SHORT COMMUNICATION

An analytical method for Fe(II) and Fe(III) determination in pharmaceutical grade iron sucrose complex and sodium ferric gluconate complex

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Abstract A robust voltammetric method has been developed and validated for the determination of Fe(II) and Fe(III) in pharmaceutical iron polysaccharidic complexes. Undesirable low molecular weight iron complexes, at concentration about 3% in the pharmaceutical formulation, can be easily determined with good accuracy and precision. This methodology can be proposed as a viable, environmentally sustainable substitute for the conventional Normal Pulse Polarographic method in US Pharmacopeia, with better analytical figures of merit, and reduced Hg consumption. A deeper insight in Fe(II) and Fe(III) composition can be gained by the combined use of a new potentiometric technique after chemical decomposition of the complex.

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1. Introduction

Most iron deficiency anemias respond well to treatment with oral or parenteral iron; in the latter case, polymeric complexes of Fe(III) with sugars, such as iron sucrose complex (ISC) and sodium ferric gluconate complex (SFGC) [1–5], are frequently used to stabilize iron hydroxide nanoparticles in the colloidal suspension [6,7]. According to USP (United State Pharmacopeia), they must contain less than 20% Fe(II) with respect to the total iron (5.3%–6.4% and 4.5%–7.0%, for ISC and SFGC respectively), and low molecular weight (MW) complexes must be undetectable by gel permeation chromatography and normal pulse polarography (NPP) [8]. The standard method reported on Pharmacopoeia, and still used, is, in fact, NPP in acetate ionic strength buffer.

In this paper, a robust voltammetric method at hanging dropping mercury electrode (HDME) for the determination of Fe(II) and Fe(III) in SFGC and ISC formulations at about 1% (w/v) concentration, is described and validated against standard USP method. This methodology combines greater sensitivity and lower limit of detection (LOD) with reduced mercury
consumptions. A redox titration method for the selective determination of Fe(II) is also reported.

2. Experimental

2.1. Reagents and chemicals

Chemicals were obtained from Aldrich and used as received. Solutions were prepared with Milli-Q grade water. Ceric ammonium sulfate was standardized against As$_2$O$_3$ by potentiometric titration. ISC and SFGC were prepared and purified according to known procedures [9,10].

2.2. Instrumentation

Electrochemical measurements were performed in a conventional three-electrode cell (volume 10 mL) with HDME as the working electrode (BASi, PWR-3 interfaced to EF-1400 controlled growth Hg electrode), Pt wire as the auxiliary electrode, and Ag/AgCl (3 M NaCl) as the reference electrode. Potentiometric titrations were performed using a Pt working electrode with an Ag/AgCl (3 M KCl) reference electrode on an Orion 520 potentiometer.

2.3. Quantitative calculations

Fe(II) in the preparations is calculated by the Eq. (1) from the NPP or differential pulse voltammetry (DPV) response ratios

\[
\left[1 - \left(\frac{2}{R}\right)^2\right] \times [Fe] = \%\text{Fe(II)}
\]

where \( R \) is Fe(II)/Fe(0) (henceforth denoted \( R_1 \)) to Fe(III)/Fe(II) (denoted \( R_2 \)) wave response ratio; [Fe] is the total iron concentration in the vial, typically 20 g/L.

According to Eq. (1), in the absence of Fe (II) in the original solution, the theoretical \( R \) value should approximate two, since Fe(III)/Fe(II) is one-electron reduction process and Fe(II)/Fe(0) is bielectronic [11], and all the Fe(II) reduced to Fe(0) derives from the reduction of the Fe(III) complex in the sample.

\[
i_{\text{lim}} = \frac{nFAD^{1/2}c}{\pi^{1/2}Dp^{1/2}}
\]

In Eq. (2), the symbols have their usual meaning, and \( i_{\text{lim}} \) is calculated from NPP wave or by integration of the DPV peak.

