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EXTRACTIVE SPECTROPHOTOMETRIC SPECIATION OF IRON(II) AND IRON(III) USING 4-(2-PYRIDYLAZO) RESORCINOL AND 1-HEXADECYLPYRIDINIUM BROMIDE WITH THE PARTIAL LEAST SQUARE METHOD

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A novel method has been developed for extractive spectrophotometric determination of Fe(II) and Fe(III) utilising the formation of the colour complexes with PAR, i.e., 4-(2-pyridylazo) resorcinol, their instantaneous ion-pairing with 1-hexadecylpyridinium counter ion (HDP^+) in aqueous solution at pH 8.1, and subsequent extraction onto the organic phase of methyl isobutyl ketone (MIBK),

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where both ion-associates can sensitively be detected spectrophotometrically. A statistical method based on the partial least squares (PLS) has then been used to define a model between calibration spectra and the corresponding concentrations. The quantitative PLS model was proposed for absorption spectra in the 350-750 nm range from the data obtained by analysing 25 various mixtures of both iron forms. Their concentration in the calibration matrix was 0.3-1.1 ppm for both Fe(II) and Fe(III); the detection limits being estimated to be 0.09 and 0.13 ppm, respectively. The performance of the model proposed has been confirmed by the determination / speciation of Fe(II) and Fe(III) in model solutions and real samples of pharmaceutical formulations.

Introduction

Iron plays an essential role in photosynthesis being a limiting growth nutrient for phyto-plankton in some open sea and ocean regions [1]. Both Fe(II) and Fe(III) act in the biosphere, serving as active centres of a wide range of protein oxidases, reductases, and dehydrogenases [2]. It can be stated that these iron species occur together in great majority of natural materials and media, representing typical environmental and biological samples. Regarding the latter, Fe(II) is important for the transport and storage of oxygen in higher animals by means of hemoglobin and myoglobin, whilst Fe(III) does not bind to oxygen [3]. Several techniques, such as measurements in flowing streams [4,5], electrochemistry [6,7], chromatography [8], atomic spectrometry [9], and spectrophotometry [10,11] are usually being used for speciation of iron — i.e., distinguishing between Fe^{II} and Fe^{III} — in various samples.

The spectrophotometric approach is often attractive alternative also in the present day's instrumental analysis thanks to its good selectivity, often acceptable sensitivity, and mainly because of its simple equipment and low operational costs compared to other instrumental techniques. Certain drawback of spectrophotometric procedures for the determination of metal ions in aqueous solutions is associated with chemical interactions of possible trace constituents in the sample solutions as well as with some colour-rising processes; both interfering in the measurements of colour intensity *via* the absorbance. Also, one should take into account that a majority of traditional spectrophotometric (colour-forming) reagents are not highly selective [12], which then requires special masking steps incorporated into the analytical procedures, making the respective methods more complicated or sensitive to operational errors and other unwanted phenomena during the detection. In fact, interest in UV/vis photometric methods has increased and undergoing certain renaissance thanks to the application of highly effective signal processing by mathematical multivariate methods; among others, the partial least squares (PLS) regression [13-17].

This tool allows simultaneous spectrophotometric determination of several elements and improves the data handling process of complex chemical systems. Application of chemometric quantitative methods for multivariate chemical data [18,19]; particularly, that one utilising the partial least squares, is becoming widespread owing to the availability of digital spectroscopic data and still more powerful software in laboratory computers.

Each method needs a calibration, where the relationship between the maxima of the spectra in the investigated wavelength region and the concentration of a substance / component is being deduced from a set of reference samples, followed by a prediction step in which the results of calibration serve to determine the respective concentrations in the sample. The theory of PLS have been widely discussed by some authors [18,19] with respect to its applicability in spectrometry [20-22] and, subsequently, a number of the corresponding methods enabling one the multicomponent determination from spectrophotometric data have been reported; see, e.g., Refs [23-30].

