

Chapter 14

Siderophores and their Applications in Wood, Textile, and Paper Conservation



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Anastasia Pournou

Abstract Since the 1950s, siderophores have been acknowledged as nature's chelating powerhouse and have been given considerable attention concerning their crucial roles in microorganisms and plants for capturing non-bioavailable iron from aquatic and terrestrial environments, as well as for their applications in agriculture, health, and materials science and environmental research. In recent years, the exceptional affinity and complexing efficacy, as well as the high selectivity of these potent chelators towards iron(III), have led to investigations by researchers aiming at understanding their capacity for removing potentially harmful and aesthetically unacceptable iron stains from organic substrates in cultural heritage objects. In the context of the conservation of cultural heritage objects, potent chelators have been proposed to remove iron from surfaces by transferring it to the more soluble complexed phase. In this review, the origins and the types of bio-environments of siderophores as well as their structure and chemistry are investigated and related to the requirements of conservation. It is evident that, given the enormous potential that these chelators have, the research for their application in cultural heritage is at a preliminary level, and has to date been within the rather narrow context of cellulosic materials such as paper and wood. The results of research conducted to date are presented in this review and questions regarding the optimal use of siderophores as iron-removing agents are posed.

Keywords Siderophores · Green chelators · EDTA (ethylene-diamino tetra-acetic acid) · DTPA (diethylene-triaminopenta-acetic or pentetic acid) · Cultural heritage · Conservation

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1 Introduction

Siderophores are among nature's successful solutions to overcome the challenges of bioavailable iron in aqueous systems (Barton and Hemming 1993; Neilands 1995; Boukhalfa and Crumbliss 2002; Crichton 2019).

The origin of the word siderophore is Greek and means an "iron carrier" (Neilands 1995; Saha et al. 2013); siderophores are natural metal-chelating agents, employed by a wide range of microorganisms and plants to mobilize iron from its least available sources (Höfte 1993; Neilands 1995; Kraemer 2004; Ali and Vidhale 2013).

They are secondary metabolites of low molecular masses (200–2000 Da) and high affinity for iron ($K_f > 10^{30}$) (Hider and Kong 2010; Saha et al. 2013; Wang et al. 2014; Ahmed and Holmström 2014; Khan et al. 2018).

Siderophores have been classified depending on the oxygen ligands for Fe(III) coordination, as catecholate (*sensu stricto*, catecholates, and phenolates; better termed as aryl caps), hydroxamate, carboxylate, and mixed types (Miethke and Marahiel 2007; Ahmed and Holmström 2014; Saha et al. 2016; Khan et al. 2018). More than 500 different types of siderophores are known, of which 270 have been structurally characterized (Hider and Kong 2010; Ali and Vidhale 2013; Wang et al. 2014; Ahmed and Holmström 2014).

They have a high biotechnological potential and a wide range of applications not only in ecology, agriculture, and medicine but also in the field of conservation of cultural heritage, mostly for removing iron corrosion products from artifacts (Albelda-Berenguer et al. 2019).

The question of removing iron from organic substrates of cultural heritage has been previously considered through the use of chelators such as citric acid, EDTA (ethylene-diaminetetraacetic acid), DTPA (diethylene-triaminepenta-acetic, or pentetic acid), etc. (Almkvist et al. 2005; Burgess 1991; Margariti 2003; Rivers and Ummey 2003; Richards et al. 2012). Water-soluble iron(III) ions are highly reactive, as they participate in Fenton-type reactions (see Sect. 14.4.1) (Koppenol 1993; Kolar and Strlic 2001; Bulska and Wagner 2002; Dunford 2002; Burkitt 2003; Jablonský et al. 2010; Corregidor et al. 2019) causing severe oxidative stress to organic substrates.

The removal of iron stains from wood, textiles, and paper substrates emerges as an important issue, which although it has been typically dealt by the above-mentioned chelators, these were shown to not fully encompass iron(III) in their complexes, and significantly, not to prevent the harmful oxidative stress on cellulosic materials (Kolar 1997; Kolar and Strlič 2004). In addition, these complexing media do not offer the best possible solution due to a lack of specificity. EDTA, for instance, complexes similarly iron, copper, manganese, cobalt, and other cultural heritage-significant ions and unwanted side-reactions (see below) which in the case of siderophores are avoided. Therefore the need for increased specificity from chelators has been recognized in the field of cultural heritage and efforts employing siderophores have been reported among some research groups (Wagner and Bulska

2003; Rapti et al. 2017; Albelda-Berenguer et al. 2019). In this review, these efforts and their results will be presented along with the questions that still need to be answered.

The enormous biodiversity among bacteria, fungi, and particular types of plants has led to optimized strategies for sequestering abundant, but minimally bioavailable iron from the environment by employing a vast palette of chelating agents, commonly termed as *siderophores*. This highly successful strategy can only be seen as a triumph of nature which produces custom-made iron chelators according to the types of organisms, the character of the environment itself, and the severity of the iron nutrient demand. Learning from nature can teach us the right approach under the right condition for our applications, including conservation. Within this review, this leads to the necessity for presenting siderophores, their biological origins, the structures, and their chemistry as a necessary background (Sects. 14.1–14.3) for effectively considering their applications in conservation regarding the removal of unwanted iron from certain types of materials (Sect. 14.4).

2 Biosynthesis, Roles, and Applications of Siderophores

Almost all microorganisms and living members of the animal and plant kingdom are dependent on iron (Renshaw et al. 2002; Hider and Kong 2010; Saha et al. 2013; Ahmed and Holmström 2014; Khan et al. 2018).

Iron's unique chemical properties, such as its ability to coordinate and activate oxygen and its ideal redox chemistry (Fe^{2+} , Fe^{3+} , Fe^{5+}), turn this element into an essential and vital nutrient of the growth and developmental processes of every living organism (Saha et al. 2013, 2016; Ahmed and Holmström 2014; Khan et al. 2018), because it is acting as a catalyst in enzymatic processes, oxygen metabolism, electron transfer, and DNA and RNA syntheses (Hider and Kong 2010; Ahmed and Holmström 2014; Saha et al. 2016).

Although iron is abundant in nature, it is not easily bioavailable because at neutral pH and in the presence of atmospheric oxygen it undergoes rapid oxidation from ferrous (Fe^{2+}) to ferric (Fe^{3+}) iron and finally forms insoluble ferric hydroxides and oxy-hydroxide, which is almost unavailable for acquisition by organisms (Renshaw et al. 2002; Saha et al. 2013; Wang et al. 2014). More specifically, the solubility and consequently the bioavailability of iron as ferric oxy-hydroxide are less than 10^{-18} M, while microbes require 10^{-5} – 10^{-8} M for carrying out vital physiological and metabolic processes and have optimal growth (Saha et al. 2013; Wang et al. 2014; Khan et al. 2018).

To solve this problem, a vast number of bacteria, fungi, plants, and even higher eukaryotes under iron-deficient conditions produce siderophores, which is an essential metabolic feature that allows them to survive (Renshaw et al. 2002; Saha et al. 2013, 2016; Ahmed and Holmström 2014; Khan et al. 2018).

Hydroxamate types of siderophores comprise the most common group of siderophores found in nature and are produced by both bacteria and fungi (Ali and

Vidhale 2013; Saha et al. 2016; Khan et al. 2018). Catecholate siderophores are found only in certain bacteria (Ali and Vidhale 2013; Saha et al. 2016; Khan et al. 2018), while the carboxylate type is produced by few bacteria such as *Rhizobium* and *Staphylococcus* and fungi such as *Mucorales* belonging to the phylum *Zygomycota* (Saha et al. 2016; Khan et al. 2018).

2.1 Biosynthesis

Siderophore biosynthesis is typically regulated by iron levels of the environment where the organism lives (Hider and Kong 2010). Although iron deficiency is the key factor, other external factors such as pH, temperature, carbon source, and the presence of other metals play an important role (Saha et al. 2013). Thus some hydroxamate siderophores are prevalent in lower or acidic pH, whereas other catecholate siderophores are produced in neutral to alkaline pH (Saha et al. 2013). The distinction of the various siderophore types and their significance regarding applications in conservation can be found in Sect. 14.3.2.

There are two biosynthesis pathways involved in siderophores' synthesis (a) the dependent on non-ribosomal peptide synthetases (NRPSs) and (b) the independent of NRPS (Miethke and Marahiel 2007; Saha et al. 2013; Khan et al. 2018). The non-ribosomal peptide synthetases are large multi-enzyme complexes responsible for the synthesis of several biologically important peptidic products without an RNA template (Miethke and Marahiel 2007; Saha et al. 2013). In general, NRPSs are essentially assembly lines of specialized domains that link amino acids via thioester intermediates. They function in a similar manner to that of fatty acid synthetase (Hider and Kong 2010). They consist of three domains required for peptide bond formation (a) adenylation domain, (b) peptidyl carrier protein domain (PCP or thiolation), and (c) condensation domain, responsible for the assembly of a wide array of amino, carboxy, and hydroxy acids in various combinations to produce macrocyclic peptidic with high structural variability (Miethke and Marahiel 2007; Hider and Kong 2010; Saha et al. 2013).

NRPSs are responsible mainly for the synthesis of aryl-capped siderophores (Miethke and Marahiel 2007). Hydroxamate and carboxylate siderophores are assembled by NRPS-independent mechanisms in the majority of cases, even though in some cases, NRPSs are partially involved in the synthesis of hydroxamate and carboxylate siderophores to build a peptidic backbone to which the iron-coordinating residues are attached (Miethke and Marahiel 2007).

Nonetheless, organisms such as bacteria, fungi, and plants have different ways to synthesize their siderophores (Khan et al. 2018; Albelda-Berenguer et al. 2019) and therefore the siderophores can be categorized based on the organism that produces them: fungal, bacterial, plant (phytosiderophores), cyanobacterial, or mammalian siderophores (Khan et al. 2018).

2.2 Roles

Fungal siderophores are mainly of hydroxamate type, belonging to the ferrichrome family (i.e., ferrichrome A), coprogen and triacetylfulvarinine families, and carboxylate type of siderophores (Renshaw et al. 2002; Hider and Kong 2010; Ahmed and Holmström 2014; Khan et al. 2018). *Aspergillus fumigatus* and *Aspergillus nidulans* have been thoroughly studied for their siderophore production (Khan et al. 2018).

Bacterial siderophores comprise extracellular forms of siderophore (Khan et al. 2018). Most of the bacterial siderophores are catecholates (i.e., enterobactin), while there are also some carboxylates (i.e., rhizobactin), hydroxamates (i.e., ferrioxamine B), and mixed types (i.e., pyoverdine) (Ahmed and Holmström 2014). The Gram-negative, facultative anaerobe *Escherichia coli*, found normally in the intestine, is the most widely studied bacterium for siderophore production. It principally produces enterobactin, with the highest affinity towards iron(III) ion, of any known siderophore (Khan et al. 2018). Members of *Actinobacteria* such as *Streptomyces* are also well-recognized for their ability to produce multiple siderophores such as the characteristic desferrioxamines G, B, and E (Wang et al. 2014).

