The Pharmaceutical Stability of Deferoxamine Mesylate

David M. Hayes, Raymond M. Reilly and May M.C. Lee

ABSTRACT

Deferoxamine mesylate (DFO) is administered using a portable IV infusion pump for the treatment of iron overload secondary to transfusions of red blood cells in patients with thalassemia. Minimizing the number of IV infusion cassettes required by using high concentrations of DFO is important for patient convenience and compliance. Our objective was to determine the maximum concentration of DFO which would be stable in IV infusion cassettes. The stability of DFO was determined at concentrations ranging from 210-370 mg/mL while stored in IV infusion cassettes or sterile polystyrene test tubes at room temperature for up to 21 days. The DFO solutions were inspected visually and assayed for DFO concentration and ferric ion binding capacity by reversed-phase HPLC. Maintenance of sterility and apyrogenicity was also evaluated for DFO stored in IV infusion cassettes. DFO solutions maintained at least 89% of their theoretical concentration for 17-21 days when stored in IV infusion cassettes or polystyrene test tubes. Physical instability was evident over this time period, however, by the gradual formation of a white, amorphous precipitate. The identity of this precipitate is unknown but its physical properties are distinct from that of DFO. DFO solutions maintained their ability to chelate ferric ion for at least eight days and were sterile and pyrogen-free up to 14 days. These results suggest that DFO solutions 210mg/mL stored at room temperature in IV infusion cassettes maintain their pharmaceutical stability for at least one week. This study further suggests that DFO solutions up to 318 mg/mL are stable for at least one week in polystyrene containers at room temperature.

Key Words: Deferoxamine, Stability, ß-Thalassemia

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RÉSUMÉ

On administre le mésylate de déféroxamine (DFO) au moyen d'une pompe à perfusion portative afin de traiter la surcharge de fer résultant de la transfusion de globules rouges aux personnes atteintes de thalassémie. Il est important de réduire le nombre de cartouches de perfusion requises au minimum en recourant à une forte concentration de DFO si l'on veut que le patient suive les instructions et lui rendre la vie plus facile. Les auteurs ont essayé de déterminer la concentration maximale de DFO qui resterait stable dans les cartouches de perfusion. Pour cela, ils ont vérifié la stabilité du produit à une concentration variant de 210 à 370 mg/mL dans les cartouches ou des éprouvettes en polystyrène stériles à température ambiante pendant jusqu'à 21 jours. La solution de DFO a été examinée visuellement et la concentration ainsi que le pouvoir de liaison avec l'ion ferrique du médicament ont été mesurés par CLHP inverse. On a également établi la stérilité et l'apyrogénicité du DFO gardee dans les cartouches. Le DFO dans la solution garde au moins 89% de sa concentration théorique de 17 à 21 jours dans les cartouches ou les éprouvettes de polystyrène. Passé ce laps de temps cependant, l'instabilité du produit est manifeste car on assiste à l'apparition graduelle d'un précipité blanc amporphe. Sans qu'on sache exactement de quoi est constitué le précipité, ses propriétés physiques diffèrent de celles du DFO. La solution de DFO garde son aptitude à lier l'ion ferrique au moins huit jours et reste stérile et apyrogène jusqu'à 14 jours. Ces résultats suggèrent qu'une solution de 210 mg de DFO par mL gardée à la température ambiante dans les cartouches de perfusion reste pharmaceutiquement stable au moins une semaine. Cette étude suggère aussi que les solutions contenant jusqu'à 318 mg de DFO par mL gardent leur stabilité pendant au moins une semaine dans des récipients en polystyrène, à température ambiante.

