DOI:10.7598/cst2018.1467
ISSN:2278-3458

Chemical Science Transactions
2018, **7(2),** 272-281

RESEARCH ARTICLE

Differential Pulse Polarography Procedure for the Estimation of Deferoxamine in Pharmaceuticals

SALAM A.H. AL-AMERI and BAYAN H.A. AL-AMERI*

Department of Chemistry, College of Science, Al-Mustansiriyah University, Baghdad, Iraq
bayanalamry@yahoo.com

Received 1 October 2017 / Accepted 19 October 2017

Abstract: A differential pulse polarography procedure has been developed and applied for the determination of deferoxamine in dosage form with DME *versus* Ag/AgCl**.** The best peak response was establish at -1.63V in 0.04 M B-R buffer at pH 2 and 0.1 M KCl as supporting electrolyte with two electrons transferred in irreversible process**.** In the best conditions**,** Beer's law was perform with in the concentration range of 0.01- 0.05 μg.mL^{-1} with a correlation coefficient**,** r=9985**.** The obtaining LOD and LOQ was 0.0024 and 0.0081 μg.mL^{-1}**,** the precision**,** %RSD ranged between 0.34-1.24%. Another DPP method has been proposed for the estimation as deferoxamine-Fe complex which gave best DDP response at -0.385V in 0.1 M acetate buffer at pH 4 and 0.01 M KCl as supporting electrolyte**.** The calibration graph was linear at a concentration range 1.25×10^{-6} - 2.5×10^{-5} M with r =9985 also a superb analytical merit numbers were found**,** LOD of 0.7×10^{-6} M and the LOQ of 2.32×10^{-6} M with RSD% ranged from 0.27- 0.66%. The formation of this colored complex at 1**:**1 mole ratio calculated by UV-Vis spectrophotometry at λ_{max} = 468 nm in aqueous medium**.** The established DPP methods offers excellent analytical figures of merits as well as applied to examine the commercial drug Desferal™ vial for the determination of deferoxamine and found to be 490**.**6 mg/unit compared to the stated value of 500 mg/unit**.**

Keywords: DPP, Deferoxamine, Fe, Complex

Introduction

Deferoxamine (DFOA), sell under the brand name desferal; deferoxamine, $C_{25}H_{48}N_6O_8$, 560.69 g.mol^{-1}, is an *N*'-[5-(acetyl-hydroxyl-amino)pentyl]-*N*-[5-[3-(5-aminopentyl-hydroxy-carbamoyl)propanoylamino]pentyl]-*N*-hydroxy-butane diamide[1] (Figure 1)**.**

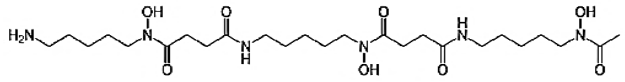

Figure 1. Chemical structure of deferoxamine

Deferoxamine effectively chelates with iron and other metals[2]**,** therefore it has been used to remove complex iron through the kidney in patients with iron excess by forming a stable complex that avoid the iron from inflowing in to more chemical reactions[3]**.** In addition**,** it's also forms a complex with aluminum[4], so this chelator has been used in the treatment

of aluminum accumulation and toxicity in dialysis patients[5]**.** Various techniques have been used for the determination of deferoxamine include spectrophotometry[6,7] HPLC[8,9]**.**

The cyclic voltammetry**,** CV at HMDE and DPP modes *versus* Ag/AgCl was applied for deferoxamine voltammetric estimation**.** The reduction potential was raises when the pH was drop off from 9.0 to 3.5. CV mode shows a one-electron reversible reduction[10] at -0.71 V in borate buffer at pH 9.0. An anodic stripping voltammetric mode for the estimation of deferoxamine, alendronate sodium and lisinopril was described; this method is based on the formation of stable drug-copper complex when mixing with copper phosphate suspension**.** The reduction peaks which related to the reduction of the copper(II) moiety of the formed complexes are obtained at -153, -74 and -111 mV respectively. The limit of detection was as low[11] as 8.6 ng**.** mL^{-1}.