Mammalian siderophores may have structural and functional similarity to bacterial siderophores (Khan et al. 2018). Studies have confirmed the existence of a mammalian siderophore and provided insight into its structure, biosynthesis, and function (Devireddy et al. 2010). Siderocalin is the only mammalian siderophore-binding protein currently known however, compounds that serve endogenously as siderophore equivalents have been identified and characterized through associations with siderocalin (Correnti and Strong 2012).

Marine organisms such as phytoplankton and cyanobacteria can also produce siderophores (Ahmed and Holmström 2014). Cyanobacterial siderophores are mainly of dihydroxamate-type (such as schizokinen and anachelin H) (Khan et al. 2018). Moreover, the coastal marine cyanobacterium *Synechococcus* sp. produces three amphiphilic siderophores, synechobactins A, B, and C (Ito and Butler 2005).

Finally, plant siderophores (phytosiderophores) are hexadentate ligands that coordinate Fe(III) with their amino and carboxyl groups (Ahmed and Holmström 2014). These are prevalent in members of *Poaceae* and belong to the mugineic acid family that form a hexadentate Fe-phytosiderophore complex (Kumar et al. 2016; Khan et al. 2018). Mugineic acid has been found to be a much better complexing agent for the ferrous ion than ligands which contain hydroxamate or catecholate (Sugiura and Nomoto 1984).

Under low iron availability, which is a critical growth-limiting factor for virtually all aerobic microorganisms, siderophores become important in several ecological niches. These are included in four major habitats: soil and surface water, marine water, plant tissue (pathogens), and animal tissue (pathogens) (Hider and Kong 2010). Therefore, mixtures of siderophores control the supply of iron in critical environments such as rivers, marine surface water, forest soils and agricultural land and pathogenic bacteria and fungi employ siderophores in a chemical competition with their plant and animal hosts (Hider and Kong 2010).

The primary role of siderophores is to chelate the ferric iron from different terrestrial and aquatic habitats and make it available for microbial and plant cells (Ahmed and Holmström 2014). Thus, they exhibit growth factor activity for nearly all known fungi and plants (Renshaw et al. 2002; Ahmed and Holmström 2014). Additionally, many siderophores have several significant roles, such as virulence in pathogens, oxidative stress tolerance, and antimicrobial properties (Renshaw et al. 2002; Khan et al. 2018).

Several studies have demonstrated the role of siderophores in mediating pathogen multiplication and development of virulence, because iron is critical for many pathogenic species (Neilands 1995; Saha et al. 2013). Moreover, siderophores have been extensively reported to reduce oxidative stress in microorganisms producing them (Khan et al. 2018). Another important role of siderophores is their contribution in biofilm formation: studies have shown that intracellular iron concentration plays an important role in the development of the complex community of microorganisms growing in various substrates in an aqueous environment (Saha et al. 2013).

Nonetheless, apart from their primary role in iron chelation and mobilization, siderophores have the ability to bind a variety of other heavy metals such as Al^{3+} , Zn^{2+} , Cu^{2+} , Pb^{2+} , and Cd^{2+} (Saha et al. 2013; Ahmed and Holmström 2014; Kumar et al. 2016; Złoch et al. 2016; Khan et al. 2018). Thus, siderophores are also seen serving in bioremediation for cadmium and lead toxicity (Khan et al. 2018) and can affect homeostasis and heavy metal tolerance of microorganisms (Złoch et al. 2016). This is the reason why siderophore-producing bacteria have been used to assist in phytoremediation of heavy metals from contaminated environments or in the scarcity of nutrients (Saha et al. 2013; Thiem et al. 2018).

It becomes thus apparent why siderophores have wide applications in various fields such as agriculture, medicine, ecology, and environmental applications (Ali and Vidhale 2013; Saha et al. 2016).

2.3 Applications

In agriculture, siderophores can be considered to be an eco-friendly alternative to hazardous chemical pesticides as they can play a significant role in the biological control mechanism against certain phytopathogens. Moreover, they can promote plant growth as iron is required for chlorophyll biosynthesis, redox reactions, and some important physiological activities in plants (Saha et al. 2016).

Medical siderophores have been exploited to deliver drugs inside the cells, a concept adopted from the naturally occurring sideromycins (daunomycins, albomycins, microsins) (Saha et al. 2013). Sideromycins are conjugates of siderophores and antibiotics, in which the antibiotic utilizes siderophores as mediators to enter the cells via the iron-uptake machinery. This process is known as the Trojan horse strategy (Ali and Vidhale 2013; Saha et al. 2013, 2016; Khan et al. 2018). They can be also used to treat diseases caused by pathogenic microorganisms

by exploiting their antimicrobial property (Khan et al. 2018). Moreover, medical siderophores have been used to treat iron overload conditions such as β -thalassemia and aluminum overload (Renshaw et al. 2002; Ali and Vidhale 2013; Saha et al. 2016; Khan et al. 2018). Desferrioxamine and other hydroxamate siderophores have also been studied for use in the treatment of other medical conditions, including cancer, infectious diseases such as malaria, due to their antimicrobial properties and for decontamination by actinides such as neptunium and plutonium (Renshaw et al. 2002; Saha et al. 2016; Khan et al. 2018).

The ability of siderophores to complex actinides, which result mainly by the production and testing of weapons and by nuclear power stations and reprocessing plants, indicates their use for reprocessing of nuclear fuel, bioremediation of metal-contaminated sites, and the treatment of industrial waste, including radioactive waste (Renshaw et al. 2002; Saha et al. 2016; Khan et al. 2018).

Another application of siderophores is in the field of microbial ecology as it has been demonstrated that a siderophore-based approach has markedly facilitated the growth and cultivation in the pure culture of many unculturable organisms (Saha et al. 2016).

Finally, some siderophores have been characterized to function as sensitive, robust, and specific Fe^{3+} biosensors. Nonetheless, most of them have not yet been characterized and thus it could be just hypothesized that some of them may also turn out to be novel and potential biosensors (Saha et al. 2016).

Besides significant ecological agricultural, environmental, medical, and biotechnological applications, siderophores have recently found a new field for applications in the conservation of cultural heritage, and in particular, paper and wood (Albelda-Berenguer et al. 2019), which will be thoroughly discussed in a following section.

3 The Structure and Chemistry of Siderophores

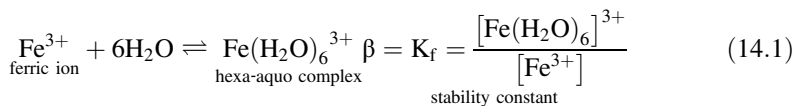
3.1 Chelation of Iron

Exceptionally stable complexes can be formed between metal ions such as iron(III) and multidentate organic Lewis bases, called chelators (from the Greek “*χηλή*,” meaning *hoof* or *claw*, suggesting two-sided, or bidentate gripping ability). Chelators are capable for two-, four-, or six-sided coordination, accordingly termed as bi-, tetra-, or hexadentate ligands, respectively (Cotton et al. 1995; Crichton 2019). This way, highly stable multidentate coordination complexes are formed.

Two forms of iron are the most common in the environment: (a) ferric or iron(III) (in its ionic form it is depicted as Fe^{3+}) which is the most abundant and (b) ferrous or iron(II) which is the least stable, often oxidizable to ferric compounds. The former is mostly the case in the discussion within this paper as it is the main species in iron corrosion products and therefore a usual target for conservators. The two forms of iron are in a dynamic relationship between each other depending on factors such as the presence of oxygen, the pH, complexing agents, the solubility of their

compounds, etc. Excellent books and reviews are available for further reading (Cornell and Schwertmann 2003; Crichton 2019). The reader also needs to have an adequate background on the acid and base character and their inter-relations, as well as the significance of pH with respect to the acidity constant of an acid; these are key to understand the siderophore action, and more generally, chelation (see for instance, Harris 2015).

Ferric ion (Fe^{3+}) is a Lewis acid capable of accepting an electron pair from functional groups in chelators, which on their part act as Lewis bases; the latter can be electron pair-rich groups such as hydroxyl ($:\text{OH}$), hydroxide ($:\text{OH}^-$), and amino ($:\text{NH}_2$) groups (Cotton et al. 1995; Marusak et al. 2006; Harris 2015; Crichton 2019). The coordination of metals by organic or inorganic molecules is a ubiquitous phenomenon in nature. For instance, metal ions, such as $\text{Fe}^{3+}_{(\text{aq})}$, can be coordinated in aqueous solutions by six water molecules arranged according to octahedral geometry; in these, the electron-rich oxygen atom in each water molecule behaves as the Lewis acid. The coordination process occurs stepwise involving ligand molecules (such as H_2O) in a sequential manner, so that most Fe^{3+} ions in their aqueous solutions exist as the hexa-aquo complex. Equation (14.1) shows the overall reaction for the complexation of Fe^{3+} by water in the formation of the iron hexa-aquo complex. This complex behaves as an acid as it further interacts with water and reduces the pH of the solutions; with pKa value 2.2, a 0.1 M aqueous iron(III) solution, assumed to be entirely in its hexa-aquo complex form, is expected to show a pH as low as 1.6.



A chelate complex forms as a ligand that approaches the ferric ion (Fe^{3+}) and arranges in its first coordination sphere; a stable hexacoordinate complex is thus formed, in which properties such as solubility, hydrolysis, and reduction are utterly controlled (Dhungana and Crumbliss 2005; Pepper and Gentry 2015; Albelda-Berenguer et al. 2019).

The overall process is governed by the combination of enthalpy and entropy factors and characterized by an equilibrium constant, which is called the *stability constant* often symbolized as $\log\beta$; the higher the value of $\log\beta$, the more stable is the coordination complex (Nakamoto 2009; Harris 2015; Crichton 2019).

The chelate complexes of ferrous ions (Fe^{2+}) are much less stable with stabilities more than ten orders of magnitude lower than those of iron(III). As a result, the reduction of iron(III) to iron(II) has enormous implications on the dechelation (or release) mechanisms of uptaken iron inside the living systems and is also a significant factor that needs to be taken into account in the various applications of siderophores (Boukhalfa et al. 2006; Hider and Kong 2010; Albelda-Berenguer et al. 2019).

The hard-soft acid base (HSAB) concept proposed by Pearson further helps to evaluate and understand the high tendency for reaction between Lewis acids and bases of similar hardness (Cotton et al. 1995; Marusak et al. 2006; Harris 2015; Crichton 2019). As a result, chelators having a hard base character can complex iron (III) (a hard acid) so efficiently that the latter can be removed from its insoluble and minimally bioavailable iron oxides, hydroxy-oxides, and hydroxide. Siderophores possess such ability, which has been developed as one of nature's successful strategies to sequester iron (Bauer and Exner 1974; Agrawal 1979; Boukhalfa and Crumbliss 2002; Kraemer 2004; Tseng et al. 2006; Reichard et al. 2007; Hider and Kong 2010; Sharpe et al. 2011; Ahmed and Holmström 2014; Albelda-Berenguer et al. 2019). In conservation, a similar issue needs to be addressed: potent chelators can be employed to remove iron from surfaces of cultural heritage objects by transferring it to the more soluble complexed phase; this way, a cleaning effect can be achieved.