Among such approaches, a spectrophotometric determination of Fe(II) and Fe(III) with 4-(2-pyridylazo) resorcinol (PAR, H_2L) has also been proposed [31-35]. This classical reagent is known to form readily intensively coloured complexes with most of transition metal ions. (Typically, in aqueous solutions, the resultant colouring is red, but it may vary between pink and red-violet; some other shades being obtainable *via* extracts with organic solvents, when one can observe also an orange or deep yellow shades [12,36].) Concerning $Fe^{II/III}$-PAR complexes and the above mentioned reports, the data on their structure(s) and acido-basic properties do not seem to be consistent. For example, Nonova *et al*. [34] reported on the formation equilibria between the $\{Fe^{II}(HL)L\}^-$ and $\{Fe^{III}(HL)L\}$ species but with no attention paid to any other iron-PAR complex(es) in the solutions studied. Russeva *et al*. [35] have confirmed the formation of Fe^{III}-PAR complexes, but without specification that the complexes of the 1 : 2 stoichiometry are predominant under the conditions used (namely, in the presence of PAR at a large excess). Finally, Hoshino *et al*. [37,38] studied in detail the extraction equilibria of both Fe^{II}- and Fe^{III}-PAR complexes paired with benzyl-dimethyl-tetradecylammonium chloride (BDTACl), noticing that the process had been hardly controllable as varying markedly with the nature of the central metal ion. The same authors continued in their spectrophotometric studies [39] but without conclusive data on possibility of the simultaneous determination of both Fe(II) and Fe(III) ions.

In this article, such an extraction followed by simultaneous determination of Fe(II) and Fe(III) in the spectrophotometric mode is proposed based on the reaction of both ions with 4-(2-pyridylazo) resorcinol (PAR and also H_2L) in the presence of 1-hexadecylpyridinium bromide (HDPB). After extraction onto organic phase of methyl isobutyl ketone (MIBK) and the subsequent spectrophotometric measurements, the data obtained are treated by the partial least squares (PLS) method. The main goal of this study is to show the usefulness of the

PLS data treatment for analysing the binary mixtures, such as Fe^{II} + Fe^{III} and their speciation in pharmaceutical formulations without any prior separation.

Experimental

Chemicals and Reagents, Solvents and Solutions

All chemicals and reagents were of analytical grade unless stated otherwise. A solution of 0.001 M 4-(2-pyridylazo) resorcinol was standardised spetrophotometrically by micro-titration with Cu(II) ions (according to a procedure described in [40]). Two stock standards of Fe(II) and Fe(III), both 100 ppm in concentration, were prepared by weighting the appropriate amounts of $Fe(NH_4)_2(SO_4)_2$A$6H_2O$ (also known as Mohr salt) and of $Fe(NO_3)_3 \cdot 9\ H_2O$, respectively. To prevent aerial oxidation of Fe(II) ions during the extraction, a small amount of L-ascorbic acid was added (at about 1×10^{-4} mol l^{-1}). The stock solution of 0.001 M HDPB was prepared from 1-hexadecylpyridinium bromide.

Had some solutions at lower concentrations been needed as well, they were prepared freshly by diluting the respective standard solutions. Triply distilled water was used throughout the experimental work.

Instrumentation, Computer Hardware and Software

Electronic absorption measurements were carried out with a spectrophotometer (model 9000, CECIL, USA), when using quartz cells with thickness of 1.00 cm. A digital pH meter (model 3345, Jenway, USA) was used for pH measurements. All absorption spectra measured in a region of 300-750 nm and registered in steps of 1 nm were digitalised, stored and then transferred in ASCII format to a PC (Dell Co., China) with Pentium III 800 MHz processor for subsequent manipulation by the PLS program. Data treatment was done in the MATLAB for Windows (Mathworks, version 6.5) in conjunction with the PLS program (for calibration/prediction and experimental design); the latter being a part of PLS-Toolbox (Eigenvector Co., USA). The data were mean-centred and scaled to the unit variance.

Preparation of the Solutions for Spectrophotometric Analysis

Typical experiment comprising the mixing of all the constituents in aqueous phase, the formation of the {HDP^+, $Fe^{II/III}$-PAR^-} ion-pairs, their extraction onto the organic solvent phase, and the subsequent spectrophotometric measurement with

the PLS analysis was carried out by the following way consisting of several consecutive sequences.

