; iron(III) chelators ; deferoxamine-based polymers ; deferoxamine-based sensors ; deferoxamine-modified electrodes ; deferoxamine-functionalized nanoparticles

1. DFO-Based Biopolymers for Chelation and Detection of Fe(III)

Deferoxamine, commercialized by Novartis with the brand name Desferal® is the first US FDA-approved clinical iron chelator. It was included in the therapy of iron-overload diseases due to its pretty good therapeutic outcomes thanks to its strong iron-binding efficacy. Since its clumsy pharmacokinetic behavior (plasma half-life of about 20 min) and several side effects, the clinical use of DFO is progressively decreasing. Several approaches, such as loading nanocarriers or functionalizing biopolymers with DFO, have been proposed to enhance the therapeutic effects of deferoxamine [1]. These strategies have focused on improving DFO's half-life reducing the administration frequency, and minimizing the side effects [2].

Initial attempts began in the 1970s with liposomal formulations [3][4][5], but since the US FDA did not approve the liposomal drugs until the 1990s, it is not surprising that these first attempts were not effectively transferred to the clinic [2].

Many papers reported the development of nanochelators with demonstrated efficiency by in vitro trials, but most of them were not yet applied as iron-overload treatments in vivo [2].

In this scenario, high DFO-loaded (of about 80%) nanoparticles assisted by polyphenols were developed with both efficient iron chelation and reactive oxygen species-scavenging properties [1]. In particular, a series of self-assembled DFO-polyphenol conjugates nanoparticles were prepared and tested for Fe(III) and ROS removal. In vitro and in vivo experiments were carried out. The best results were obtained with DFO-gallic acid nanoparticles thanks to the formation of iron complexes with both DFO and polyphenol. Herein, it seems to be a promising strategy for improving the therapeutic effect on iron(III)-overload patients.

With the aim of developing an oral drug delivery system for deferoxamine, polymeric micelles were prepared and characterized [6]. The micelles formulation was optimized by an experimental design. All polymeric micelles increased the iron(III) complexing ability compared to the free DFO drug. Optimized polymeric micelle consisted of Tween 80 and Span 20 surfactants and Poloxamer® as polymer, demonstrating more than 97% iron(III) chelation. Moreover, they showed perfect rat intestine permeation and good stability, making them up-and-coming as DFO's drug delivery system.

Another kind of self-assembled polymeric micelle was developed for chelating and selectively detecting Fe(III) both in vitro and in iron-overloaded cells [7]. The micelles comprised Pluronics F127 polymer and Pluronics F127–DFO polymer conjugates, and they could encapsulate tetraphenylethylene (TPE). The fluorescence of TPE, determined by the aggregation-induced state in the micelle, can be quenched in the presence of iron(III). The DFO–based polymeric micelles demonstrated the ability to retain the iron-chelation properties of the free deferoxamine, exhibiting slight cytotoxicity compared to the free drug. Moreover, the fluorescence "turn-off" mechanism can detect the presence of iron(III) and

monitor the chelation process in iron-overload cell models. It could be a proof of concept for a future design of an effective in vivo therapy system.

As bio-polymeric materials, polysaccharides, such as starch, dextrose and chitosans, were conjugated with DFO mainly to extend the drug's half-life. Clinical trials with starch-conjugated DFO as a long-lasting formulation started in 2007 [8]. This work evaluated the pharmacokinetics, iron excretion and safety of starch-conjugated DFO in patients with β-thalassemia. However, the clinical development of the formulation stopped after the current time.

Recently, two kinds of chitosan nanoparticles loaded with DFO for the slow release of the drug were suggested [9][10]. In both cases, nanoparticles were prepared by the ionotropic gelation method [11], involving a solution of DFO and chitosan (i.e., a polycation polymer) in which tripolyphosphate (i.e., a polyanionic counter ion) was added dropwise. The DFO release and the iron-chelation efficiency were tested in vitro in cell culture models, and other studies are required to evaluate the pharmacological performances of this formulation in vivo.

Chitosan/alginate hydrogels were also proposed as lengthy delivery systems of deferoxamine. In particular, DFO-based chitosan/alginate hydrogel alone or DFO-encapsulated into a composite of hydrogel and poly(d,l-lactide-co-glycolide, PLGA) biodegradable microspheres were studied in vitro [12]. The composite resulted in the most effective delivery system since the DFO is strongly entrapped in the hydrogel network and is gradually released by diffusion. The formulation, wholly biodegradable and biocompatible, coupled with excellent results, seems promising for clinical applications.

Cyclodextrin-based polyrotaxanes were also reported as efficient carriers for several drug delivery systems [13]. Polyrotaxanes are supramolecular materials constituted by a linear polymer chain, and many cyclic molecules screwed on its linear axis stopped with two large end groups at the extremes of the chain. The main advantage of preparing polyrotaxanes-drug conjugates is that despite their small dimension, they can extend the blood circulation of several drugs [14]. In 2016, Liu et al. [15] synthesized polyrotaxanes by threading multiple α-cyclodextrin rings onto poly(ethylene glycol) bis(amine) chains capped with enzymatically cleavable bulky Z-L phenylalanine. Then the hydroxy groups of the cyclodextrin moieties were oxidized into aldehydes and conjugated with the terminal amine of DFO to obtain the polyrotaxane-DFO.