The purpose of this work is to confirm the polarographic behaviour of the mentioned drug and to improve a simple and sensitive polarographic method for their determination in pharmaceuticals**.**

Experimental

Electrochemical measurements were performed by a 797VA computrace metrohm, Herisau, Switzerland polarographic analyser**.** It was used with DME mode as a working electrode and Ag**/**AgCl as a reference electrode with Pt wire while auxiliary electrode**.** All spectral and absorbance measurements during this study were done using a digital recording double–beam LABOMED**,** INC (USA) UV-Visible 2960 spectrophotometer operation at wavelength of 190-1100 nm equipped with 1cm optical path quartz cell**.** All experiments were performed at 25 °C**.**

Materials and reagents

All experiments were achieved with analytical grade reagent**,** chemicals and solvents**.** Deionised water was employ for preparation the standard and samples**.** Deferoxamine standard material was provided from the Novartis pharma AG**,** deferral vial 500 mg was obtained from local pharmacies**.**

The standard solution of deferoxamine at 250 $\mu g.mL^{-1}$ and 10^{-3} M for the direct and deferoxamine-Fe complex analysis was prepared by disolving of 12.5 and 32.8 mg of standard deferoxamine in a minimum amount of deionised water in 50 mL volummetric flask and completed to mark with deionised water**.** Diluted 50, 5 and 1 $\mu g.mL^{-1}$ standard solution deferoxamine was prepared by transferring 10 mL from 250 $\mu g.mL^{-1}$ standard solution, 5 mL from 50 $\mu g.mL^{-1}$ standard solution to 50 mL volummetric flask and diluted to the mark with deionised water. Diluted 1 $\mu g.mL^{-1}$ standard solution of deferoxamine was prepared by transferring 10 mL from 5 $\mu g.mL^{-1}$ standard solution to 50 mL volummetric flask and diluted to the mark with deionised water. Diluted 10^{-4} M standard solution of deferoxamine was prepared by transferring 5 mL from 10^{-3} M solution to 50 mL volummetric flask and completed to the mark with deionised water.

A 10^{-3} M standard solutions of ferric nitrate was prepared by disolving 5**.**9 mg of standard ferric chlorid in 50 mL deionised water**.** Diluted 10^{-4} M solutions of ferric nitrate was prepared by transferring 5 mL from 10^{-3} M solution to 50 mL volummetric flask and completed to the mark with deionised water. 1 M potassium chlorid**,** lithum chloride and potassium nitrate solutions was prepared by dissolved 7**.**45 g, 4**.**239 g and 10**.**1 g respectively in 100 mL of deionized water**.**

Acetate buffer solution, 0.1 M was prepared by mixing 9 mL of 0.1 M sodium acetate with 41 mL of 0.1 M acetic acid and completed to volume 100 mL with deionized water[12]. B-R buffer, 0.04 M solution was prepared by disolving 2.47 g of boric acid in 500 mL deionised water in 1000 mL volummetric flask then added 2.3 mL acetic acid and 2.7 mL phosphoric acid and complete the volume to the mark with deionised water.

General DPP

Deferoxamine

An aliquot volume of deferoxamine samples was transferred to 25 mL volumetric flasks, then 2 mL of 0.04 M B-R buffer at pH 2 was added with 2.5 mL of KCl as supporting electrolyte and diluted to the mark with deionized water. Each sample was transfered to a polarographic cell and degassed with high purity nitrogen for 300 s to purge the oxygen and analysis at scan rate 5 mVs^{-1} with pulse amplitude 50.

Deferoxamine-Fe complex

An aliquot volume of deferoxamine was transferred to 25 mL volumetric flasks then 5 mL 10^{-4} M ferric nitrate solution was added and 2 mL of 0.1 M acetate buffer at pH 4 with 0.250 mL of KCl as supporting electrolyte and diluted to the mark with deionized water. Each sample was transfered to a polarographic cell and degassed with high purity nitrogen for 300 s to purge the oxygen and analysis at scan rate 5 mV s^{-1} with pulse amplitude 50.