Into a 25 ml volumetric flask, a mixture of 0.175 ml 100 ppm Fe(II) + 0.175 ml 100 ppm Fe(III) + 0.135 ml 255 ppm PAR + 5 ml 0.5 M Na_2SO_4 was prepared and a volume of 0.250 ml of 385 ppm HDPB added, slight alkalinity adjusted — usually, at pH 8.1 — and the flask filled up to the volume with distilled water. (The individual concentrations of all the components of the aqueous phase had been selected empirically, when the iron-to-PAR ratio was expected to be 1:2 as found out by the molar-ratio method [35,37-39]. The ionic strength of the mixture was kept constant by adding a small portion of sodium sulphate and optimum pH controlled by adding diluted HNO_3 and/or NaOH, whenever needed.)

Afterwards, a volume of 2 ml aqueous solution was transferred into separation funnel and MIBK solvent added at a volume of 2 ml. Extraction was intensified by mechanical shaking for (usually) 12 min, followed by short quiet period to complete the separation of both phases.

Finally, the organic phase was carefully taken out, its appropriate volume transferred into the photometric cuvette, and the absorbance measured against the blank — extract without Fe^{II} and Fe^{III} — in the reference cell at a wavelength of 495 nm for Fe(II) and of 500nm for Fe(III). All the operations during the preoparation of solutions for spectrophotometric measurements were made at a constant temperature of 20 ± 0.5 °C.

The Samples and Their Analysis

Model Solutions. A mixture of the reagents spiked with 2 ppm Fe(II) and Fe(III) was prepared as described in the previous section. The proper photometric analysis was carried out in the combination with the proposed calibration method incorporating the PLS evaluation (see below). The results were calculated *via* the recovery rates, R_r.

Real Samples. Specimens with real matrix were represented by three pharmaceutical products: hematinic (i) *capsule* (manufactured by Razak Co., Iran) and (ii) *tablet* (Rouz Darou Co. Iran), together with commercially marketed (iii) *oral drops* (Kharazmi Co., Iran). The respective solutions were prepared by dissolving either capsule or tablet in 10 ml 0.1 M H_2SO_4, the resultant solutions then filtered (using Whatman No.1 paper), and both filtrates collected in 1000 ml volumetric flasks, and diluted with water up to the mark. Finally, selected aliquots of these solutions were mixed with the reagents and extracts in MIBK prepared and measured in the same way as described above. Regarding the oral drop samples, a volume of 1.0 ml of the sample was pipetted and diluted with water in a 250-ml volumetric flask, and the aliquot of 1.0 ml of so diluted solution was diluted again with water to 100 ml; the extraction and measuring steps being again the same as above.

Results and Discussion

In the following sections, spectrophotometric studies of extractive simultaneous determination of Fe(II) and Fe(III) with PAR using 1-hexadeclpyridinium bromide as counter ion in MIBK by partial least squares method are described.

Among principal experimental conditions, slightly alkaline media were chosen based on the results from analysis by the molar-ratio method; see e.g., Ref. [36]: (i) at pH 8.0 (for studying the Fe^{II}-PAR chelate) and (ii) pH 8.2 (for the parent Fe^{III}-PAR complex); see also below. The formation / stability constants, $\beta_{FeL} = [\{FeL^2\}]/[Fe]\,[L^{2-}]^2$, for both chelates are so high — namely: $\beta_{Fe^{II}PAR} = 10^{31.4}$ and $\beta_{Fe^{III}PAR} = 10^{34.2}$ (according to Ref. [41]) — that hydrolysis of the PAR chelates in such slightly alkaline solutions has been almost negligible if one ensures the concentration excess of the PAR reagent in the solution(s).

Other concurrent equilibria in common matrices (e.g., the formation of sulphate, chloride or ascorbate complexes) can also be neglected under the conditions used.

Figure 1 shows absorption spectra of Fe(II) and Fe(III) complexes with PAR and the free PAR in MIBK. Sufficiently high values of the formation constants for both chelates and the individual behaviour of PAR with Fe(II) and Fe(III) ions in extraction equilibria give rise to the spectra with well-developed absorption maxima; however, with a high degree of their overlapping.