3. Results and discussion

3.1. Determination of Fe(II) by NPP

In the USP polarographic method for the determination of Fe(II) in ISC aqueous solutions, the half-wave potential, \( E_{1/2} \), is related to the MW of the complex [8]. The two waves at −750 mV and −1400 mV correspond to Fe(III)/Fe(II) and Fe(II)/Fe(0) reductions, respectively. The absence of additional peaks may, therefore, exclude the presence in the sample of low MW complexes at concentration higher than 5% of the total iron complex. According to Eq. (1), \( R \) values greater than two (i.e. the second wave height greater than twice the first), must be observed whenever Fe(II) is present in the initial solution. However, the waves often show poor reproducibility, in terms of current intensity and \( E_{1/2} \) values. As a consequence, the calculated concentration of Fe(II) tends to be extremely high or even negative, with no analytical significance. Such large \( R \) values are likely to be related to systematic overestimation of Fe(II), which is, instead, unlikely to occur during the preparation of ISC and SFGC, where the highly alkaline melt comprising mixture of ferric oxide and sucrose or glucose would result in a fast oxidation of Fe(II) by air. This prompted us to undertake an investigation of the electrochemical behavior of the two Fe complexes with the final aim to devise an alternative analytical method that could ensure adequate reproducibility and precision, as required in Quality Control and Quality Assurance protocols, reducing, at the same time, Hg consumption during the analysis.

3.2. Determination of Fe(II) by DPV

DPV was initially carried out using the instrumental parameters reported in the USP methodology [8], and successively optimized. The final working conditions were: LiClO$_4$ 50 g/L, SFGC or ISC 330 mg/L, step 30 mV; pulse width 100 ms; pulse period 325 ms, pulse amplitude 100 mV; scan rate 92 mV/s; \( E_{p}=0 \) V; \( E_{p}=-1700 \) mV. A two-peak voltammetric profile (Fig. 1) is observed for ISC, and peak height is shown to be dependent on ISC concentration. The peak around −700 mV is due to [Fe(III)]/[Fe(II)] reduction, whereas the second peak, around −1400 mV, is ascribed to [Fe(II)] reduction to zerovalent iron. Analogous results were observed with the SFGC.

A good linearity between current and concentration was found in the range 0.05–50 mg/L Fe for both iron.

![Figure 1](image-url)
formulations, using lithium perchlorate as a non-complexing supporting electrolyte (Table 1).

The determination of Fe(II) and Fe(III) complexes in real samples can be obtained, without any pre-treatment or separation, by standard addition method using ISC and SFGC standard solutions. Noteworthy, this method can allow the analysis of samples even if the standard solutions are not available. In fact, the $R_1/R_2$ ratio can be used to quantify Fe(II) selectively and, after determination of the total iron content by alternative methods, typically by spectroscopic techniques (e.g. ICP-OES, AAS), Fe(III) is calculated from the difference between these two values. With this simple approach, Fe(II) concentrations as low as 1% of total iron can be easily quantified.

The effect of the analytical parameters, in particular the composition of the supporting electrolyte, was found to have a deep influence on the electrochemical behavior, leading to modifications of the shape and of the ratio of the two voltammetric peaks. The best results were obtained with lithium perchlorate at a concentration of 50 g/L. The effect of pH on the electrochemical behavior was also investigated in the lithium perchlorate supporting electrolyte. As the result, no buffering of the electrolyte solution was required, provided that the pH was greater than 4.

### 3.3. Influence of free Fe(III) and Fe(II) and weaker iron complexes

In order to evaluate the effect of free Fe ions on the composition of the complexes, ferric and ferrous ammonium sulfate were added to the solution of each iron formulation and voltamograms were recorded subsequently. It was verified that the addition of free Fe(II) and Fe(III) shifted the equilibria of SFGC and ISC toward new complexes with intermediate MW [2]. Interestingly, an analogous behavior was also observed with labile Fe(II) complexes, e.g. the gluconate complex [12].