An addition, most iron(III) chelate complexes are colored due to specific electron transitions facilitated by charge transfer between the iron(III) and the ligand. This offers an extra asset for visual observation, and additionally, a widely followed methodology for titrating of such complexes (Boukhalfa et al. 2006; Harris 2015).

3.2 *The Tool: Siderophores*

Siderophores have been developed by living systems based on organic functional groups (phenolic hydroxyls, carboxyl groups, hydroxamic and sulfhydryl groups) capable of efficient complexation of metals. Their development is also based on the number and the stereochemical arrangement that these groups possess for more efficient complexation in order to overcome the limited bioavailability of iron as a micronutrient (Kraemer 2004; Kalinowski and Richardson 2005; Bertrand et al. 2009; Hider and Kong 2010).

Siderophores, as most chelators, form thermodynamically stable hexacoordinate complexes with iron(III) accommodated in the most favorable molecular geometry (see, for instance, Fig. 14.1b) (Crumbliss 1990; Kraemer 2004); this along with the high affinity of siderophores for iron(III) produce strong driving forces towards complexation. The stability constants for iron(III) are generally higher than iron (II) which means that ferric complexes are generally much easier to form as compared to ferrous. This preference is more significant in the case of siderophores, as compared to other chelators such as EDTA. This accordingly plays a major role in stability of ferric complexes (see Sect. 14.3.3.3); in the context of conservation applications, it has the significance that in the presence of the wrong chelator, or even more, in the absence of any chelator, ferric ions can be reduced to ferrous with detrimental effects on many organic substrates (see Sect. 14.4.1).

The action of chelation by siderophores implies multiple acidic functional groups (such as OH, COOH) capable of chelating metals which are involved in successive deprotonation schemes (Edwards et al. 2005; Boukhalfa et al. 2006; Reichard et al.

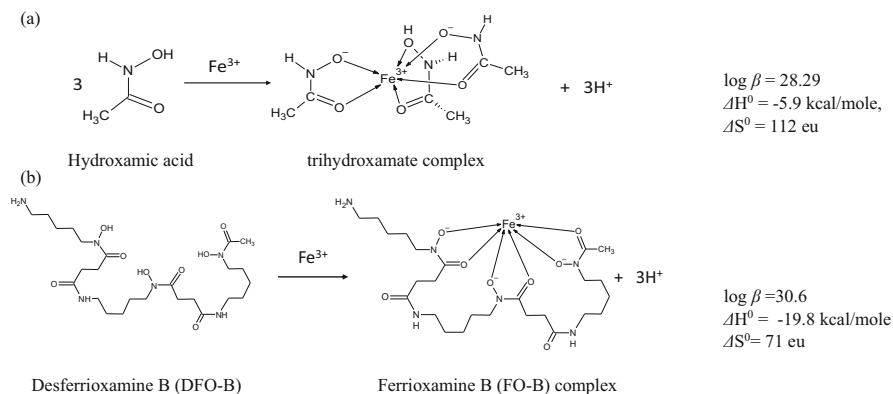


Fig. 14.1 Chelation schemes: (a) model chelate complex: iron(III) trihydroxamate; (b) ferrioxamine (or desferrioxamine B complex with iron); and (c) ferrioxamine E (or desferrioxamine E complex with iron); modified from Crumbliss (1990)

2007). As acids, they tend to dissociate in their aqueous solutions releasing free hydronium ions (H_3O^+), thus decreasing the pH of the solution. Their tendency to dissociate is characterized by their pK_a values; the lower the pK_a value, the stronger the acid. As the deprotonated (mostly anionic) forms are generally capable of metal complexation, their pK_a values are important as they control the range for their most efficient action.

Siderophores can be categorized according to their functional groups as carboxylates, catecholates, hydroxamates, and mixed type (Neilands 1995; Boukhalfa and Crumbliss 2002; Kraemer 2004; Crichton 2019). A siderophore database can be found on the web (Bertrand 2010); chemical structures of selected siderophores are shown in Fig. 14.2. A brief presentation of the main types of siderophores follows.

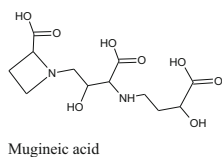
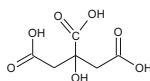
3.2.1 Carboxylate Siderophores

The carboxylate siderophores contain α -hydroxycarboxylic acid moieties, which renders them as acids with a broad range of acidities as seen through their pK_a values. Citric acid has been considered as a model compound (Fig. 14.2a) with pK_a values 3.13, 4.76, and 6.40 (Harris 2015) the lowest value assigned to the α -OH carboxyl group (Silva et al. 2009; Heller et al. 2012). The pK_a values of carboxyl groups in the siderophore molecules range between 3.5 and 5 (Miethke and Marahiel 2007) and are therefore active for complexation in relatively low pH values.

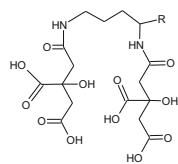
These siderophores can coordinate with the metal ion through their electron-rich α -OH carboxylate oxygens; as a result, they can form chelates at low pH values, which offer an asset for applications that require an acidic environment. Nature has taken advantage of this fact by utilizing these agents in acidic microbial

(a)
Carboxylates

model: citric acid



Mugineic acid

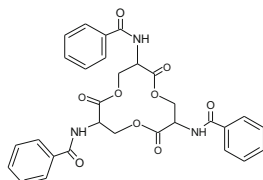


Rhizoferrin (R=H)

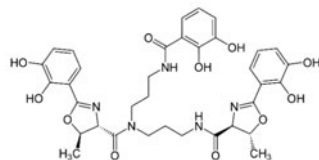
Staphyloferrin (R=COOH)

(b)
Catecholates

Model: catechol (1,2 diphenol)



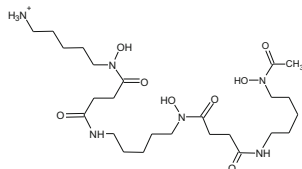
Enterobactin



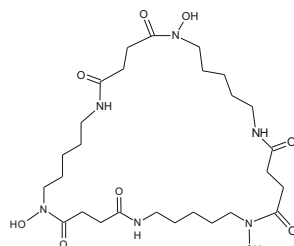
Vibriobactin

(c)
Hydroxamates

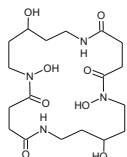
Model: Hydroxamic acid



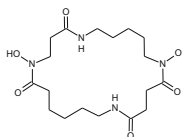
Desferrioxamine B



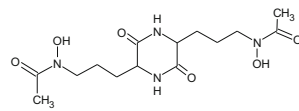
Desferrioxamine E



Alcaligin



Bisucaberin



Rhodotorulic acid

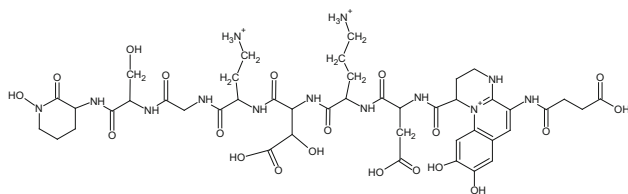
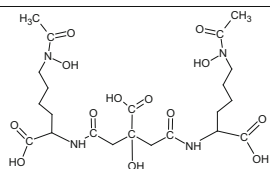
(d) MixedPyoverdine (*Pseudomonas putida*) Catecholate + hydroxamate + citrateAerobactin (*Aerobacter aerogenes*) Hydroxamate + citrate

Fig. 14.2 Chemical structures of typical multidentate chelator classes and corresponding siderophores: **(a)** carboxylates model: citric acid; **(b)** catecholates model: catechol (1,2 diphenol); **(c)** hydroxamates model: hydroxamic acid; and **(d)** mixed

environments (Harris et al. 1979). Their $\log\beta$ values are among the lowest in siderophores, typically around 20–30, suggesting that they are among the least stable siderophore complexes; their relatively low chelate stabilities are compensated in nature by achieving concentrations in the rhizosphere as high as 1 mM, while the more potent desferrioxamine chelators (see below) can be successful at three orders of magnitude lower concentration (approximately, 1 μ M) (Kraemer 2004; Hider and Kong 2010; Ahmed and Holmström 2014). These concentration ranges are also expected to play a similar role in other applications of siderophores in conservation.

3.2.2 Catecholate Siderophores

This siderophore type is based on catechols, or 1,2-dihydroxybenzenes (Avdeef et al. 1978; Romero et al. 2018) (Fig. 14.2b), which due to the geometrically favorable acidic phenolic groups can efficiently coordinate to iron. Catechols have been extensively studied due to their antioxidant, as well as pro-oxidant properties (i.e., the indirect oxidative action, by enabling the formation of reactive oxygen species, also suggesting potential antimicrobial action), depending on the conditions (Iwahashi et al. 1989; Moran et al. 1997; Nakamura 2000; Salgado et al. 2013). Especially the pro-oxidant activities occur by increasing the activity of Fenton reagent for reducing Fe(III) to Fe(II) and O₂ to peroxides (\bullet OOH). These highly energetic and reactive oxygen species play significant roles in wood biodegradation by white and brown rot fungi (Goodell et al. 2006; Romero et al. 2018). These activities are related to their ability for chelation through their catechol phenolate ions, which are formed over pH 9–10 (Avdeef et al. 1978; Hynes and Ó Coinceannainn 2001). This issue will be further discussed in the following sections.

Catechol-based siderophores show among the highest stability constants for iron chelation; specifically, enterobactin, which is produced by *Escherichia coli* and other bacteria (Harris et al. 1979; Kraemer 2004) shows the highest known $\log\beta$ value, 39.5 (Table 14.1), meaning that it is the most stable siderophore complex known; in other words, ferric species in contact with this siderophore will prefer the chelation route over any other, leading to the enterobactin–Fe(III) complex. Enterobactin forms hexadentate chelates by coordinating to iron in a “salicylate mode,” i.e., using a carbonyl adjacent to a phenolic OH (Correnti and Strong 2012).