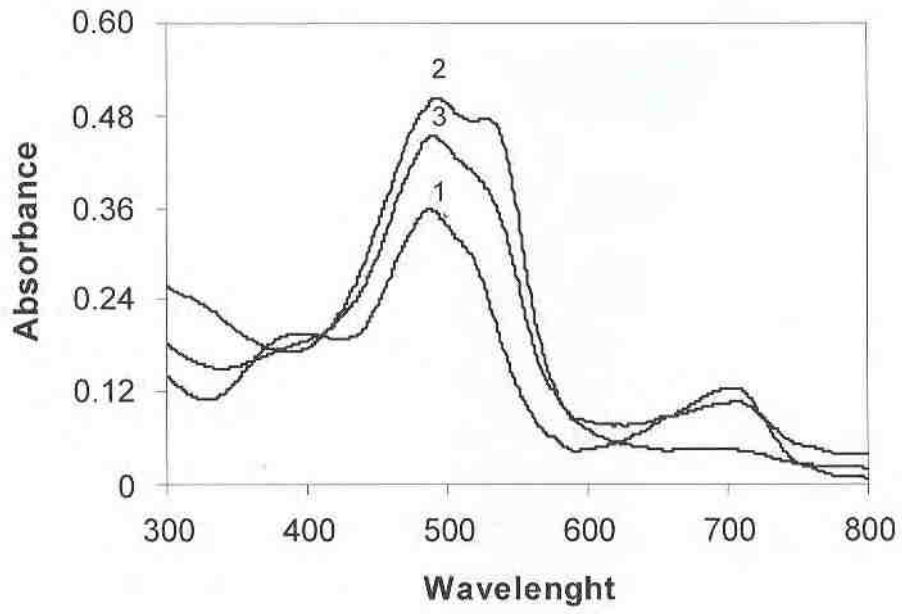

Fig. 1 Absorption spectra of the extracts in MIBK. Experimental conditions: 2×10^{-5} M PAR, 0.001 M HDPB, 1×10^{-5} M Fe^{II}; 1×10^{-5} Fe^{III}; 1) pH 8.0, 2) pH 8.2, 3) mixture of 5×10^{-6} M Fe^{II} + Fe^{III}. Legend: Wavelength [nm], Absorbance [--], enlarged scale (originally: A = 0.04-0.20)

This was also the main reason — or even necessity — for incorporating the PLS method into the procedure, thus enabling the determination of iron in its two common oxidation states; in other words — the speciation/differentiation of both forms.

Stoichiometry of the $Fe^{II/III}$-Complexes and Effect of pH on the Extraction Process

For both Fe(II) and Fe(III), the"Fe-to-PAR" ratio was found to be 1 : 2, which can be symbolised as "ML_2", with the aid of the molar-ratio method. The optimum alkalinity was found equal to pH 8.0 for Fe(II)and pH 8.2 for Fe(III), respectively, the value of pH 8.1 being applied to the simultaneous determination. The maximum wavelength was chosen at 495 nm for Fe(II) and 500 nm for Fe(III), respectively. The literature data [34,39] indicate that the proper formulas for the resultant complexes of the two iron species are: (i) $H\{Fe^{II}(HL)L\}$ and (ii) $H\{Fe^{III}L_2\}$, where L represents the active moiety of the PAR reagent.

Selection of Experimental Conditions

By considering the possibility of determining Fe(II) and Fe(III) in mixtures, optimum working conditions were separately studied for each species. Apart from the choice of pH that has already been discussed above, the effect(s) of the type of solvent, HDPB concentration, solvent volume, and the shaking time had to be investigated.

Choice of Solvent. Various extracting solvents, such as chloroform, nitrobenzene, toluene, and MIBK were investigated. The results showed that the last named was the most convenient because of high efficiency and good rate of extraction of the complexes onto MIBK and, mainly, due to the fact that the absorption background of MIBK was notably lower than those of other solvents. Hence, MIBK was the solvent of choice and used in all the subsequent experiments.

Effect of Solvent Volume. The effect of volume of MIBK on the extraction was also studied. Volumes of 0.5, 1.0, 2.0 … up to 5ml were examined, always for 2ml samples of Fe(II) and Fe(III). It was noticed the absorbance was constant in the range of (volume) 1.5-3.0 ml and, therefore, 2ml MIBK was used further on.

Effect of HDPB Concentration on Extraction of Fe(II) and Fe(III). By performing the reaction / extraction experiment, the optimum volume of the HDPB stock solution was sought in an interval of 0.182-1.365ml; the volume of 0.250 ml being found to be the value of choice. Further, as found out, the absorbance was almost constant at the concentration excess of HDPB higher than 16:1 with respect to Fe(II) and 24:1 for Fe(III); the latter corresponding to 3×10^{-5} M HDPB and

being used for simultaneous determination of both cations.