### 3.4. Validation of the proposed DPV method

The analytical method was validated according to International Conference on Harmonization (ICH) guidelines [13]. The linearity, assessed by linear regression determinations, was calculated by the least-square regression method. The calibration graphs were obtained with 10 standard solutions in the concentration range 0.05–50 mg/L as Fe, both on ISC and SFGC. The correlation coefficient ($r$) value was found to be 0.998. A set of 6 samples containing 20 µg/mL as Fe, of each pharmaceutical formulation was analyzed to assess repeatability and precision. The repeatability was evaluated by assaying samples during the same day, whereas the intermediate precision was investigated by comparing results on two different days. LOD and LOQ values for the complexes evaluated from the linear regression were 15 µg/L and 50 µg/L, for ISC and SFGC (as Fe), respectively. Recovery was evaluated by addition of known amounts of standard solutions of each drug to the commercial formulations. The spiked solutions were then analyzed by the proposed DPV method and the results were in the range 94%–117%. Similarly, the intraday and interday precisions showed good results, with percentage errors ranging between 3.2% and 4.5%.

The proposed method was then validated against the USP NPP method. The same standard samples, with an expected $R_1/R_2$ ratio of 2.0, have been analyzed by both (NPP and DPV) methods. The comparative results are shown in Table 2.

The proposed DPV method has been found statistically more precise (RSD=3.6% against 11.5% for SFGC and RDS=3.8% against 12.4% for ISC) and accurate than NPP, and, therefore, it can be directly applied to the analysis of the pharmaceutical preparations.

### 3.5. Potentiometric determination of Fe(II) in ISC and SFGC

In order to assess the total Fe(II) concentration in the samples, a potentiometric determination using Ce(IV) and ferroine as indicator, was undertaken after the complete chemical decomposition, evidenced by disappearance of color, of the complexes (200 mg) with 2 mL of a 1:1 mixture of commercial 96% H$_2$SO$_4$/85% H$_3$PO$_4$. Phosphoric acid stabilizes Fe(III) ions and leads to a sharp titration end point. Hence, Fe(II) concentration as low as 1% with respect to the total iron, could easily be quantified. This potentiometric method was compared with the DPV Fe(II) analysis, using standard ISC and SFGC samples, as well as a ISC preparation contaminated with Fe(II). Fe(II) concentration was found to be below LOD in the standard samples, and 0.061 mmol/g ISC (DPV) and 0.066 mmol/g ISC (potentiometric) in the contaminated sample. The good agreement of the results obtained by the two methods confirmed the reliability and the accuracy of the DPV proposed method for Fe(II) and Fe(III) quantification in iron sucrose and sodium ferric gluconate complexes.

| Table 1 | Voltammetric results obtained in the determination of Fe(III) and Fe(II) by calibration curves. |
|----------------------|---------------------------------------------------------------|
| Complex | Fe(III) peak | Fe(II) peak (total iron quantification) |
| ISC | $I$(µA)=0.172(2) mg/ | $I$(µA)=0.035(2) mg/ |
| | $L_{Fe(III)}=0.1(1)$ | $L_{Fe}=0.2(3)$ |
| | $R^2=0.998$ (10 data points) | $R^2=0.997$ (10 data points) |
| SFGC | $I$(µA)=0.015(2) mg/ | $I$(µA)=0.032(1) mg/ |
| | $L_{Fe(III)}=0.1(1)$ | $L_{Fe}=0.1(2)$ |
| | $R^2=0.998$ (10 data points) | $R^2=0.996$ (10 data points) |

| Table 2 | Comparison of $R_1/R_2$ values obtained by DPV and NPP methods ($n=3$). |
|----------------|---------------|---------------|---------------|---------------|
| Complex | DPV RSD (%) | NPP RSD (%) |
| ISC | 2.03 | 3.8 | 1.86 | 12.4 |
| SFGC | 2.09 | 3.6 | 1.77 | 11.5 |
| Expected value | 2.0 | 2.0 |
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