3.2.3 Hydroxamate Siderophores

Hydroxamate siderophores have drawn a lot of attention as they compose one of the largest and most efficient categories (Ahmed and Holmström 2014). They contain hydroxamic acid moieties (Fig. 14.2c) which bear relatively high acidities (comparable to phenols), thus allowing the formation of hydroxamate ion, which is the actual chelator. Hydroxamic acids have been systematically studied since the 1970s, based on their “unexpectedly” not-so-low acidities (Agrawal 1979; Crumbliss 1990; Yang et al. 2006). Aceto-hydroxamic acid has been used as a model compound for

Table 14.1 Selected iron(III)chelators with some of their most important properties

Chelator	Origin	$\log(\beta\text{Fe}^{\text{III}})$	$\log(\beta\text{Fe}^{\text{II}})$	$E^0_{(\text{Fe}^{\text{II}}/\text{Fe}^{\text{3+}})^{\text{a}}}$	$[\beta(\text{Fe}^{\text{3+}})/\beta]$	$E_{1/2, \text{mV}}$ (vs NHE)	pFe	$\log(\text{K}_{\text{sol}})^{\text{a}}$	Denticity of chelate
<i>Siderophores</i>									
Enterobactin	<i>Escherichia coli</i>	49	23.91	-0.71		-750	35.5	14.62	Hexadentate
Pyoverdine	<i>Pseudomonas</i> spp.	30.8	9.78	-0.47		-510	27	6.97	Hexadentate
Ferrichrome	<i>Aspergillus</i> spp. <i>Penicillium</i> spp. <i>Ustilago sphaerogena</i>	32.0	9.91	-0.53		-440	25.2	5.45	Hexadentate
Desferrioxamine B	<i>Streptomyces pilosus</i>	30.6	10.29	-0.43		-468	26.6	6.83	Hexadentate
Desferrioxamine E	<i>Streptomyces</i> spp.	32.5	11.16	-0.49		-477	27.7	7.87	Hexadentate
Aerobactin	<i>Escherichia coli</i>	22.5	4.86	-0.27		-336	23.3	3.67	Tetradentate
Alcaligin	<i>Rhodotorula pilimanae</i>	32.3	12.3	-0.41		-	23.0	8.28	Binuclear tetradentate
Rhodotorulic acid	<i>Rhodotorula pilimanae</i>	31.2	10.6	-0.45		-	21.8	7.6	Binuclear tetradentate
<i>Model hydroxamate chelators</i> ^b									
Aceto-hydroxamic acid		28.29	11.2	-0.24		-293	12.5	3.68	Hexadentate
N-methylaceto-hydroxamic acid		29.4	11.2	-0.3		-348	16.2	6.87	Hexadentate
Hydroxamic acid- iron		46.3	26.3	-0.41		< -450	20.94		Hexadentate

Compiled data from (Schwertmann 1991; Boukhalfa and Crumbliss 2002; Kraemer 2004; Hider and Kong 2010; Cézar et al. 2014)

^aValues calculated from Eq. (14.6)

^bRefer to text, in “The stability of siderophore–iron complexes”

the study of related siderophores; its iron(III) complex shows significant stability with a $\log\beta$ value of 28.3 (Fig. 14.1a) (Boukhalfa and Crumbliss 2002).

The *desferrioxamines* (often known as deferoxamines) A, B, and E are among the most well-studied hydroxamate siderophores, carrying three such groups which form 1:1 hexadentate complexes with iron(III), called *ferrioxamines* (Fig. 14.1b); these show very high $\log\beta$ values 32.0, 30.6, and 32.5, respectively (Konetschny-Rapp et al. 1992; Edwards et al. 2005; Sharpe et al. 2011).

Acid dissociation plays here, too, a significant role. The typical pK_a values of hydroxamate siderophores are generally at 8.5–11 (Singh et al. 2015). In particular, the pK_a values of desferrioxamine B, one of the most studied, are 8.32, 9.16, 9.94, and 11.44, meaning that their anionic forms (hydroxamate ions) are gradually produced above the corresponding pH values, and therefore effective complexation may occur in this region.

Denticity is another ruling factor. Rhodotulic acid, alcaligin, and bisucaberin form tetradentate chelates; the former has relatively lower stability (β value 31.2), while alcaligin and bisucaberin possess higher stabilities (β values 32.3 and 32.3, respectively) due to their closed ring molecular geometries which are pre-arranged for efficient coordination to iron (Crumbliss 1990; Konetschny-Rapp et al. 1992; Hider and Kong 2010). This closed ring effect is more pronounced for the hexadentate desferrioxamine E, ($\log\beta$ value 32.5). Possibly, the most studied hydroxamate siderophore is desferrioxamine B, an open-ring, hexadentate chelator with $\log\beta$ value of 30.6, which has also been considered for conservation-related applications (Rapti et al. 2017; Albelda-Berenguer et al. 2019). All above structures are listed in Fig. 14.2.

3.2.4 Mixed Type Siderophores

A large number of siderophores carry more than one type of functional group. For instance, aerobactin (see structure in Fig. 14.2d) contains three carboxylate and two hydroxamate groups, which allow complexation with $\log\beta$ of 22.5 (Neilands 1995; Miethke and Marahiel 2007), which is considered as a relatively low stability constant (cf. $\log\beta_{Fe(III)}$ values in Table 14.1). The participation of the groups in complexation varies significantly over pH, with three protonation steps below 4.5 (carboxylates) and two steps at over 9 (hydroxamates) It is significant to note that complexation starts from pH value as low as 1, where half of the total iron is complexed (Harris et al. 1979).

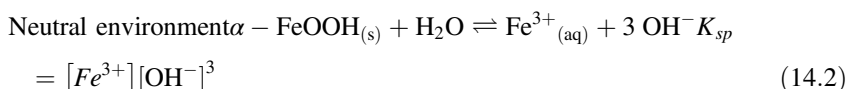
Quite often, mixed type siderophores contain amino acids. For instance, pyoverdine incorporates glycine, serine, aspartic acid, ornithine, and diaminobutyric acid, as well as succinic acid and catechol moieties; in these, α -hydroxy-carboxylate, catecholate, and hydroxamate groups are located, all participating in complexation (Boukhalfa and Crumbliss 2002; Boukhalfa et al. 2006; Albelda-Berenguer et al. 2019). Pyoverdine has pK_a values 2.72, 3.48, 4.97, 6.40, 8.12, 9.56, 10.24, and 12.88. As a result, complexation may start at low pH values, while at neutral pH almost all iron is complexed (Boukhalfa et al. 2006).

3.3 The Action: Dissolution of Iron Oxides and Hydroxy-Oxides

3.3.1 Acidic Dissolution of Iron Species

Dissolution of iron oxides and hydroxy-oxides or their transformation into a water-soluble form is necessary for the cleaning of iron-stained objects. The following discussion attempts to present the scientific basis on which the action of siderophores is critical for the dissolution of iron and allowing its availability for nature's nutrition systems as well as for our applications.

In the absence of a chelator, the solubilities of iron oxides, hydroxy-oxides, and hydroxides are characterized by their ion dissociation in water and depend significantly on the pH. The soluble form is the iron(III) aquo complex, $\text{Fe}^{3+}(\text{H}_2\text{O})_6$, sometimes described as $\text{Fe}^{3+}_{(\text{aq})}$. At neutral pH, the ionic solubility of iron oxides, hydroxy-oxides, and hydroxide can be expressed by using goethite ($\alpha\text{-FeOOH}$) as example, according to Eq. (14.2) (Cornell and Schwertmann 2003).



From Eq. (14.2), the solubility of iron (expressed as $[\text{Fe}^{3+}]$) can be calculated; for this, selected values can be found in Table 14.2, which can be as low as 10^{-13} mol/L (Crumbliss 1990; Cornell and Schwertmann 2003; Kraemer 2004). The lowest the $[\text{Fe}^{3+}]$ concentration, the least soluble and bioavailable is the oxide or hydroxy-oxide. The low solubilities of iron oxides and hydroxy-oxides at neutral pH consequently demand strong dissolution strategies in applications aiming at iron uptake. Specifically, goethite and hematite are the least soluble compounds and despite their

Table 14.2 Selected properties of some iron compounds considered targets for siderophores

Name	Chemical structure	Iron oxidation state	Crystal structure	Solubility, [Fe] at pH 7 ^a
Hematite	$\alpha\text{-Fe}_2\text{O}_3$	III	Rhombohedral hexagonal	$10^{-8.8}$ – $10^{-5.8}$
Maghemite	$\gamma\text{-Fe}_2\text{O}_3$	III	Cubic/tetragonal	$10^{-5.0}$
Wüstite	FeO	II	Cubic	–
Magnetite	Fe_3O_4	Mixed II+III	Cubic	–
Bernalite	$\text{Fe}(\text{OH})_3$	III	Orthorhombic	–
Ferrihydrite	$\text{Fe}_5\text{O}_7(\text{OH})_4 \cdot \text{H}_2\text{O}$	III	Hexagonal	$10^{-3.1}$
Goethite	$\alpha\text{-FeOOH}$	III	Orthorhombic	$10^{-10.9}$ – $10^{-5.9}$
Akaganeite	$\beta\text{-FeOOH}$	III	Monoclinic	–
Lepidocrocite	$\gamma\text{-FeOOH}$	III	Orthorhombic	$10^{-5.2}$
Ferroxhyte	$\delta\text{-FeOOH}$	III	Hexagonal	–

^aValues from Schwertmann (1991)

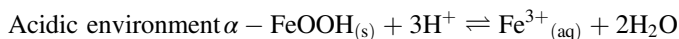
high abundance in the environment, their bioavailability is extremely low because of their low solubilities; as a consequence, their mobilization (or bioavailability through dissolution) becomes a real problem. Also, the same compounds, either as corrosion products or as unwanted iron stain factors, are among the most common iron species, and drastic tools for their removal through dissolution, such as citrate and cystine have been proposed (Kolar et al. 2006; Henniges et al. 2008; Sharpe et al. 2011; Liu et al. 2017).

At acidic pH, the solubility for goethite can be expressed according to Eq. (14.3). The acidic dissolution constant (K^H_s) values are relatively high as compared to neutral dissolution ones (expressed by K_{sp} values); this means that in order to effectively solubilize such iron species and formation of the water-soluble Fe^{3+} ion, the pH needs to be significantly lowered ($\text{pH} < 5$). However, this can be quite prohibitive for substrates in many cultural heritage objects for which pH values of 6–8 are generally acceptable; on the other hand, this may be a workable region in the case of materials, such as East Asian lacquer, which are stable in acidic pH. The aquo complex of iron [$\text{Fe}(\text{HO}_2)_6^{3+}$] is also very acidic (Siddall and Vosburgh 1951; Milburn and Vosburgh 1955; Sapieszko et al. 1977; Wilkins 1991; Cotton et al. 1995), and therefore, its presence may lower the solution pH. Moreover, iron(III) can initiate Fenton reactions with detrimental effects on organic substrates (see below).

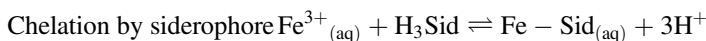
To complete the picture, the kinetics of dissolution (studying *how fast* the phenomenon occurs) is equally important as the thermodynamics (studying *how stable* the chemical species are). Kinetics is directly linked to the mechanism of iron dissolution, which has been studied and has been shown that it is heavily proton dependent. This happens because, as H^+ ions are adsorbed on the mineral surface, the surface Fe^{3+} -OH bonds weaken and the detachment of iron ions is facilitated (Stumm and Furrer 1987; Cornell and Schwertmann 2003). In particular, the initial dissolution of iron(III) is very fast and is subsequently followed by a slower step (Samson and Eggleston 1998; Reichard et al. 2007). This affects the way siderophore molecules work which is able to capture a “burst” of liberated Fe^{3+} ions during this first step. This proton-mediated process has been found to control all types of dissolution, including the action of siderophores (see below).