Effect of Shaking Time on Extraction of Fe(II) and Fe(III). Again, the same mixture and procedure described in the Experimental section was applied, when the shaking time for extraction was varied in an interval from 2 to 14 min. Then, as above, the aliquots (of 2 ml) of these solutions were subjected extraction and the MIBK phase analysed spectrophotometrically. The maximal absorbance was obtained at shaking time for 12 min for Fe(II) and 10 min for Fe(III), respectively. Based on this, these periods were then chosen for further experiments.

Calibration Studies

Univariate Calibration. Both calibration curves depicted in Fig. 2 and plotted as absorbance *vs*. metal ion concentration were constructed at optimum conditions in the range of 0.3-1.1 ppm for both iron cationic forms. The detection limits (LODs, 3σ) were estimated to be 0.095 and 0.128 ppm for Fe(II) and Fe(III), respectively. Linear regression equations, $y = kx + q$, and the correlation coefficients, R^2, are also shown as the respective insets.

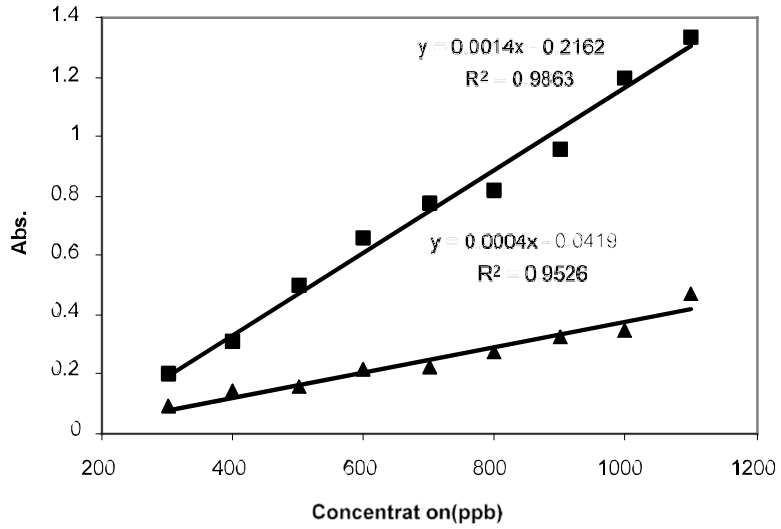

Fig. 2 Calibration curves for univariate extractive determination of Fe^{II} (▲) and Fe^{III} (■). Exp. conditions: Fe^{II} – λ_{max} = 500 nm, $C_{HDPB} = 2.9\times10^{-5}$ mol l^{-1}, shaking time, t_{sh} = 12 min, pH 8.0; Fe^{III} – λ_{max} = 495 nm, $C_{HDPB} = 3.1\times10^{-5}$ mol l^{-1}, t_{sh} = 10 min, pH = 8.2

Multivariate Calibration. To perform the determination of Fe(II) and Fe(III) in mixtures, the calibration models were proposed by using the PLS1 algorithm, two PLS models for each iron ion. To carry out this, a series of 25 solutions containing various Fe(II) + Fe(III) mixtures had been prepared as a special calibration set (see Table I) and the individual distribution of the $Fe^{II/III}$-PAR complexes extracted onto MIBK studied again *via* their absorption photometric spectra.

Table I Calibration set of solutions. Specification

Sol. No.	Fe(II), ppb	Fe(III), ppb	Sol. No.	Fe(II), ppb	Fe(III), ppb
1	300	300	14	700	900
2	300	500	15	700	1100
3	300	700	16	900	300
4	300	900	17	900	500
5	300	1100	18	900	700
6	500	300	19	900	900
7	500	500	20	900	1100
8	500	700	21	1100	300
9	500	900	22	1100	500
10	500	1100	23	1100	700
11	700	300	24	1100	900
12	700	500	25	1100	1100
13	700	700			

Selection of the Optimum Number of Factors. To select the number of factors in the PLS1 algorithm for modelling the system without overfitting the concentration data, a cross-validation method [16] was used as being capable of leaving out one sample at desired time. Given a set of 25 calibration spectra, the calibration model was constructed from 24 spectra and the corresponding calibration data, always using one particular calibration (with the concentration of both Fe^{II} and Fe^{III}) left out during the predicted calibration. This process was repeated 25×, until each calibrated sample had been left out for one time.