Determination of Deferoxamine : Metal ratio

The deferoxamine : metal ratio was determined in deionized water using Job method[13]. A series of solutions was prepared, each one containing the similar total number of deferioxamine and Fe(III) moles but each one contain a different ratio of deferoxamine to Fe(III) moles.

Preparation of the calibration curve of deferoxamine

A series of nine standard solutions ranged between 0.01-0.05 $\mu g.mL^{-1}$ daily prepared by transfer volumes 0.25-1.25 mL of 1 $\mu g.mL^{-1}$ deferoxamine standard solution in 25 mL volumetric flask with 2 mL of 0.04 M B-R buffer at pH 2 and 2.5 mL of 1 M KCl as supporting electrolyte, then diluted to the mark with deionized water. Each standard solution was analysis using suggested DPP method in the optimal conditions. A standard calibration graph were prepared between i_d obtained for deferoxamine against the concentration using the least squares method[14].

Preparation the calibration curve of Deferoxamine-Fe complex

A series of 1.25×10^{-6}, 2.5×10^{-6}, 5×10^{-6}, 1×10^{-5}, 1.5×10^{-5}, 2×10^{-5} and 2.5×10^{-5} M of stansard solutions were daily prepared by transferring 0.25, 0.5, 1, 2, 3, 4 and 5 mL of 10^{-4} M deferoxamine solution with 5 mL 10^{-4} M ferric nitrate solution in 25 mL volummetic flasks and 2 mL of 0.1 M acetate buffer at pH 4 and 0.250 mL of 1 M KCl as supporting electrolyte then diluted to the mark with deionized water. Each solution was transfered to a polarographic cell and analysis using developed DPP method in the optimum setting. A standard calibration graph for deferoxamine in the concentration range from 1.25×10^{-6} to 2.5×10^{-5} M were prepared between i_d find beside the concentration using the least squares method[14].

Analysis of desferal vial samples

A 5 vial contents were mixed and 25 µg were weighed and transferred to a 25 mL volumetric flask and diluted to the mark with deionized water. Appropriate solutions were

prepared by taking suitable aliquots and diluting them with deionized water**.** Each sample were analysed using developed DPP.

Results and Discussion

Optimization of DPP

The effects of a number of experimental factors which impact the DPP effectiveness were carried out by one variable at a time optimization, whereas just one factor is changed and the others are kept at a steady rank. The effect of pH solutions, buffers and supporting electrolyte were chosen in this work**.**

Polarographic measurments

DPP technique was applied for the analysis of deferoxamine**.** The DPP technique was the most sensitive than other technique. The effect of pH at valuese ranged between 2 to 9 was examined with different buffer solutions**,** its essential to buffer solutions in organic voltammetry, since mainly organic electrode process create hydrogen ions**,** if not buffered solutions are used, pH value changes can occur as the reaction proceed[13]**.** Deferoxamine has been appear one reduction peak in acidic, natural and alkali media, this peak shifted to additional negative potential with pH increase, Figure 2.

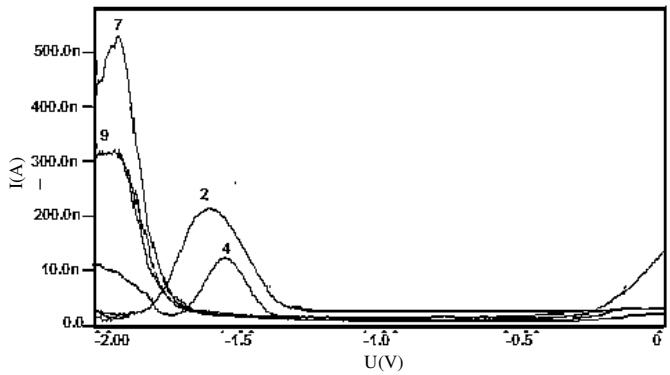

Figure 2. DPP polarograms of deferoxamine at different pH solutions

Deferoxamine showed a distinguished peak at -1.63V applied potential *versus* Ag/AgCl in 0**.**04 M B-R buffer at pH 2 as a best buffer solution, also 0**.**1 M KCl was found to be the most excellent supporting electrolyte compared with KNO_3 and LiCl, Figure 3.