Mots clés: déféroxamine, stabilité, ß thalassémie

David Hayes, BScPhm, is a Team Area Coordinator, Department of Pharmacy Services, The Toronto Hospital, Toronto, Ontario.
Raymond Reilly, MScPhm, is the Radiopharmacy Coordinator, Division of Nuclear Medicine, The Toronto Hospital and Assistant Professor, Faculty of Pharmacy, University of Toronto.
May Lee, Pharm.D., a Clinical Coordinator, Department of Pharmacy Services, The Toronto Hospital and Lecturer, Faculty of Pharmacy, University of Toronto.
Address Correspondence to: Raymond M. Reilly, Division of Nuclear Medicine, The Toronto Hospital, 200 Elizabeth St., Toronto, Ontario, M5G 2C4.
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INTRODUCTION

Thalassemia, a group of disorders that accounts for more than 30 molecular defects, is characterized by defective synthesis of one or more haemoglobin chains [1]. The haemoglobin chain that is affected is denoted by a prefix, as in ß-thalassemia [2]. Untreated patients with thalassemia suffer from severe anemia, massive hepatosplenomegaly, marked growth retardation, and bony deformations such as frontal bossing and anaxillary prominence[1]. Due to the defect in haemoglobin synthesis, these patients must receive regular red blood cell transfusions. Iron overload remains a serious long term complication of these transfusions [1].

Deferoxamine (DFO) effectively chelates ferric ion and, when administered on a regular basis, prevents the long term effects of iron overload including cardiac, endocrine and hepatic complications [3]. DFO is the primary drug used in the treatment of iron overload in patients with thalassemia[1].

In the past, initial iron chelation therapy involved nightly subcutaneous infusions of DFO; however, these were found to be cumbersome, irritating and unacceptable to many patients [2]. As an alternative, iron overload in thalassemia patients may be treated using a regimen of continuous intravenous (IV) infusions of DFO administered through central venous catheters [3]. IV infusion permits uninterrupted administration of DFO via a portable infusion pump.

The required IV DFO solutions are prepared, as follows: A 50 mL IV infusion cassette is filled with a solution of DFO in Sterile Water for Injection USP. The cassette is attached to the portable infusion pump and the required dose is administered over a period of 3.5 days. The total dose of DFO administered over this time period ranges from 10-16 g (50-70 mg/kg/24 h) [4]. Minimization of the required number of IV infusion cassettes by using high concentrations of DFO is important for patient convenience and compliance. Our objective, therefore, was to determine the maximum concentration of DFO which would be stable in IV infusion cassettes. In particular, we were interested in determining if the concentration and ferric ion binding capacity of DFO was unchanged and if the solutions were physically stable in terms of clarity, sterility and apyrogenicity over the study period.

METHODS

Reagents

All reagents used in this study were purchased from commercial sources: deferoxamine mesylate (DFO) sterile powder for injection (Ciba-Geigy Canada Ltd., Mississauga, Ont., lot no. 199900), the internal standard ciprofloxacin (Miles Pharmaceuticals, Etobicoke, Ont., Bay q3939, lot no. 2427), sodium phosphate monobasic (Fisher Scientific Co., Toronto, Ont.), methanol (HPLC grade with UV cutoff of 205 nm, Fisher Scientific), acetonitrile (HPLC grade with UV cutoff of 190 nm, Fisher Scientific), ferric chloride hexahydrate (BDH Chemicals, Poole, England, lot no. 91980/7410), and diethylenetriaminepenta acetic acid (DTPA) calcium trisodium salt (Caledon Laboratories Ltd., Caledon, Ont., lot no. 282383889). ^{59}Fe ferric chloride (specific activity >600 kBq/ug) was obtained from Frosst Radiopharmaceuticals, Dorval, Que.

Stability studies

DFO sterile lyophilized powder was reconstituted with Sterile Water for Injection USP to a concentration of either 210 mg/mL (manufacturer's recommended concentration for reconstitution), 285 mg/mL or 370 mg/mL. The DFO solutions were then stored either in polyvinyl chloride (PVC) IV infusion cassettes (Pharmacia Deltec Inc., St. Paul, MN) or sterile polystyrene test tubes at room temperature (20-23°C). Samples were analysed for DFO concentration by high pressure liquid chromatography (HPLC) at various intervals over 17-21 days.

Since reference standards of possible breakdown products of DFO were not available, an accelerated degradation study was also carried out in order to qualitatively identify these products in our HPLC analysis. DFO (210 mg/mL in Sterile Water for Injection USP) contained in an IV infusion cassette was heated to 60 °C for 20 days for this purpose.