The Fe(III) chelating properties of the developed materials were checked by UV–vis spectrophotometry. In vitro studies considering iron-overloaded macrophages demonstrated the ability of polyrotaxane-DFO to decrease the drug's cytotoxicity while keeping its chelating properties unaltered. The same research group had recently proposed an improved version of polyrotaxane-DFO, incorporating (ROS)-sensitive thioketal groups into the polyrotaxane platform [16]. In vivo experiments demonstrated how ROS-induced dissociation of the chelator into small parts of 2 nm size drastically increased fecal and urine elimination of excess iron(III). Moreover, this nano-drug showed excellent biocompatibility since no adverse effects were detected in the organs analyzed, proving to be a promising alternative to free DFO.

In the last 20 years, many attempts have been devoted to conjugating DFO to a water-soluble polymer, such as polyethylene glycol (PEG), to enhance its pharmacokinetics. For example, DFO was conjugated to PEG-containing copolymers to develop high blood compatible and long-circulating macromolecular chelator [17][18]. PEG has been applied in several formulations since the FDA approved it as a pharmaceutical polymer material [19][20][21]. However, the main drawbacks of PEG-containing copolymers are the wide molecular weight and low loading efficacy of the active pharmaceutical ingredients; thus, multi-armed PEGs were recently exploited thanks to their higher efficiency in drug loading compared to the classical linear polymers [22][23]. In this field, Yu et al. [24] proposed a star-like 8-arm-polyethylene glycol conjugate with DFO, demonstrating that it can be a promising candidate as a long-circulating, less toxic iron chelator to be used in the treatment of Fe(III)-overload patients.

2. Solid Phase Chelating Materials for Fe(III) Sensing

The ability of deferoxamine to strongly complex Fe(III), its physical–chemical proprieties and the presence in its structure of a terminal amino group allowed the development of DFO-based solid sorbents for iron sensing. Several strategies have been applied to anchor deferoxamine to different solid substrates, which will be summarized and commented on in this paragraph.

Among various materials, mesoporous silica was the most widely employed since it was particularly attractive for its large specific surface area, cost-effective production, biocompatibility and ease of functionalization [25].

DFO was generally grafted on the silica particles' surface by covalent bonding. For example, Biesuz et al. presented the synthesis of DFO self-assembled monolayers (DFO-SAM) on the mesoporous silica MCM-41 by a one-pot strategy [26].

This synthetic approach was optimized by an experimental design, and the procedure consisted of DFO's dissolution in dimethyl sulfoxide followed by a reaction with (3-glycidyloxypropyl)trimethoxysilane (GPTMS) at 90 °C under overnight stirring. MCM-41 is then added to the mixture obtaining the final product.

The characterization of the material revealed that the iron uptake kinetic at pH 2.5 was relatively fast, less than 2 h. From the sorption isotherms, the maximum sorption capacity of iron(III), obtained in optimized conditions, was about 0.3 mmol/g. The promising results, coupled with those obtained by applying the material to urine samples spiked with the Fe(III) [27], were confident that this functionalized mesoporous silica could be used for iron detection in environmental and biological samples.

Recently, stellate mesoporous silica nanoparticles grafted with deferoxamine were developed [28]. Nanoparticles were synthesized by a sol-gel approach and then functionalized with deferoxamine B (DfoB) by a three-step procedure: firstly, the nanoparticles' surface was modified with aminopropyltriethoxysilane through a condensation reaction in ethanol; then, the amine groups previously inserted were converted into carboxylic groups by a reaction with succinic anhydride (SA) and finally, the carboxylic functionalities, after activation with 1-ethyl-3,3-dimethylaminopropylcarbodiimide hydrochloride reacted with the terminal amino group of DFO.

The material obtained showed performances similar to those of the DFO-SAM previously described [26], confirming the efficient and selective removal of Fe(III) from biological samples.

Pawlaczyk and Schroeder reported a similar approach [29]. In that case, two commercial amorphous silica with microparticles' surfaces modified by isocyanate and maleimide groups were functionalized with DFO by a reaction between the terminal free amino group of deferoxamine with the isocyanate or maleimide group of the silica. The obtained materials presented a good sorption capacity for Fe(III) of about 1.5–2 mmol/g at pH 2.45, indicating the high adsorptive potential of the DFO-functionalized materials. The process was spontaneous and endothermic, but the iron(III) uptake required at least 5–10 h. In the same paper, other kinds of DFO-based hybrid material were proposed; in particular, the most promising was that of magnetite encapsulated in silica nanoparticles. Since the good performance, probably due to the nanostructured texture, the material was applied to a competitive test in vitro to evaluate its iron(III) scavenging properties from the biological complex protoporphyrin IX−Fe(III) (hemin). The excellent results can lead to a further study of the material for its application in clinical or biological fields.

Polysaccharides such as agarose and alginates were also functionalized with deferoxamine for developing biocompatible sorbents with high porosity, good chemical stability and high loading capacity. For example, Yehuda et al. [30] immobilized DFO on pre-activated Sepharose gels aiming to develop a slow-release Fe(III) fertilizer. The most promising product was achieved by functionalizing (p-nitrophenyl)chloroformate activated Sepharose in the presence of (Dimethylamino)pyridine, obtaining a good affinity for iron(III) and a maximum sorption capacity of 0.14 mmol/g at pH 4.5.

More recently, deferoxamine-grafted alginate hydrogel was synthesized by an amidation reaction between sodium alginate and DFO; its application as a wound cover material was considered [31].

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