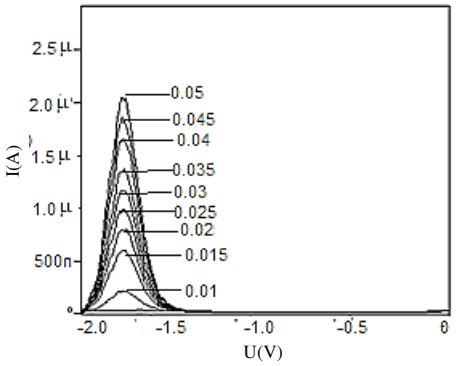

Figure 3. DPP polarograms of deferoxamine at different concentrations

DPP analysis of deferoxamine-Fe(III) complex illustrate a various peak reduction potential as pH solution increased from 2 to 9 (Figure 4), also the reduction peak was shifted to more negative potential which means more difficult than in acidic and neutral media since the organic reduction including hydrogen ion which is a little sum in the alkali media compared with other media[15].

Deferoxamine-Fe complex showed a distinguished peak at -0.385 V applied potential in 0**.**1 M acetate buffer at pH 4 as establish to be the finest buffer solution and 0**.**01 M KCl was prove to be the best supporting electrolyte compared with KNO_3 and LiCl (Figure 5). The results showed that well-defined polarograms and relatively high peak currents were obtained when scan rate 5 mVs^{-1} with pulse amplitude 50. The results demonstrate that well-defined polarograms and quite high peak currents were obtained.

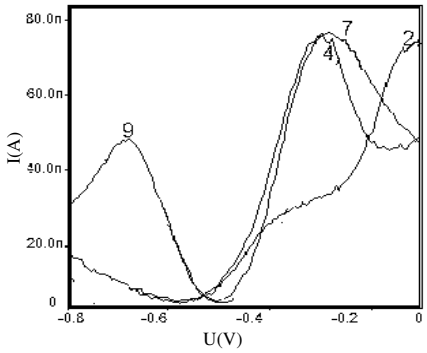

Figure 4. DPP polarograms of deferoxamine-Fe complex in different pH solutions

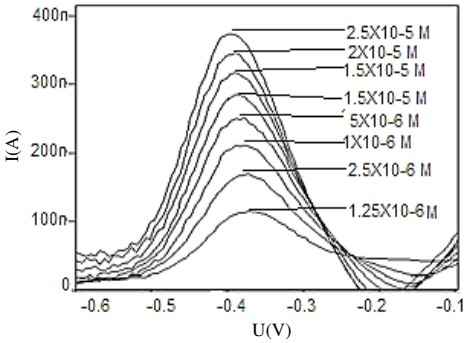

Figure 5. DPP polarograms of deferoxamine-Fe complex at different concentrations

The absorption spectra for the deferoxamine drug and its Fe^{3+}- complex against blank solution prepared under comparable conditions illustrate that the absorption maximum, λ_{max} of deferoxamine drug alone occurs in 252 nm while the Fe(III) reagent alone displays an λ_{max} at 330 nm and deferoxamine-Fe complex appeared λ_{max} at 468 nm**,** then a wavelength maximum at 468 nm for the complex was used all over this work (Figure 6).

The deferoxamine : metal ratio was determined using Job method**,** the results of L**:**M showed that the ratio was 1**:**1 which obtained by plotting the absorbance of the complex solution at 468 nm varies the mole fraction of the metal, VM /(VM +VL) (Figures 7 and 8).