Deferoxamine Analysis

DFO concentrations were analysed by a modified HPLC method as previously reported by Tesoro et al [5]. The mobile phase consisted of 50 parts of 10 mM sodium phosphate monobasic buffer pH 3.5, 35 parts methanol and 15 parts acetonitrile. The stationary phase consisted of a reversed phase LKB SuperPac column (Spherisorb ODS-2, 0.4 cm ID x 25 cm, LKB, Bromma, Sweden) attached to a guard column. The mobile phase was pumped through the column at a flow rate of 1 mL/min using an LKB Model 2150 HPLC pump. The column eluate was monitored at 210 nm (0-2 AUFS) by a variable wavelength UV monitor (LKB Model 2141) interfaced to a computerized integrator (Chromjet, Spectra-Physics, San Jose, CA).

Standard solutions of DFO (0.25-2.0 mg/mL in Sterile Water for Injection) were prepared fresh daily by mixing equal volumes of

DFO solutions of known concentration with the internal standard ciprofloxacin (1 mg/mL in Sterile Water for Injection USP). Samples (50 µL) of these standard solutions were immediately injected into the HPLC system using a manual injector (Rheodyne Model 7125, Cotati, CA). At the completion of the analysis, the peak area ratio of DFO: ciprofloxacin was determined from the integrator. All analyses were completed in triplicate and the mean peak area ratio plotted versus the DFO concentration in order to construct a standard curve.

Samples taken from the IV infusion cassettes or polystyrene test tubes were diluted with Sterile Water for Injection and then analysed in an identical manner to the standards. The concentration of DFO was determined by reference to the standard curve.

Ferric ion binding analysis

The iron binding capacity of the DFO solutions was determined by the ability to bind ferric chloride containing the radiotracer ^{59}Fe. DFO chelates the ferric ion in a 1:1 molar ratio and is converted in the process to ferrioxamine [2,5].

Samples of a DFO solution (250 mg/mL) stored in polystyrene test tubes for up to eight days at room temperature were mixed with an equimolar quantity of ferric chloride hexahydrate solution (0.4 mg/mL) containing 185 kBq (0.3µg) of ^{59}Fe ferric chloride. The mixture was incubated for one hour at room temperature. A 50 µL sample was then removed and mixed with 50 µL of a 1 mM solution of DTPA. The amount of DTPA was sufficient to chelate the total amount of ferric ion (regardless of the amount bound by DFO). The solution was immediately analysed by HPLC for radioactivity bound to DFO (as ^{59}Fe-ferrioxamine) or free (as ^{59}Fe-DTPA). The HPLC analysis was conducted as previously described with the omission of the internal standard ciprofloxacin and with the use of a radioactivity in-line flow through detector (Beckman Model 170, Beckman, Fullerton, CA) instead of the UV monitor. Control samples in this assay consisted of a mixture of Sterile Water for Injection and ^{59}Fe ferric chloride solution.

Physical stability

The DFO solutions contained in IV infusion cassettes or sterile polystyrene test tubes were inspected daily for 21 days for colour and clarity. The presence of particulate matter was determined by visual inspection against a black and white background. Samples were aseptically removed from a single cassette containing DFO (210 mg/mL) which was stored at room temperature for 14 days. These samples were then tested for sterility by the USP Sterility Test and for pyrogens by the USP Pyrogen Test.

Data analysis

DFO concentrations were determined as the mean ± SD of at least three replicate measurements of a single test solution. Reproducibility of the HPLC assay was assessed by the coefficient of variation in measuring the concentration of DFO. Linearity of the assay was determined by least squares regression on the peak area ratio of DFO: ciprofloxacin versus DFO concentration. Maintenance of DFO concentration was defined as a measured concentration which was within ± 10% of the theoretical DFO concentration.

RESULTS

Chemical Stability

A sample chromatogram is shown in Figure 1. The HPLC assay for deferoxamine was reproducible (coefficient of variation <6%, Table I) provided that a new calibration curve was generated daily by analysis of freshly prepared standards of DFO. The assay was also linear over the range of concentrations studied (Figure 2).