Prediction of the PRESS Values. The predicted concentration of Fe(II) and Fe(III) in each sample was compared to the known concentration of both cationic forms in the reference sample and the prediction of the so-called residual error sum of squares (*PRESS*) calculated in the same way and every time added as a new factor to the PLS1 model. One reasonable choice for optimal number of factors would be that number which yielded the minimum *PRESS*. However, the use of the number of factors (h^*) resulted in a minimum in the *PRESS*, which usually led to some overfitting. A better criterion for selecting the optimum number of factors had involved the comparison of the *PRESS* from model that was not significantly greater than the *PRESS* value from modelling with the h^* factors.

Finally, the *F*-statistics was used to evaluate the significance of determinations. Haaland and Thomas [16] empirically proved that an *F*-ratio

probability of 0.75 is sufficient and good choice. Thus, also herein, we had selected the number of factors for the first *PRESS* values by means of the *F*-ratio probability, according to which are the *PRESS* values minimum at the number of 5 for both Fe(II) and Fe(III) and due to this, these numbers of factors were also selected as the optimum for our calibration models.

The results obtained by applying the PLS1 algorithm to the prediction set of 12 samples are surveyed in Table II, whereas the plots of these predicted concentrations *vs*. actual concentration for optimal model depicted in Figs 3 and 4.

Table II Composition of prediction set, predicted values, and relative errors

No.	Actual value		Predicted value		Relative error, %	
	Fe^{2+}, ppb	Fe^{3+}, ppb	Fe^{2+}, ppb	Fe^{3+}, ppb	Fe^{2+}, ppb	Fe^{3+}, ppb
1	400	400	418	383	4	–4.0
2	400	600	413	595	3	–0.8
3	400	800	414	810	3	0.2
4	600	1000	588	986	–2.0	–1.0
5	600	400	612	421	2	5
6	600	600	598	584	–0.3	–3.0
7	800	800	817	789	2	–1.0
8	800	1000	805	1014	0.6	1
9	800	400	811	393	1	–2.0
10	1000	600	998	607	–0.2	1
11	1000	800	999	802	0.1	0.2
12	1000	1000	1021	1016	2	2

The respective correlation coefficients then were $R^2(Fe^{II}) = 0.9982$ and $R^2(Fe^{III}) = 0.9969$, which, again, verified good performance of the PLS1 models in predicting the concentrations of Fe(II) and Fe(III) in the mixture analysed.

Statistical Analysis. For the optimised models, two parameters were selected to evaluate the prediction ability for the simultaneous determination of Fe(II) and Fe(III) in the studied set. The predictive ability of a multivariate calibration models is usually expressed in terms of root mean square error of prediction (*RMSEP*) determined for an independent test set. And it is apparent that the adequate validation of the multivariate model required a certain minimum number of test samples.

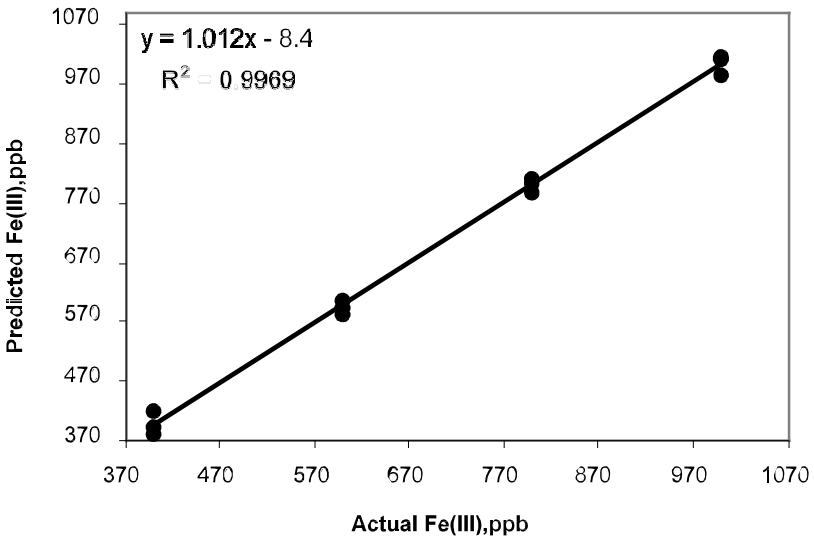

Fig. 3 Predicted concentration vs. actual concentration for Fe(II) in the prediction set