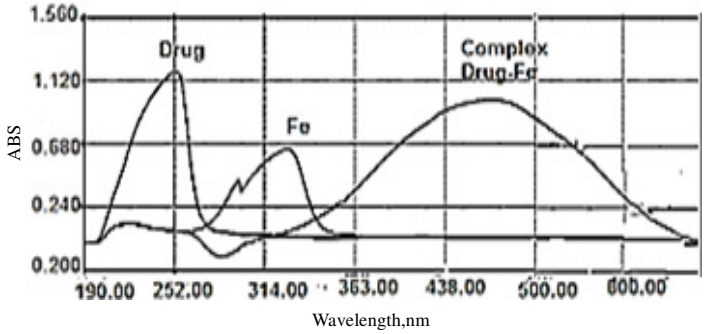

Figure 6. Absorption spectra of deferoxamine, Fe(III) and deferoxamine-Fe complex solutions

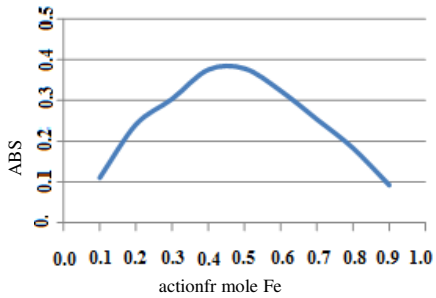

Figure 7. Job's plot of deferoxamine-Fe complex

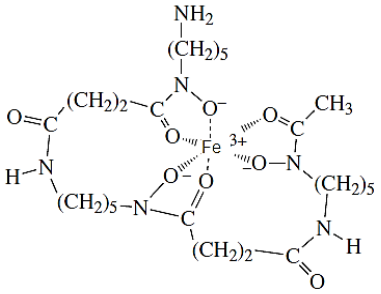

Figure 8. Suggested deferoxamine-Fe complex structure

Method validation

Under the optimized conditions, the graphical management of the peak current obtained for deferoxamine and deferoxamine-Fe complex was achieve by plotting the peak current beside the concentration of analyte solutions (Figures 9 and 10).

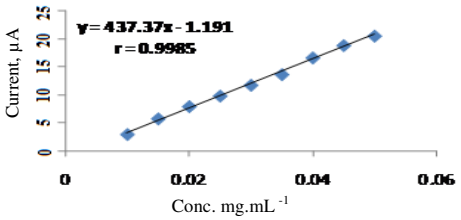

Figure 9. Standard calibration graph for Deferoxamine

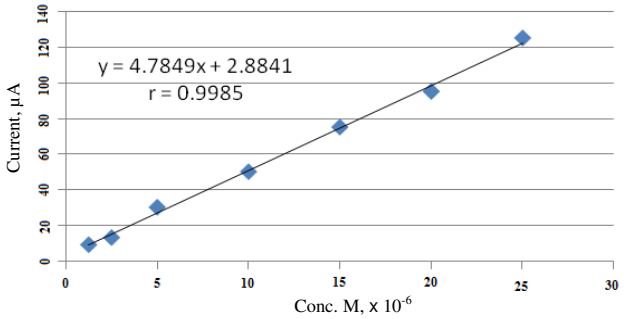

Figure 10. Standard calibration graph for deferoxamine-Fe complex

The numerical estimation for the calibration graph exposed that the linear regression equations for analyte are statistically suitable**.** The prediction based on the regression line is acceptable as listed in Tables 1 and 2; this regression line is used to estimate the deferoxamine concentrations in the selected samples which appear justified on statistical basis.

The limit of detection (LOD) and the limit of quantification (LOQ) for deferoxamine and deferoxamine-Fe complex estimated based on the standard deviation of the response**,** residual standard deviation $S_{y/x}$ and the slope b of the calibration curve using the equations $(a + 3S_{y/x})$ and $(a + 10S_{y/x})$ respectively[14]. The results obtained by the projected methods is in agreement with a few but greatly better than obtained with mainly methods in literature, the results show that the LOD and LOQ found for deferoxamine was equal to 0**.**005 and 0**.**0165 µg.mL^{-1} while 0**.**1×10^{-6} and 0**.**3×10^{-6} M for deferoxamine-Fe complex**.**