3.3.2 Dissolution of Iron Species in the Presence of Siderophores

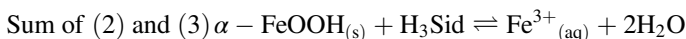
The stability of siderophore–iron complexes: In the presence of a siderophore, the complexation of iron occurs, which is expressed in Eq. (14.4); this involves an acidic environment as protons (H^+) are produced during complexation; however, summing these two equations lead to the combined dissolution reaction, shown in Eq. (14.5) (Kraemer 2004).



$$K^H_s = \frac{[\text{Fe}^{3+}]}{[\text{H}^+]^3} \quad (14.3)$$



$$K_{\text{Sid}} = \frac{[\text{Fe} - \text{Sid}][\text{H}^+]^3}{[\text{H}_3\text{Sid}][\text{Fe}^{3+}]} \quad (14.4)$$



$$K^H_{s,\text{Sid}} = \frac{[\text{Fe} - \text{Sid}]}{[\text{H}_3\text{Sid}]} \quad (14.5)$$

where Sid is siderophore, K^H_s the acidic dissolution constant, K_{Sid} the complex stability constant (often symbolized as $\log\beta$), and $K^H_{s,\text{Sid}}$ the combined dissolution constant. It appears that the overall process in Eq. (14.5) for siderophores, such as desferrioxamine B is thermodynamically stable over a wide range of pH (Kraemer 2004; Kraemer et al. 2005); this has obvious consequences for the usage of such siderophores in their applications.

Focusing on the hydroxamate siderophores, smaller hydroxamic acids, often called “model molecules,” are typically used in chemical methodology to better understand the action of more complex, but similar molecules. In this context, chelate complexation by model hydroxamic acids, as compared to that of desferrioxamine B siderophores, can be shown in Fig. 14.1.

For complexation to occur successfully, all energy (or *enthalpy*) of molecules and ions involved in the destruction of goethite Eq. (14.2) and the formation of the siderophore complex Eq. (14.3) need to decrease. On the other hand, the very strict arrangement of the complex formed in Eq. (14.3) involves increase of molecular ordering (or decrease in *entropy*); keeping in mind that entropy reflects the disordering of all molecules and ions involved, its decrease imposes a geometric difficulty to complexation. In other words, the decrease in enthalpy is favorable for the reaction, while the decrease in entropy is disfavoring; however, it appears that the contribution of enthalpy is much bigger, and therefore, the complexation occurs successfully.

However, the contribution of entropy is not negligible, and this can explain the more favorable action of cyclic or corona-type siderophores, such as DFO-E, which is more stable (higher $\log\beta$ value) than the non-cyclic DFO-B (Crumbliss 1990). Figure 14.1 shows the chelation reactions of a model hydroxamic acid (Fig. 14.1a) in comparison to DFO-B (Fig. 14.1b) and DFO-E (Fig. 14.1c); in the latter case, the entropy factor is comparatively more favorable. Of the three, desferrioxamine E (a corona-type, or cyclical molecule) is “pre-arranged” for a favorable chelation; therefore, the final complex has similar cyclic geometry to the precursor and therefore, entropy only *slightly* decreases. The stability constants of chelation are mathematically linked to both enthalpy and entropy (Crichton 2019), the combined

consideration of enthalpy and entropy finally explains why DFO-E ($\log\beta = 32.5$) is better complexing agent toward iron(III) than DFO-B ($\log\beta = 30.6$), by almost two orders of magnitude, or 100 times.

The role of pH: As has already been mentioned above, carboxylate siderophores (Sect. 14.3.2.1) can form complexes in relatively low pH values, while hydroxamates (Sect. 14.3.2.3) at generally are higher than 8–9. Finally, mixed siderophores, such as pyoverdine (Sect. 14.3.2.4) may form their anionic forms at a much larger span, ranging from 2.7 to 12.9, meaning that complexes can be produced in significantly lower pH. Therefore, optimal complexation is clearly depending on the acidities in each case. As a result, the simultaneous consideration of the $\log\beta$ values and the deprotonation constants may not allow a straightforward evaluation of the chelation effectiveness of siderophores towards iron(III).

As a more practical approach, the concentration of dissolved iron (expressed as pFe) has been proposed to reflect more objectively the effectiveness of complexation (Harris et al. 1979; Albelda-Berenguer et al. 2019). The pFe, defined as $-\log[\text{Fe}_{\text{free}}]$, has been introduced for iron (and accordingly, pM for metals in general), at certain pH, metal and ligand concentrations. High pFe values mean low free iron concentration and therefore effective complexation. For instance, the high $\log\beta$ value of desferrioxamine B (30.6) corresponds to reasonably high pFe value (26.6); on the other hand, aerobactin, although with significantly lower $\log\beta$ (22.5), has relatively high pFe (23.3) suggesting that it can still adequately complex iron(III). On the other hand, aceto-hydroxamic acid with $\log\beta = 28.3$ has low pFe (12.5). Values of pFe for various siderophores are shown in Table 14.1.

Selectivity of siderophores towards iron (III): The selectivity of complexation regarding metal ions is an obvious demand for several fields, including that of conservation. Cultural heritage objects often contain metal ions as pigments, corrosion products, depositions, etc., where the selective removal of iron(III) calls for drastic chelating strategies; these generally involve considerable differences for stability constants of chelators towards ferric compounds as compared to other metal ions. In other words, selective cleaning action is needed where the conservator aims to remove a specific metal ion/stain/corrosion product without affecting others.

The drive for choosing iron(III) chelators better than EDTA and DTPA emanates from the fact that these two widely used reagents complex effectively a wide range of metals, such as aluminum, cobalt, nickel, copper, and manganese, which all are present in cultural heritage objects. On the other hand, siderophores show a comparative preference towards iron, which makes these chelators a suitable choice for selective complexation of iron(III) towards other metals. Ferric ion (Fe^{3+}) is a hard Lewis acid (see Sect. 14.3.2 for a brief introduction of the hard-soft acid base concept) and therefore shows a high affinity for hard Lewis bases such as most siderophores (Pearson 1973; Crumbliss 1990; Jensen 1991).

In addition, it has been shown that siderophores are particularly sensitive to the electronegativity (χ_M) of metal ions as well as their charge (Z); the value of $\chi_M \cdot Z$ for Fe^{3+} is 5.9, while for Al^{3+} and Cu^{2+} is 4.9 and 4.0, respectively. As a result of the above, the stability constants of DFO-B towards iron(III) is by far higher than any other metal ion ($\log\beta = 30.7$), followed by aluminum ($\log\beta = 23.1$), Cu^{2+}

Table 14.3 Comparison of overall stability constants (β) between representative chelators towards common metal ions

Metal	Stability constants (β)		
	DFO ^a	EDTA ^b	DTPA ^c
Fe ³⁺	30.7	25.1	27.5
Fe ²⁺	7.2	14.3	16.0
Ca ²⁺	9.0 ^d	10.6	10.9
Ni ²⁺	10.9	18.4	20.2
Cu ²⁺	14.1	18.8	21.1
Zn ²⁺	10.1	16.5	18.4
Cd ²⁺	7.9	16.5	19.9
Al ³⁺	23.1	16.4	–

^aAdapted from (Shenker et al. 1996; Hernlem et al. 1996; Harris 2015)

^bHarris (2015)

^cValues from Rivers and Ummey (2003)

^dValue from Keberle (2006)

⁺($\log\beta = 14.1$), Zn²⁺ ($\log\beta = 10.1$) (Shenker et al. 1996; Hernlem et al. 1996). For comparison reasons, values of stability constants for other chelators towards common metal ions are given in Table 14.3. These values can be used to predict the effectiveness of cleaning solutions to selectively chelate metals.

3.3.3 Effects of Siderophores on the Reduction of Iron(III)

A significant issue underlying why we mostly care about the presence of iron(III) in cultural heritage artifacts is the fact that it is readily reduced generally to iron (II) through complex chemical cycles involving deleterious reactive intermediates; because of this, iron(III) is often termed as “active iron” (Bulska and Wagner 2005), responsible for significant damage in cellulosic materials (see Sect. 14.4.1). Therefore, from the conservator’s point, stabilizing iron(III) may prevent deleterious cycles as the Fenton reaction on this type of substrates.

Iron(III) species may readily be reduced to iron(II); these two forms represent a couple involving the oxidizing agent and the corresponding reducing agent, respectively; these are termed the *redox couple* iron(III) /iron(II). As a measure of the tendency for the iron(III) \rightarrow iron(II) reaction, the reduction potential (E_0) measured in Volts (25°C, 1.0 M aqueous solution) is a critical factor. In aqueous solution, the reduction potential of Fe³⁺ \rightarrow Fe²⁺ is $E_0 = +0.77$ V, which means that this reduction is a spontaneous process in solution (Cotton et al. 1995, Cornell and Schwertmann 2003, Crichton 2019). Generally, the higher the E_0 value, the higher the tendency for reduction of iron(III) to iron(II). On the other hand, lower E_0 values mean lower tendency for reduction; even more, when these values are negative, the tendency is minimal and the iron(III) form is stabilized (Cotton et al. 1995; Pepper and Gentry 2015).

Controlling iron(III) reduction through siderophore chelation: In the light of the above, the tendency for reduction of iron(III) may change for other forms such as

iron oxides and chelate complexes. In particular, siderophore chelates of iron(III) show negative reduction potentials, meaning that iron(III) is practically not reduced to iron(II); for instance, values for DFO-B and enterobactin are -478 and -750 mV, respectively, meaning that enterobactin is a superior stabilizing agent and that reduction of iron(III) is no longer spontaneous, unless a very strong reducing agent is available (Dhungana and Crumbliss 2005).

Microorganisms and plants after complexing non-bioavailable iron(III) in its oxides and hydroxy-oxides, they need to utilize it as nutrient, often by reducing it to a more available form (see Sect. 14.3.3.4). For this, exceptionally strong reducing agents are needed, with negative reduction potentials (for instance, NADH, or hydrogen-nicotinamide dinucleotide, with $E_0 = -320$ mV) to override the complex stability and finally rendering it in the more bioavailable iron(II). This strategy succeeds in many cases, but fails for the more stable complexes, such as the enterobactin–iron(III) complex (Boukhalfa and Crumbliss 2002). This is a desired situation for applications such as conservation, because mild reducing agents (such as polyphenols) are typically present in cellulosic materials, which consequently are not strong enough to reduce iron from its siderophore complexes.

Very negative E_0 values, such as those mentioned, do not allow unwanted redox reactions, such as the Haber–Weiss cycle and more particular the Fenton reaction (see Sect. 14.4.1) which produces hydroxyl radical ($\bullet\text{OH}$) and the superoxide ion ($\bullet\text{O}^{2-}$), which are highly reactive oxygen species, harmful to organic substrates (Hider and Kong 2010). For the benefit of conservation practice, a conservator needs to be aware of processes such as the Fenton reaction, which is responsible for most of damage in cellulosic materials; because of these, strategies for the decrease for unwanted redox reactions are direly needed (Dhungana and Crumbliss 2005; Albelda-Berenguer et al. 2019). The importance of this process in conservation will be discussed in the following section.