The measured DFO concentration in solutions stored either in IV infusion cassettes or sterile polystyrene test tubes at room temperature was 89-112% of the theoretical concentration for 17-21 days after reconstitution (Table II). However, when the IV infusion cassette was heated to 60°C for a period of 20 days, multiple additional peaks were observed on the chromatogram (Figure 3). These additional peaks likely represent breakdown products of the DFO.

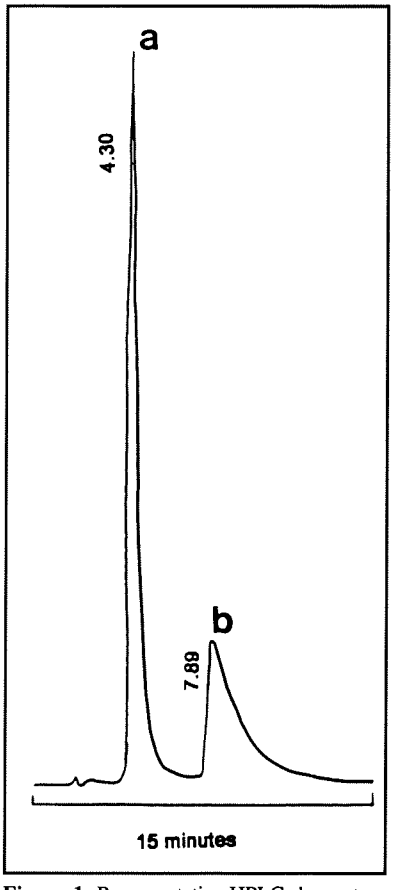

Figure 1: Representative HPLC chromatogram showing peaks for deferoxamine (**a**) and the internal standard ciprofloxacin (**b**).

Table I: Accuracy and Reproducibility of HPLC Assay

Concentration of DFO (mg/mL)	[a]Measured Concentration of DFO (mg/mL)	Coefficient of Variation (%)
210.0	202.8±11.3	5.6
285.0	253.5 ± 3.4	1.3
370.0	364.7 ± 3.6	1.0

[a]Mean ± SD, n=3

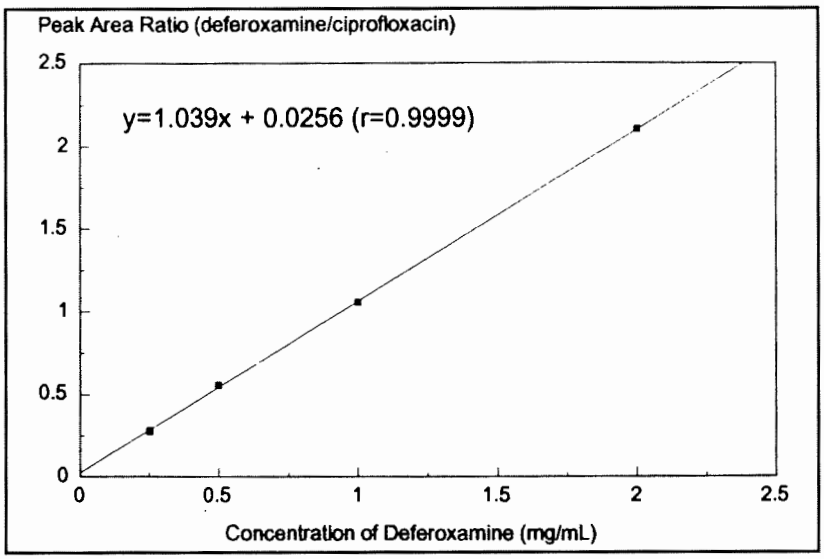

Figure 2: Representative calibration curve for HPLC assay of deferoxamine demonstrating linearity over the range of concentrations assayed.

Table II: Chemical stability of DFO Solutions (percent of theoretical concentration).