Table 1. Analytical numbers of merit of the deferoxamine estimation using DPP methods

Parameters	Values
Peak potential, E_p(V)	-1.63
Concentration range, μg.mL^{-1}	0.01-0.05
Regression equation y=bx-a	Y=437.37x+1.191
Correlation coefficient ®	0.9985
Linearity (R^2)	0.9971
Slop (b)	437.37
Intercept (a)	1.191
Standard deviation of regression line ($S_{y/x}$)	0.3537
Standard deviation of intercept (S_a)	0.262
Standard deviation of slope (S_b)	9.057
C.L. for the intercept (a ± ts_a) at 95%	1.191±0.619
C.L. for the slope (b ± ts_b) at 95%	437.37±21.374
Limit of detection- LOD, μg.mL^{-1}	0.005
Limit of quantitation – LOQ, μg.mL^{-1}	0.0165

Table 2. Analytical numbers of merit of the deferoxamine-Fe complex estimation using DPP methods

Parameters	Values
Peak potential, E_p(V)	- 0.385
Complex M:L ratio	1:1
Concentration range (M)	1.25×0^{-6} – 2.5×10^{-5}
Regression equation y=bx-a	Y= 4.7849X +2.8841
Correlation coefficient ®	0.9985
Linearity (R^2)	0.9971
Slop (b)	4.7849
Intercept (a)	2.8841
Standard deviation of regression line ($S_{y/x}$)	1.11
Standard deviation of intercept (S_a)	0.6998
Standard deviation of slope (S_b)	0.0497
C.L. for the intercept (a ± ts_a) at 95%	2.8841±1.798
C.L. for the slope (b ± ts_b) at 95%	4.7849±0.127
Limit of detection- LOD, M	0.1×0^{-6}
Limit of quantitation – LOQ, M	0.3×0^{-6}

The accuracy and precision of the method for the determination of deferoxamine and deferoxamine-Fe complex were confirmed**.** Various standard samples were prepared and analysis (n=5), Table 3 and 4 respectively**.**

Table 3. Analysis of standard deferoxamine

Initial conc. Mg.mL^{-1}	Found conc. Mg.mL^{-1}	Absolute error	%RE	%Rec	SD	$\frac{SD}{\sqrt{n}}$	C.L of the mean	%RSD
0.01	0.0096	-0.0004	-4.00	96.0	0.0008	3.5×10^{-4}	0.0096 ±0.0008	0.87
0.03	0.0286	-0.0014	-4.66	95.3	0.0003	1.3×10^{-4}	0.0286 ±0.0003	1.24
0.05	0.0491	-0.0009	-1.80	98.2	0.0001	4.4×10^{-5}	0.0491 ±0.0001	0.34

**n=5, t= 2.57*

Table 4. Analysis of deferoxamine-Fe complex

Initial conc. $\mu g.mL^{-1}$	Found conc. $\mu g.mL^{-1}$	Absolute error	%RE	%Rec	SD	$\frac{SD}{\sqrt{n}}$	C.L of the mean	%RSD
2×10^{-6}	1.97×10^{-6}	-0.03	-1.50	99.00	0.013	5.8×10^{-3}	1.97×10^{-6} ±0.01	0.66
3×10^{-6}	2.94×10^{-6}	-0.06	-2	98.00	0.008	3.5×10^{-3}	2.94×10^{-6} ±0.009	0.27
4×10^{-6}	3.93×10^{-6}	-0.07	-1.75	98.25	0.012	5.3×10^{-3}	3.93×10^{-6} ±0.013	0.31

**n=5, t= 2.57*

The proposed DPP method was applied to the determination of deferoxamine and deferoxamine-Fe complex in commercial desferal vial, 500 mg. Each sample was treated according to experimental work explained by recommended DPP.

The results show that the actual deferoxamine amounts in commercial desferal vial, 500 mg ranged between 486 to 495 mg, which are actually equal to the amount fixed in the original products. The results are presented in Table 5.