Careful choice of complexing agents towards iron involves the selection of chelators with suitable β values for their iron(III) and (II) chelates. It has been proposed that the higher the ratio of the β value of siderophore complex of iron (III) ($\beta^{\text{Fe(III)-Sid}}$) with respect to that of iron(II) ($\beta^{\text{Fe(II)-Sid}}$), the better the suppression of the unwanted redox cycles (Boukhalfa et al. 2006; Albelda-Berenguer et al. 2019).

$$E_{\text{Fe(III)-Sid}} = E_{aq}^0 - 59 \log \frac{\beta^{\text{Fe(III)-Sid}}}{\beta^{\text{Fe(II)-Sid}}} \quad (14.6)$$

Selected $\log\beta$ values towards iron(III) and iron(II) are shown in Table 14.3. Other strong iron chelators, such as EDTA notoriously fail to prevent Fenton reactions, as the β values between the two iron species are not very different. This is a strong motif towards a better practice concerning the removal of iron from organic substrates.

3.3.4 The Kinetics and Mechanisms of Siderophore Action

The thermodynamic stability of complexes (often called thermodynamic stability) and the rates of reactions such as dissolution and complexation have been already encountered in Sect. 14.3.3.1. The thermodynamics of siderophore-mediated iron(III) and the corresponding chemical equilibria of complexation do not offer a complete picture of the process and the conditions that favor it unless the kinetics are equally considered (Römheld 1991). As already mentioned in Sect. 14.3.3.1, understanding goethite removal from substrates is critical to comprehend the necessity for potent chelators. This has been recognized in conservation, as it has been recognized in living systems.

The rates of goethite dissolution in the absence of chelator are depending on acidity. It has been shown that the detachment of iron from its mineral surface is triggered by an H^+ -depending mechanism (Samson et al. 2000). It appears that pH plays a significant role in dissolution rates, which are related to the mechanisms of iron removal from its crystal lattice sites on goethite and other minerals (Monzyk and Crumbliss 1979; Brink and Crumbliss 1984).

A mechanism has been proposed, generally involving three steps. It has been found that during the first step, which is the faster, siderophore molecules of the solution are consumed, thus decreasing their local concentration around iron spots; this may negatively influence the overall effectiveness of complexation in an application, unless high concentrations of siderophores are employed (Hider and Kong 2010). In the subsequent steps, gradual complexation occurs, involving bidentate and hexadentate complexes of iron(II) in a progressive mode (Furrer and Stumm 1986; Kraemer 2004; Reichard et al. 2007).

Specifically in the first step, the complexation process involves competition between the chelator and protons provided by the solution towards iron(III). A similar competition has been observed when a second chelator (or a co-chelator) is added. This is an interesting outcome at the application level: as the rates of complexation by siderophores are favored by acidity, as well as, synergistically to other agents as co-chelators, such as oxalic acid (Brink and Crumbliss 1984; Crumbliss 1990; Cheah et al. 2003; Kraemer 2004).

Regarding applications in cultural heritage, the above mechanistic considerations, in combination with what has been presented in the context of thermodynamic stability of siderophore–iron complexes may impose extra difficulties regarding the effect of acidity, and they consequently demand for careful design of siderophore-based strategies.

Dechelation of captured iron(III): The stability of siderophore complexes does not allow for their easy destruction (often called *dechelation*). The iron-uptake process by microorganisms and plant roots from their immediate geo-environment eventually involves a later stage for releasing chelated iron. This involves specific mechanisms, as the exceptionally high stabilities of the chelate complexes do not allow for easy complex dissociation, often called *dechelation* (Boukhalfa and Crumbliss 2002; Dhungana and Crumbliss 2005).

Although this is a desired property regarding conservation—related applications, living systems need (and finally succeed) to achieve dechelation, because complexed iron, although soluble, is still non-bioavailable. The reduction strategy by bacteria involving potent reducing agents (such as NADH) has been presented above; in conservation, although reducing agents in substrates such as wood are generally milder, this needs to be taken into account for targeted cleaning strategies.

This is achieved by proton-assisted mechanisms and/or by reducing iron(III) to iron(II), where the corresponding complex of the latter is less stable by many orders of magnitude (Table 14.3). The proton-assisted dechelation mechanism has been studied extensively and showed that dechelation rates significantly increase at lower pH values. Although the siderophore complexes are extremely stable, in low pH can be labile, this factor has been found to favorably affect dechelation pathways for the benefit of living systems. In the case of iron stain cleaning, pH needs to be seriously considered regarding the balance between iron capture by chelation and release by dechelation (Boukhalfa and Crumbliss 2002).

3.3.5 Side Reactions, Synergistic Effects, and Light Sensitivity

The Fenton-type reactions that were mentioned in the Sect. 14.1 are of significant concern for the various applications as they act detrimentally to most substrates. While chelators such as EDTA do not prevent Fenton reactivity of iron(III) (Albelda-Berenguer et al. 2019), it has been reported that siderophores, such as desferrioxamine B and enterobactin do not favor this type of unwanted side-reactions.

A synergistic effect between dicarboxylic acids and siderophores has been observed; increase of iron(III) dissolution rates through complexation by desferrioxamine B in the presence of oxalate was observed (Reichard et al. 2007; Loring et al. 2008; Albelda-Berenguer et al. 2019). The similar accelerating effect was observed with other dicarboxylic acids, such as fumaric, malonic, and succinic; on the other hand, citric acid was found to inhibit the rates, possibly due to adsorption competition effects (Reichard et al. 2007).

Light also seems to play a significant role in the final dechelation stages and the bioavailability of iron in marine systems. In particular, some iron(III) complexes with marine siderophores have been found to dechelate the metal through a photolysis mechanism which leads to the final release of ferrous ions (Hider and Kong 2010). Studies using aquachelin (a mixed type siderophore contains hydroxamate and α -hydroxy-carboxylate groups) have been shown to react photochemically towards releasing an iron(III) photoproduct complex, which undergoes a ligand-to-metal charge-transfer process, resulting in the reduction of iron(III) to iron (II) (Barbeau et al. 2001, 2003). The latter is easily released and therefore, available to the benefit of iron-starving organisms. On the reasonable basis that photolytic processes may affect the effectiveness of applications employing iron(III) chelation by siderophores, this issue merits further investigation for other siderophore types as well.

4 Application of Siderophores in Conservation

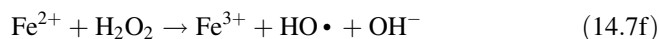
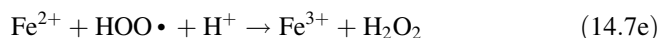
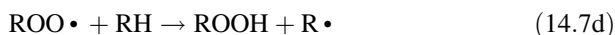
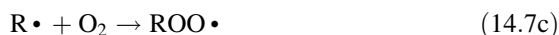
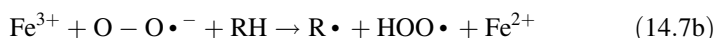
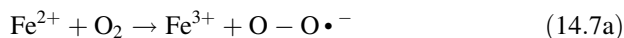
4.1 *The Detrimental Effect of Iron*

Museum artifacts such as furniture, toys, musical instruments, tools, household utensils, manuscripts, and religious items are often consisted of organic materials, such as wood, textile, paper, leather, and elements made of iron. When these composite objects are become wet or are exposed to humid environments, their iron elements corrode, and the formed corrosion products may accumulate on the surface of the organic substrate or they may impregnate its matrix.

The most common iron corrosion products formed are iron(III) (ferric) and iron (II) (ferrous) oxides, such as Fe_2O_3 , Fe_3O_4 , and FeO , hydroxy-oxides such as FeOOH , occurring in various polymorphs, often also called oxy-hydroxides and iron hydroxide $\text{Fe}(\text{OH})_3$. Iron corrosion products generally exhibit very low solubilities, which depend on their grain size, since the smaller the size the higher the solubility. Therefore, for some compounds such as hematite and goethite, solubility increases, as their grain size can be extremely small ranging between 10 and 150 nm. In addition, other hydroxy-oxides of iron(III), such as ferrihydrite ($\text{Fe}_5\text{O}_7(\text{OH})\cdot 4\text{H}_2\text{O}$), may also exist as corrosion products and are considered significant participants in the overall iron corrosion process (Kraemer 2004). Hematite (red), magnetite (black), and goethite (pale brown-yellow, beige) have also been historically used as pigments. In Table 14.2, a list of the most abundant forms of iron compounds found on cultural heritage objects is presented (Cornell and Schwertmann 2003; Scott and Eggert 2009; Richards et al. 2012).

The destructive effect of iron on organic substrates, which can be either cellulosic or proteinaceous, has been the subject of several studies. Metal ions, when found in proximity to organic substrates, take part in redox reactions and act as catalysts to oxidation processes of these substrates. These processes are a wide variety of chemical reactions leading to the formation of various deterioration products and to depolymerization of main chemical components of organic substrates and consequently to the reduction of their mechanical strength (Baker 1980; Timár-Balázs and Eastop 1998; Strlič et al. 2001; Scott and Eggert 2009; Badillo-Sanchez et al. 2019).

The oxidation of organic materials is probably caused by the free hydroxyl radical formation and hydrogen peroxide, which allow the Fenton-type reactions to occur. Thus, during the oxidation of ferrous iron to its ferric state, the free radical formation might be enhanced if organic substrates are present and this in turn leads to the formation of organic radicals, as seen in Eqs. (14.7a)–(14.7d), or to the formation of hydrogen peroxide, as seen in Eqs. (14.7e)–(14.7f) (Neevel 1995). The hydrogen peroxide produced during oxidation of organic substrates is decomposed by reacting further with ferrous ions and forms additional hydroxyl radicals, according to Eqs. (14.7e) and (14.7f).



The latter equation is often referred to as “*Fenton reaction*” (Gupta et al. 2016), although there is still considerable controversy among chemists in the reaction mechanism and the products obtained from that reaction. Some researchers claim that the *Fenton mechanism* involves hydroxyl radicals (OH•) formation, whereas others report the generation of other oxidizing iron species, such as ferryl ion (FeO²⁺) (Wardman and Candeias 1996; Strlič et al. 2001; Barbusiński 2009). Nevertheless, the mixture of hydrogen peroxide and the ferrous ion is called the *Fenton reagent* and it is a strong oxidant of organic materials, such as wood, textile, and paper. Its oxidative properties depend on the hydrogen peroxide concentration, the ratio of ferrous ion to hydrogen peroxide, the pH, and the reaction time (Barbusiński 2009). The severe oxidation mechanism of organic substrates has been intensely studied and the catastrophic role of hydroperoxides and the hydroxyl free radicals (OH•) has been also demonstrated (Emery and Schroeder 1974; Zeronian and Inglesby 1995; Kolar 1997).

Based on the above it becomes clear that the removal of iron corrosion products from museum artifact combining organic substrates, such as wood, textile, paper is an urgent issue during the conservation processes.