Storage Period (days)	Concentration of Deferoxamine (% of theoretical concentration; mean ± SD)		
	[a]210 mg/mL	[b]285 mg/mL	[b]370mg/mL
0	[c]96.6 ± 5.4	88.9 ± 1.2	98.6 ± 1.0
1	93.9 ± 2.3	91.7 ± 2.9	106.6 ± 11.1
3	93.8 ± 3.3	89.8 ± 1.2	[c]nd
9	112.2 ± 1.4	103.2 ± 1.1	nd
17	104.3 ± 5.1	nd	100.4 ± 1.4
20	nd	98.9 ± 7.4	nd
21	101.7 ± 2.8	nd	nd

a Deferoxamine stored in IV infusion cassettes
b Deferoxamine stored in polystyrene test tubes
c nd: no data available.

Ferric ion binding assay

There was no apparent change in the ability of DFO (250 mg/mL) to complex ferric chloride containing the radiotracer ^{59}Fe when stored for eight days at room temperature in polystyrene test tubes. The chromatogram for the mixture of DFO and ^{59}Fe ferric chloride at day 0 and day 8 showed only a single peak with retention time (t_R) of 5.6 minutes (Figure 4A and B). The t_R of this peak is different than that of DFO (t_R=4.3-4.4 minutes) and likely represents ^{59}Fe-ferrioxamine. The chromatogram for the control sample (a mixture of sterile water and ^{59}Fe ferric chloride) is shown in Figure 4C. This chromatogram also demonstrates a single peak with t_R of 2.4 minutes. Since excess DTPA was added to all samples to chelate any free ^{59}Fe ferric chloride, this peak likely represents ^{59}Fe-DTPA. The area under the ^{59}Fe-ferrioxamine peak was within 10% of that under the ^{59}Fe-DTPA peak which indicated that all of the added free ferric chloride was complexed by the DFO.

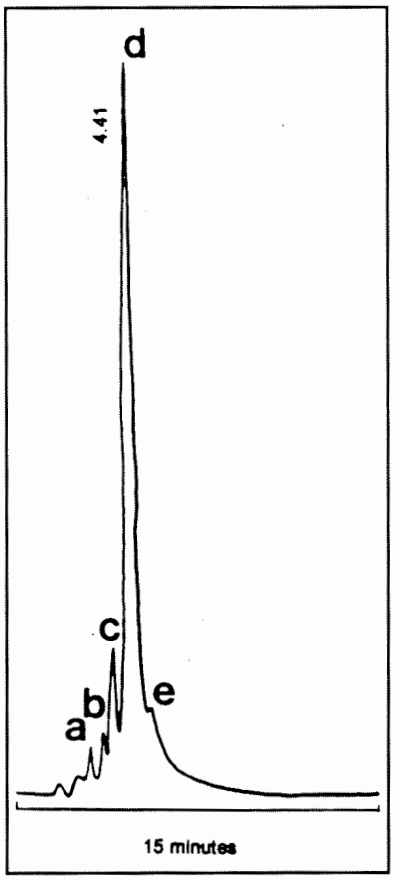

Figure 3: Chromatogram of deferoxamine solution (210 mg/mL) stored for 20 days in an IV infusion cassette at 60 °C. This accelerated stability study demonstrates the presence of possible breakdown products (peaks **a, b, c** and **e**) as well as intact deferoxamine (peak **d**).

Physical stability

Physical instability was observed for all DFO solutions stored in IV infusion cassettes and polystyrene test tubes. This was exhibited by the formation of a white, amorphous precipitate. The time taken for a noticeable amount of precipitate to form was inversely proportional to the concentration of DFO (Table III). A sample of this precipitate was isolated from the DFO solution and subjected to