Table 5. Analysis of commercial pharmaceuticals deferoxamine sample

Desferal vial-500 mg					
Initial conc. $\mu g.mL^{-1}$	Measured Conc. $\mu g.mL^{-1}$	Measurement of drug	%Rec.	SD	%RSD
0.05	0.0495	495	99.00	0.0004	0.92
	0.0491	491	98.20		
	0.0486	486	97.35		
	0.0495	495	99		
	0.0486	486	97.35		
	av.=0.0491	av.=490.6	98.18		

Number of transferred electrons and actual $E_{1/2}$

The definite number of transfer electrons in a reversible/irrevrsible electrode procedure and the real value of E½ was designed using Heyrovsky–Ilkovic equation which clarify the cathode reduction wave as reversible process at 25 °C[16].

$$E_{applied} = E_{1/2} - (0.0591/n) \log (i/id - i) \quad (1)$$

This equation describes the relationship between diffusion current and applied potential for a reversible/irrevrsible reaction**.** Number of electrons (n) can be proved from the plot of log (i/id -i) *versus* applied voltage (E) at set group concentrations**.** For a reversible process**,** (n) appear to be a exact number, while an incomplete number for (n) show an irreversible process[17]. The actual peaks voltage $E_{1/2}$ calculated of deferoxamine and deferoxamine-Fe complex were -1.623 and -0.384 V and two electrons were required for the reduction (Figures 11 and 12)**.**

Deferoxamine has five carbonyl groups while deferoxamine-Fe complex has only two free carbonyl groups, as the results showed**,** the more easily carbonyl group reduced to give hydroxyl group with two electrons transfer (Figure 13)**.**

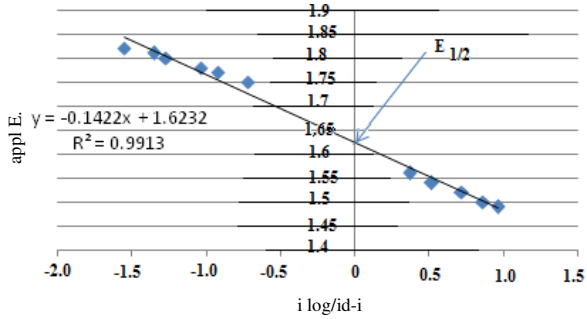

Figure 11. Effect of E applied on the variant log (i/id-i) by Heyrovsky-Ilkovic equation at 0.05 µg.mL^{-1} deferoxamine

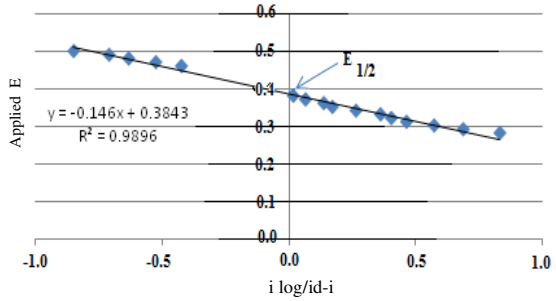

Figure 12. Effect of E applied on the difference log (i/id-i) with Heyrovsky-Ilkovic equation at 2.5×10^{-5}M deferoxamine-Fe complex