4.2 *The Problem of Conventional Chelators’ Use in Conservation*

There are many published studies on the removal of iron corrosion products from substrates, such as paper, textile, stone, metal, paintings, waterlogged wood by using chelating agents such as citric acid, di-ammonium and tri-ammonium citrate, EDTA (ethylene-diaminetetraacetic acid), DTPA (diethylene-triaminepenta-acetic, or pentetic acid) (Hart 1981; Hofenk de Graaff 1982; Banik and Ponahlo 1983; Slavin 1990; Burgess 1991; Häkärä 1992; Phenix and Burnstock 1992; Chapman 1997; Margariti 2003; Rivers and Ummey 2003; Almkvist et al. 2005; Fors 2008). However, those substances are not always giving consistent results concerning their efficacy in removing iron corrosion products and as mentioned previously they lack specificity for iron and promote unwanted side reactions due to their tendency for the production of hydroxyl radicals. Furthermore, there are several

studies giving considerable attention to the environmental impact and the biodegradability of conventional chelators, such as EDTA and DTPA, which are extensively used, in the conservation field, especially on waterlogged objects (Sillanpää 1997; Nörtemann 1999; Grčman et al. 2001; Nörtemann 2005; Kołodzyńska 2011). EDTA, however, has been found to be more persistent than DTPA in degradation by conventional biological and physicochemical methods (Nörtemann 1999; Grčman et al. 2001; Nörtemann 2005). Although DTPA is more biodegradable, it has been reported that EDTA can be one of its decomposition products (Sillanpää 1997). Nonetheless, both EDTA and DTPA may result in several risks when released to the aquatic environment, as they may contribute to toxicity at significantly low concentrations (Sillanpää 1997; Grčman et al. 2001). Additionally, their degradation products can be used as a nitrogen source for algae growth and thus EDTA and DTPA have the potential to contribute to eutrophication (Sillanpää 1997; Kołodzyńska 2011).

4.3 *Applications of Siderophores in Conservation of Cultural Heritage Objects*

The tendency nowadays is to find safer alternative materials and methods for conservation purposes. This tendency though, becomes a necessity, considering the established national legislations and international environmental restrictions (Balliana et al. 2016) and therefore the replacement of conventional chelators with new ones with improved biodegradability, is critical.

There are a few studies regarding the removal of iron corrosion products from cultural heritage objects, with “green” compounds such as siderophores (Wagner and Bulska 2003; Rapti et al. 2015, 2017; Albelda-Berenguer et al. 2019). These studies have investigated the application of siderophores, mainly desferrioxamine B (DFO-B), on different organic substrates and preservation states. The application methodologies followed by both siderophores and conventional chelators were not similar and therefore comparative analysis of the obtained results cannot be done.

4.3.1 **Extraction of Iron from Historical Manuscripts With Iron Gall Ink**

Bulska and Wagner (2005) dealt with historic manuscripts written with iron gall inks¹ that contained a substantial amount of “active” iron(II) ions, which can act as

¹Historical recipes for gall ink production involve the use of aqueous solutions of iron(II) sulfate with extracts of gall nuts to form complexes of oxidized iron(III) with gallic acid. However, most of historical manuscripts contain an excess of iron(II) ions that were not complexed and thus are active iron(II) ions that can act as catalysts to oxidative degradation processes of paper.

catalysts and are responsible for promoting the degradation processes of paper cellulose via Fenton reactions. In order to prevent this paper degradation process, which is often accompanied by acid hydrolysis among other deteriorative mechanisms, Bulska and Wagner (2005) tried to find a cleaning treatment to remove the excess of active iron ions. The treatment was expected, without altering the iron gall ink color, to impede both Fenton reaction and acid hydrolysis leaving the paper of the manuscripts unaffected by the cleaning procedure. Therefore, they comparatively evaluated conventional chelators versus siderophores for their efficiency of iron extraction on mock-ups mimicking historical manuscripts with iron gall inks and on mock-ups simulating only the amount of active iron ions. Chelators were required to demonstrate low cleaning efficacy on the first mock-up type and concurrently high on the second type of mock-ups. Moreover, they attempted to simultaneously neutralize the acidity of papers (deacidification) during the cleaning treatment.

Several physicochemical analyses were implemented in order to design the cleaning methodology and to evaluate the efficacy of the cleaning treatments, including Scanning Electron Microscopy (SEM), X-ray Fluorescence Spectrometry (XRF), Graphite Furnace Atomic Absorption Spectrometry (GFAAS), Inductively Coupled Plasma Spectrometry (ICM-MS), Molecular spectroscopy (UV/VIS), X-ray Absorption Near-Edge Structure (XANES) (Bulska et al. 2001; Wagner et al. 2001a, 2001b; Wagner and Bulska 2003; Bulska and Wagner 2004; Wagner and Bulska 2004).

The chelators used for iron extraction and iron complex formation included bidentate ligand acetylacetone, dibenzoylmethane, diethylene-triaminepenta-acetic acid (DTPA), the potassium-magnesium salt of phytic acid and the mesylate salt of DFO-B. All compounds were selected due to their inhibition effect of further formation of hydroxyl radicals and to their high stability constants of complexes with iron as it was expected to be more effective and phytate, a known compound for its beneficial iron deactivation effect, as it reduces iron ability to undergo further reactions and occupy all coordination sites of iron (Neevel 1995). The solutions of the examined chelators were prepared at various concentrations (0.001, 0.005, and 0.01 M) and pH values in the range of 7–9, in addition to that obtained after the chelators' dissolution in water (low pH < 3). Ethanol/water solutions (10, 25, and 50%) of DTPA, phytic acid, and DFO-B were also examined at 0.005 M. The mock-ups were immersed in the various chelators' solutions for 20 min (Bulska et al. 2001; Wagner et al. 2001b; Wagner and Bulska 2003).

Results obtained by physicochemical analyses showed that iron–DFO-B complex formation at both types of mock-ups was above 80%. Moreover, on mock-ups mimicking the excess of active iron ions, DFO-B revealed an increase of iron extraction, which depended mainly on the pH of solution. The concentration of all prepared solutions of chelators was pronouncedly higher than the amount of iron ions need to be extracted. In particular, at pH 8, a value typically used in paper conservation to enhance deacidification, and solution concentration of 0.005 M, iron extraction reached ca. 91%. Among all the tested pH, the lowest extraction of 67–72% was observed at the highest concentration 0.01 M. Concerning applications on mock-ups simulating manuscripts with iron gall ink, DFO-B with a concentration

of 0.005 M also showed high extraction capacity which reached 83 and 85%, at pH 8 and 9, respectively (Wagner and Bulska 2003). However, this was considered a drawback because iron extraction was not selective, as the color of iron gall ink was severely affected. Consequently, the most effective solution for removing excess iron was proved unsuitable for iron gall ink mock-ups.

The influence of pH on siderophores' efficacy has been also the subject of several environmental studies unrelated to conservation. Borer et al. (2009) among others (Cheah et al. 2003; Borer et al. 2005; Reichard et al. 2005; Loring et al. 2008) investigated DFO-B and aerobactin in order to improve the understanding of these siderophores biogeochemistry and to gain information about siderophores reactivity towards the dissolution and mobilization of iron hydroxy-oxides, such as goethite and lepidocrocite, which are often found in terrestrial and aquatic environments. Their study revealed that pH influences the iron adsorption in ferric compounds, for instance, in lepidocrocite via DFO-B and that was also associated with siderophores' functional group and their deprotonation stage. More specifically, they showed that the maximum absorption of lepidocrocite by DFO-B occurred at pH 8.6, which is close to its first pKa (8.3), whereas at lower or higher pH values, the adsorption was decreased.

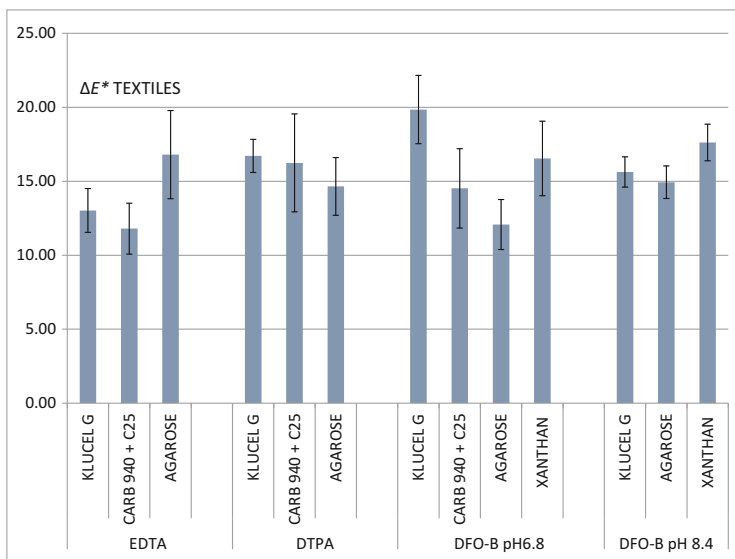
4.3.2 Extraction of Iron Compounds from Composite Museum Objects

Rapti et al. (2015) investigated in a preliminary study the removal of iron ions from dry wooden substrates, which cannot tolerate waterborne treatments, due to the hygroscopic and anisotropic nature of wood. Desferrioxamine, in the form of a commercial injectable drug, Desferal® manufactured by Novartis, was compared with the conventional chelators EDTA and DTPA to treat wooden mock-ups artificially stained with iron corrosion products. To avoid immersion in aqueous solutions and minimize the amount of water that would penetrate into the wood and at the same time maintain the cleaning potency of the solution applied, the chelators were applied by cotton swab and carboxymethyl cellulose gel (CMC) 4.5% w/v. The effectiveness of the two conventional chelators was also examined with the addition of a reducing agent, sodium dithionite (SDT) (Selwyn and Tse 2009). The solutions of EDTA and DTPA were prepared at 2.5% w/v with or without the addition of SDT (5% w/v) at pH 6.2, and 6.8, respectively, whereas DFO-B concentration was 5×10^{-5} M, at pH 8.5. Colorimetry and elemental analysis by Energy Dispersive X-ray (EDX) spectroscopy were implemented before and after cleaning for evaluating the efficacy of the chelators. Results showed that DFO-B was an effective chelator, as efficient as EDTA when SDT was added to it. However, residues of Na and S ions were detected, owed to SDT, which have the potential to promote future deterioration of substrates and therefore, this suggest the need to avoid SDT as part of the chelators solutions on this type of substrate; thus, siderophore was considered more appropriate. Conventional chelators when applied without the reducing agent were shown clearly less efficient.