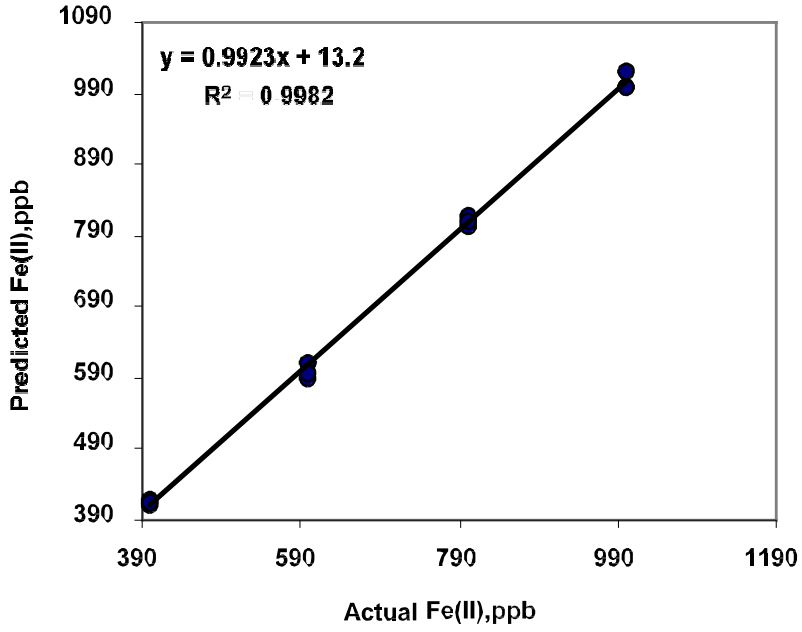

Fig. 4 Predicted concentration *vs*. actual concentration for Fe(III) in the prediction set

Reasonable criterion for the number of test samples was a demand that the resultant estimate of the *RMSEP* would be sufficiently precise for an application at hand. Thus, the *RMSEP* values are an estimate of the absolute error/deviation from the actual amount for each component [42]

$$RMSEP = \left[\frac{1}{n} \sum_{i=1}^{n} \left(\hat{x}_i - x_i \right)^2 \right]^{0.5} \tag{1}$$

The second parameter was then relative error of prediction (*REP*), given in % and showing the predictive ability of model for each component, when defined as

$$REP(\%) = \left[\frac{100}{\bar{x}} \sum_{i=1}^{n} (\hat{x}_i - x_i)^2 \right]^{0.5} \quad (2)$$

where x_i denotes the true concentration of the analyte in the sample, $\hat{x}_i$ is the estimated concentration of the analyte in the sample, $\bar{x}$ the mean of true concentrations in the prediction set, and n is the total number of samples used in the prediction sets. The values of *RMSEP* and *REP* as well as the number of factors in optimum number of factors are gathered in Table III, being calculated for the prediction set and the concentration of Cu(II) as a typical matrix constituent [36,42]:

Table II PLS model: Statistical parameters of the test matrix

Iron cations	No. of factors	*RMSEP*	*REP*, %
Fe(II)	5	0.041	4
Fe(III)	5	0.036	4

Effect of Foreign/Interfering Ions

Interferences from a number of cations and anions were studied in detail. For this purpose, different amounts of the individual ion species were mixed at the selected ratio(s) with 500 ppb Fe(II) + Fe(III). The results are summarized in Table IV.

Table IV The effect of various ions on simultaneous determination of Fe(II) and Fe(III)

Interfering/foreign ions	Tolerance limit, ppb
K^+, Na^+, Li^+, Ca^{2+}, Mg^{2+}, F^-, NH_4^+, Ba^{2+}, Cl^-, CO_3^{2-}, NO_2^-	400
Ag^+, Hg^{2+}, CN^-, Br^-, CO_3^{2-}	200
Bi^{3+}, Cu^{2+}, V^{4+}, Ni^{2+}, Zn^{2+}, $S_2O_3^{2-}$, CH_3COO^-, ascorbate	100
Co^{2+}, Ni^{2+}, S^{2-}, SCN^-, SO_4^{2-}, NO_3^-, HPO_4^{2-}	30
Co^{3+}, La^{3+}, Pb^{2+}, Mn^{2+}, VO_4^{3-}, $HCrO_4^-$	< 10 *

* seriously interfering sp.