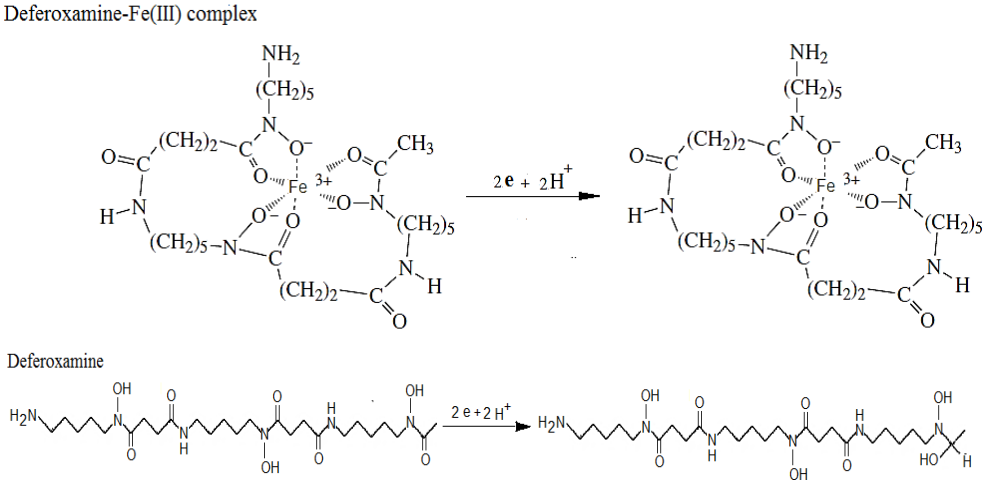

Figure 13. Suggested reduction mechanism for deferoxamine and deferoxamine-Fe complex

Conclusion

This method has clarified that DPP technique has various advantage found suitable for the determination of deferoxamine in the pharmaceutical preparations. The technique proved to be accurate, fast and precise thereby it may be considered as an other techniqe for pharmaceutical analysis.

References

1. Deferoxamine Mesylate; Retrieved on February 2018, http://www.genome.jp/dbget-bin/www_bget?dr:D01186
2. Michael L Ott, Desferrioxamine, 311C, Chemistry Botany, Department of Chemistry, University of Iowa, Iowa City, IA 52242, 12, February, 2001.
3. McLaren G D, Muir W A and Keller Meyer RW, *Crit Rev Clin Lab Sci*., 1983, **19**, 205-266; DOI:10.3109/10408368309165764
4. Snow G A, *Biochem J*., 1969, **115(2),** 199-205; DOI:10.1042/bj1150199
5. Yokel Robert A, *Coord Chem Rev*., 2002, **228(2)**, 97-113.
6. Randal J Keller, James D Rush and Thomas A Grover, *J Inorg Biochem*., 1991, **41(4)**, 269-276; DOI:10.1016/0162-0134(91)80019-E
7. Nascimento, Paulo C *et al*., *Talanta*, 2006, **70(3)**, 540-545.
8. Marcin K, Arthur S, Tomasz B, Michał P M and Ewa Z, *J Sep Sci*., 2013, **36(4),** 665-669; DOI:10.1002/jssc.201200683
9. Lehmannl W D and Heinrich H C, *Anal Biochem*., 1990, **184(12),** 219-227; DOI:10.1016/0003-2697(90)90672-V
10. Robert H and Glen D, *Bioelectrochem and Bioenergetics*, 1989, **22(3)**, 187-196; DOI:10.1016/0302-4598(89)87038-2
11. Abdel Razak O, Belal S F, Bedair M M and Haggag R S, *Talanta*, 2003, **59(5),** 1061-1069; DOI:10.1016/S0039-9140(03)00013-4
12 Chandra Mohan, Buffers- A guide for the preparation and use of Buffers in biological systems, EMD Bioscience, Germany, 3rd Edn., 2006.
13. Douglas A Skoog, Donald M West, James F Holler, Stanley R Crouch, *Fundamental of Analytical Chemistry*, 9th Ed, 2013.
14. Salam A.H. *Global Journal of Science Frontier Research: B Chemistry*, 2017, **17(1),** 27-32**.**
15. James N M and Jane C M, Statistics and chemometrics for analytical chemistry, Pearson Education Limited, Prentice Hall, 5th Ed, Person Education Limited, Edinburgh Gate, Harlow, England, 2005.
16. Salam A H Al-Ameri and Najlah M H Al-Waeli, *Int J Bioanal Methods Bioequival Stud*., 2016, **3(1),** 47-54; DOI:10.19070/2470-4490-150006
17. Joseph Wang, Analytical Electrochemistry, John Wiley and Sons, Inc., pp. 64, 2nd Ed., 2000.