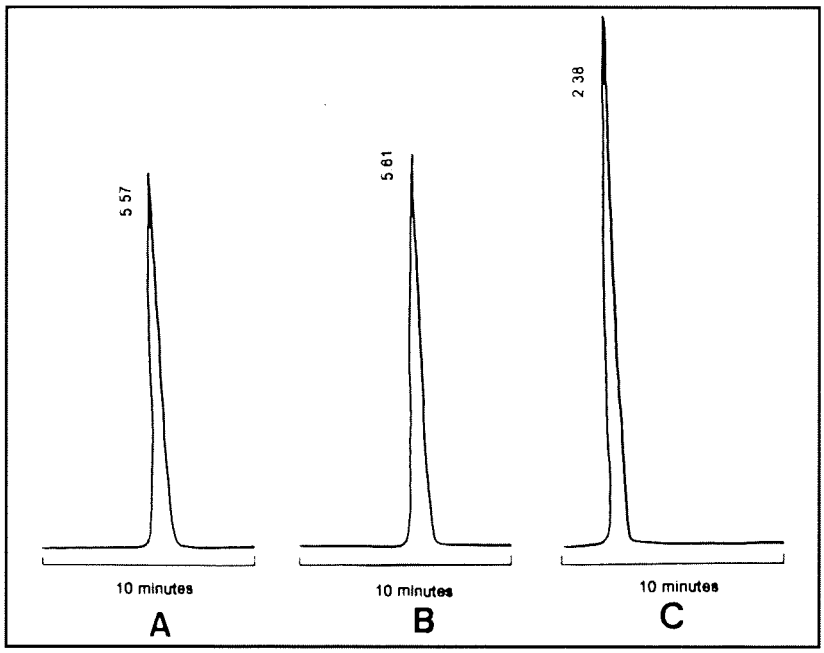

Figure 4: Chromatograms of DFO solutions (**A** and **B**) or controls (**C**) incubated with ^{59}Fe ferric chloride. There was no difference in the ability of DFO to bind ferric ion over a period of 8 days (ie. peak area in **A** is the same as in **B**). The retention time of the peaks in **A** and **B** corresponds to ^{59}Fe-ferrioxamine whereas the retention time in **C** corresponds to ^{59}Fe-DTPA. DTPA was added to all samples to complex any free ferric ion.

Table III: Physical Instability of DFO Solutions

DFO Concentration (mg/mL)	Minimum Time for Precipitation to Occur (days)
210	17
221-233	14
234-244	12
245-284	11
285-305	9
306-317	8
318-349	5
370	1

further analysis. We have previously reported the results of this analysis [7]. The precipitate was found to be insoluble in ether, chloroform and mineral oil. The melting point of the precipitate was 117-119°C which is substantially different than that of DFO (148-149 °C) [6]. The infrared spectrum of the precipitate (Figure 5B) also did not exhibit some of the absorption bands associated with DFO (Figure 5A).

Samples taken from a cassette containing DFO (210 mg/mL) which was stored for 14 days at room temperature were sterile and pyrogen-free.

DISCUSSION

The results of this study suggest that DFO, reconstituted with Sterile Water for Injection to concentrations of 210-370 mg/mL and stored at room temperature (20-23 °C) is chemically stable for 17-21 days. At the end of this time period, at least 89% of the theoretical concentration of DFO was present in solutions stored in IV infusion cassettes or sterile polystyrene tubes. Koren et al[8] also studied the stability of DFO at various temperatures and pH. They concluded that at a pH between 4 and 6 and at a temperature of 23°C or less, DFO solutions retained more than 90% of their initial concentration after a period of 30 days. However, the solutions of DFO studied were much more dilute (10 µg/mL) than those we evaluated (210-370 mg/mL). The temperature (20-23°C) and pH (5.0) of DFO solutions in this study were similar to that reported by Koren et al. [8]

DFO solutions maintained their ability to bind ferric ion for eight days when stored at room temperature. This was assessed in an ^{59}Fe binding assay which utilized the chelating agent DTPA to scavenge any free ^{59}Fe ferric chloride. The addition of DTPA was necessary to prevent precipitation of free ferric chloride on the HPLC column when exposed to the phosphate elution buffer. Others [5] have also reported problems in assaying DFO caused by binding of ferric ion in the chromatographic syringe before injection onto the HPLC system. This problem can be minimized by injecting the sample immediately after it is drawn up. We further determined that due to occasional but unpredictable changes in the chromatographic characteristics of the ciprofloxacin internal standard, it was necessary to run calibration curves daily in order to obtain accurate results in the measurement of DFO. The reproducibility of our assay was sufficient to permit a single experimental determination of DFO stability although multiple determinations have been recommended [9].