The concentrations that have not caused evident changes in absorption spectra — i.e., max. ±5 % of the observed signal — are given *via* the

corresponding limits of tolerance. The remaining ions could then be classified as seriously interfering.

When looking within this group gathered in the last row, it can be stated that none of these ions represents a common matrix constituent in pharmaceutical samples and therefore, the determination of Fe(II) and Fe(III) can be characterised as fairly selective.

Analysis of Model and Real Samples

The method proposed was applied to determine iron and differentiate both Fe^{II} and Fe^{III} forms in a set of model solutions or three different pharmaceutical products, respectively, when all the results are gathered in Table V.

Table V Analyses of model solutions and real samples. Survey of results

Sample (specification)	Bivalent form, Fe^{II}, ppm		
	Added	Found	Recovery, %
Model solutions No. 1-5 (n = 5)	2	2.02-2.06	101-103
Hematinic capsule	2	1.64	82
Hematinic tablet	2	1.8	90
Oral drops	2	1.96	98
Sample (specification)	Trivalent form, Fe^{III}, ppm		
	Added	Found	Recovery, %
Model solutions No. 1-5 (n = 5)	2	1.88-1.92	94-96
Hematinic capsule	2	1.61	80
Hematinic tablet	2	1.89	87
Oral drops	2	1.91	95

At first, the accuracy and precision of the method was checked on analyses of model solutions made in five replicates (n = 5), when the recovery had varied within an interval of 101-103 % for Fe^{II} and of 94-96 % for Fe^{III}. Second, three selected pharmaceutical samples were analysed. Due to the fact that the original content(s) of iron had not been known, all three samples served as specific model solutions with real matrices [43] and the method was verified again by means of the recovery measurements.

In all the cases, the solutions of real samples were prepared according to the description in Section Experimental and additionally spiked with 2 ppm Fe(II) +

2 ppm Fe(III) and both forms determined in single analyses.

The recoveries have varied within 82-98 % for Fe^{II} and 80-95% for Fe^{III}, which can be considered as acceptable; nevertheless — for first two samples — revealing notably lower values than those chosen for the spike(s). Because the previous analyses of model solutions have not exhibited marked losses — e.g., during extraction — this suggests some suppression effect in matrices of capsule- and tablet real samples.

Conclusion

A new spectrophotometric method has been developed and tested enabling to determine iron in the form of Fe^{II}- and Fe^{III}-PAR complexes extractable onto the MIBK phase and capable of differentiating between the two oxidation states, Fe^{II} and Fe^{III}. The study has confirmed that the Fe^{II}-PAR + Fe^{III}-PAR system represents rather uneasy case for quantification of both individual components whose absorption spectra had revealed a high degree of mutual overlap. To overcome this interference, the PLS1 multivariate calibration method was chosen undergoing a detailed study. Its results have confirmed the suitability of this approach for mixtures of Fe(II) + Fe(III), offering a very good predictive ability of the model(s) proposed.

From a practical point of view, the method has shown certain potential for extractive spectrophotometric determination of Fe(II) and Fe(III) in pharmaceutical samples, when using simple, inexpensive, and non-sophisticated technique, and could be satisfactorily applied to pharmaceutical samples without tedious and time-consuming pre-separation steps. In these aspects, spectrophotometric method presented herein resembles some electroanalytical procedures with chemically modified electrodes, enabling also speciation of Fe(II) and Fe(III) in mixtures; see, e.g., Ref. [44] and references therein.

Such features are still attractive despite somewhat poorer performance of the method revealed *via* the recovery measurements and apparently caused by negative effect of some matrix constituent(s), probably those of organic origin that had not been included in the interference studies. Such residual compounds cannot usually be ascertained by spectrophotometric measurements and have to be identified with the aid of highly sophisticated techniques, such as ion chromatography with chemiluminiscence detection, as shown recently for selective determination of iron with speciation of both Fe^{II} and Fe^{III} forms [45].

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