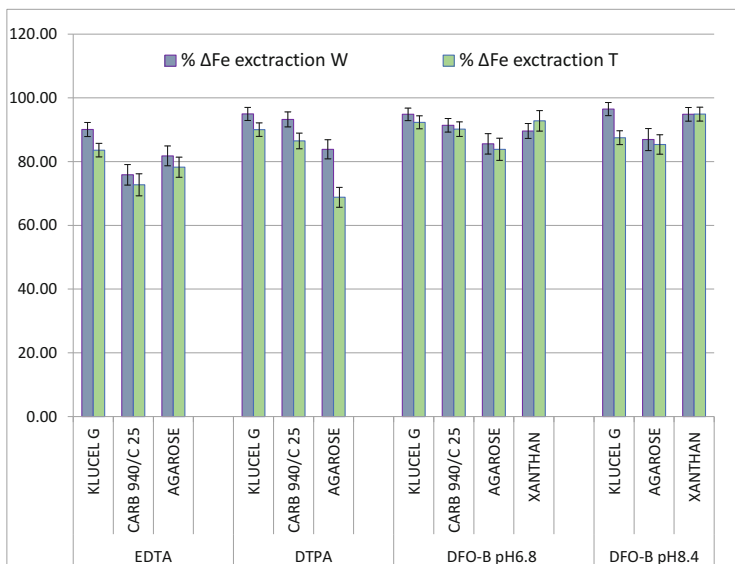
Further comparative investigation of Desferal®, EDTA, and DTPA was undertaken by Rapti et al. (2017). In this study, the cleaning potential of chelators combined with gel formulations when applied on dry composite objects comprising of wood, textiles, and iron was examined. Gel formulations were selected as they could be easily applied, clean the substrate in a more controlled and selective way and at the same time minimize the diffusion of the aqueous solution within the wood without though reducing its cleaning efficiency. Moreover, the use of gels has the advantage of avoiding dismantling the composite object, as cleaning can be achieved selectively at the stained substrate. For that purpose, wood and textile mock-ups were artificially stained with iron corrosion products. Chelator solutions (Desferal®, EDTA, and DTPA) were prepared (3×10^{-2} M), and accordingly applied with several gel formulations (Klucel G 10% w/v, agarose A0701 4% w/v, Carbopol 940 2.5 w/v neutralized by Ethomeen C25 15 v/v and xanthan gum 2.2 w/v). The pH of Desferal® was adjusted to 8.45 and 6.8, of EDTA to 6.2 and DTPA 6.8, according to their acid dissociation constants and to the substrate compatibility in order to avoid deterioration processes caused by the cleaning procedure. The time of application was not set, since it varied depending on the color change caused by complexation. The gel was removed after colored complexes were formed (EDTA ferric complex is pale yellow, DTPA complex is yellow, while DFO-B is orange-red) (Almkvist et al. 2005; Dominguez-Vera 2004); the process was repeated with fresh identical gels until complexation was no longer observed, followed by a rinse with swab wetted in 50% v/v ethanol aqueous solution. The effectiveness of all chelators was assessed by colorimetry and energy dispersive X-ray (EDX) spectroscopy before and after the cleaning procedure.

The colorimetry measurements (L^* , a^* , b^*) showed that the L^* and b^* coordinates increased in all samples after cleaning, indicating that samples became brighter and yellower. In contrast, a^* factor was reduced due to the partial removal of the red-brown iron corrosion products. The total color difference (ΔE^*) indicates the cleaning efficacy; the higher the difference the more effective cleaning of mock-ups. Regarding the textile mock-ups, DFO-B at pH 6.8 showed the greatest change when gelled with Klucel G, followed by xanthan gum (Fig. 14.3a). In contrast, DFO-B at pH 8.4 exhibited the second highest ΔE^* of all the cleaning methods employed, when applied with xanthan gum instead of Klucel G. Moreover, the DTPA application provided similar ΔE^* to DFO at pH 8.4, whereas EDTA was the least effective, with the exception of the agarose gel. Although the results of ΔE^* values of both substrates suggested that the gelling agents were actually involved in the effectiveness in cleaning efficacy, it was not possible to draw any conclusions, as the results obtained were not consistent for all chelators. Analogous results on the chelators' efficacy were obtained for wooden mock-ups.

Furthermore, elemental analysis by Energy Dispersive X-ray spectroscopy (EDX) demonstrated the weight percent of the detected iron before and after the cleaning of wood and textile mock-ups (Fig. 14.3b). More specifically, DFO-B at both pHs (6.8 and 8.45) proved superior to EDTA and DTPA in its performance for removing iron corrosion products, and this is consistent with its higher stability constant. In contrast to colorimetry measurements, EDX results were more



(a)



(b)

Fig. 14.3 (a) ΔE^* colorimetry values obtained after cleaning textile mock-ups with different chelators and gel formulations; (b) EDX results of iron extraction demonstrated by the weight percentages of iron difference (ΔFe) before and after the cleaning of wooden and textile mock-ups with different chelators and gel formulations, based on Rapti et al. (2017). *W* wood; *T* textile

consistent and showed that the efficacy of the chelators in iron extraction was influenced by the gelling agents. In the case of DFO-B at both pH values, EDTA and DTPA, gels prepared with Klucel G seemed to be more effective than other gelling agents.

Finally, as observed by EDX and confirmed by colorimetry results, wood was cleaned more effectively than the textile and this is possibly due to the fact that iron oxides have been impregnated deeper within the textile fibers (Glenn et al. 2015). Although iron corrosion products were not completely removed from either substrate, the overall appearance of both mock-ups was improved.

4.3.3 Extraction of Iron Oxides and Iron Sulfides From Waterlogged Wood

Albelda-Berenguer et al. (2019) under the framework of the MICMAC project (microbes for archeological wood conservation) investigated the production and subsequent application of siderophores in extraction and stabilization of sulfur and iron compounds found in waterlogged archeological wood. Pyoverdine (produced from the fluorescent *Pseudomonas putida*) was employed as the selected siderophore, due to the fact that its chromophore (see Fig. 14.2d) offers an additional asset for its spectrophotometric detection. This was studied along with Desferal® and EDTA on mock-ups made of balsa wood that were artificially impregnated either with iron oxides or iron sulfides through immersion for 24 h in each chelator solution (20 mM, pH 7). The formation of iron complexes was visually evaluated, based on the fact that each iron–chelator complex acquires a unique color upon formation (Dominguez-Vera 2004; Almkvist et al. 2005). More specifically, pyoverdine changes from yellow-green to red-brown upon iron(III) complexation (Hohnadel and Meyer 1986). Repetition of the process with new solutions is needed to be applied to promote further iron extraction. Colorimetry measurements were conducted on the wooden mock-ups after the cleaning process. Results showed that mock-ups impregnated with iron sulfides were more efficiently cleaned than those prepared with iron oxides, which remained almost unchanged. Pyoverdine was showed to be the most effective for the iron sulfides removal during the cleaning application.

Albelda-Berenguer et al. (2019) ongoing study is focusing on the development of an application protocol employing siderophores for extracting iron oxides or iron sulfides found in waterlogged archeological wood of shipwreck timbers. Moreover, the extraction of iron from known iron minerals by the use of siderophores is also a target of this ongoing work in order to demonstrate their ability in complexing iron. In this regard, iron sulfides present in three mineral phases, mackinawite (FeS), pyrite (FeS₂), and mineral sulfur α -S8 along with iron oxides (hematite) and oxy-hydroxides (goethite) are being tested with pyoverdine, Desferal®, and EDTA at different pH. Preliminary results revealed that pyoverdine could extract iron from mackinawite, pyrite, and goethite, but is not capable to extract it from hematite (Monachon et al. 2019).

4.3.4 Other Studies with Siderophores

Besides the above-mentioned studies, other disciplines employing siderophores as possible preventive antioxidants of cellulose deterioration can be considered as indirect approaches to conservation issues (Strlič et al. 2001). The use of siderophores as antioxidants has been investigated in the food industry (Todokoro et al. 2016; Albelda-Berenguer et al. 2019). Strlič et al. (2001) studied whether DFO-B, phytate, EDTA and DTPA chelators were possible preventive antioxidants of cellulose degradation, as it typically constitutes several museum objects. Their antioxidant role was investigated concerning the formation of stable complexes and the occupation of all the available coordination sites of iron. The inhibition of hydroxyl radical production during degradation of cellulose was comparatively investigated in aqueous systems containing the above-mentioned chelators by calculating their rate constants. Their efficiency concerning their antioxidant behavior at 20°C (range tested: 20–80°C) and in the pH range from 6.5 to 8 was found to be higher for DFO-B followed by phytate and DTPA.

5 Conclusions

The research investigations on siderophores were initially conducted mostly due to their significant roles in making iron available for living systems by chelating ferric compounds from terrestrial and aquatic habitats. These roles have provided an impressive body of biological, chemical, and biochemical data which have greatly helped in introducing those agents for several applications in fields such as medicine, agriculture, environmental research, and materials science. However, despite their high potential, these materials have only recently been introduced in fields such as the conservation of cultural heritage. Nonetheless, some promising research results in this field show that the siderophores are highly useful materials due to their chelating abilities which allow for efficient and safe practices in conservation.

Siderophores, as naturally occurring chelators, have been considered as “green” chemical substances with low environmental risk. Their high affinity to iron and the potential for minimum harm to museum objects may rank them among the most promising novel materials with potentially significant uses in the conservation field. However, factors such as their complex stability constants, the working conditions (such as the pH range, interfering metal ions and light), along with kinetic considerations regarding chelation and dechelation need to be examined and balanced before these compounds are recommended for a specific use. So far, only desferrioxamine B and pyoverdine have been investigated for cleaning cultural heritage objects. Nonetheless their application although limited to few organic substrates seems to provide promising results for the effective removal of iron corrosion products. In order to develop widely accepted strategies employing these powerful agents for conservation treatments further investigation efforts are needed, mainly

concerning the understanding of their properties and optimizing their application. The following issues are among those that need to be considered:

- The formation of highly stable complexes requires a certain pH range, depending on the nature of siderophores; therefore, pH needs to be adjusted to avoid both iron dechelation and the harmful side-reactions to the object. These demands are typically ad hoc defined, based on the specific properties of each cultural heritage substrate, so that the risk for further damage due to a siderophore-based application is minimized.
- Selectivity issues towards interfering metals need also to be investigated. Balancing acidity and redox conditions can potentially increase selectivity. Moreover selectivity of one type of iron compound over others based on their solubilities, redox behavior, etc., need also to be addressed.
- Application on complex or composite materials, in particular, those incorporating inorganic components need some attention as siderophores can be beneficial for one substrate and damaging for another.
- The significant synergistic effect of similarly chelating dicarboxylic acids present can be further exploited for enhanced and optimized action. The parallel functioning of some of these acids (such as malonic and succinic) as pH buffering agents may also offer an additional asset.
- The incorporation of siderophores in gel systems needs also to be addressed and their specific action towards various substrates needs to be further investigated.
- Sensitivity to UV and visible light is a clearly underestimated issue. In addition, the fluorescence chromophores that are included in the structures of some siderophores may point toward additional applications, as well as for some extra caution.

Taking into account these issues, the optimization and fine-tuning of siderophore-based applications on wood, textile, paper, etc. are possible. The information about siderophores' efficacy, in combination with different application methodologies, should be a starting point for their future broader application in conservation.

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