Despite the evidence of chemical stability of DFO, all of the solutions studied exhibited physical instability with the formation of a white, amorphous precipitate. The time required for this precipitate to form was dependent on the concentration of DFO: higher concentrations required less time for precipitation to occur. Since the concentration of DFO in these solutions was relatively un-

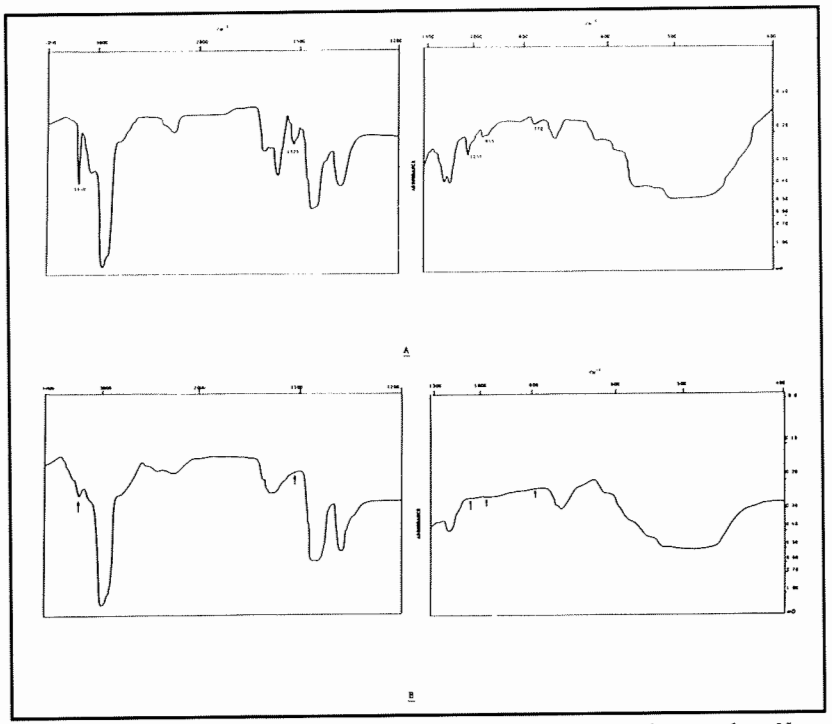

Figure 5: Infrared spectrum (mineral oil dispersion) of **A**. Deferoxamine mesylate. Note absorption bands at 700, 950, 1030 and 1525 cm^{-1} as well as sharp absorption at 3350 cm^{-1}. **B**. Unidentified precipitate. Note absence of absorption bands at 770, 950, 1030 and 1525 cm^{-1} as well as much weaker absorption at 3350 cm^{-1} (arrows).

changed despite precipitate formation, this precipitate may not be DFO but rather a chemical impurity. Further analysis of this precipitate by infrared spectroscopy and other physical tests supports this hypothesis. Due to the possibility of precipitation, caution must be exercised in establishing an expiry for DFO solutions intended for IV infusion. The data presented in Table III may be useful for this purpose.

We conclude that DFO in Sterile Water for Injection is chemically stable for at least 17-21 days when stored at room temperature. However, it is physically unstable over this time period due to the formation of an unidentified precipitate. The formation of this precipitate is the limiting factor in establishing an expiry for DFO solutions intended for IV infusion.

The results of this study suggest that solutions of DFO 210 mg/mL maintain their pharmaceutical stability for at least one week when stored in IV infusion cassettes. This study further suggests that DFO solutions up to 318 mg/mL are stable for at least one week in polystyrene containers at room temperature. However, further investigation is required to determine if this stability is also exhibited in IV infusion cassettes.

Even though DFO remains the primary drug used in the treatment of iron overload[1], numerous efforts have been made to develop a safe, less expensive, more effective and more easily administered iron chelator. A number of oral iron chelators under investigation have shown promise. Isoniazid pyridoxal hydrazone and derivatives of hydroxypyrid-4-ones have demonstrated their ability to effectively chelate iron when administered orally[10-12]. It therefore appears that an effective oral iron chelator will probably supercede DFO in the future. In the meantime, DFO will likely remain the primary drug for treatment of transfusion-related iron overload.

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