Research article

Drug Testing and Analysis

Received: 11 November 2010 Revised: 3 January 2011 Accepted: 4 January 2011 Published online in Wiley Online Library: 21 February 2011

(www.drugtestinganalysis.com) DOI 10.1002/dta.267

Colourimetric solid-phase extraction coupled with fibre optic reflectance spectroscopy for determination of ascorbic acid in pharmaceutical formulations

Hayati Filik,* Duygu Aksu, Derya Giray and Reşat Apak

A redox colourimetric solid-phase extraction (C-SPE) procedure for the determination of ascorbic acid (AA) in pharmaceutical formulations was proposed. Iron (III)-2,2′-dipyridyl (Fe(III)-Bpy) reagent solution was used as a colouring reagent for AA and the immobilization of the redox product onto Amberlite XAD-16 resin was achieved. The analyte in the sample reacted with a solid sorbent loaded with the colourimetric reagent (Fe(III)-Bpy) and then quantified directly on the sorbent surface by using a fibre optic reflectance spectrometer (FORS). The amount of AA was reflectometrically determined in a few seconds with a total sample workup and readout time of ∼10 min using only 10-ml sample volumes. The limit of detection (LOD) and quantification (LOQ) values were 0.18 and 0.6 mg L^{-1}, respectively, and the linear dynamic range for AA extended up to 8.8 mg L^{-1}. The C-SPE for different extractions (n = 5) gave a relative standard deviation (RSD) of 2.9% at 5.28 mg L^{-1} AA level.

Keywords: ascorbic acid sensing; solid-phase extraction; 2,2′-dipyridyl; iron complex; reflectance spectrometry.

Introduction

Ascorbic acid (AA) is an important vitamin which occurs in different concentrations in a variety of natural samples. It is added to several pharmaceutical products as an essential ingredient, a stabilizer for vitamin B complex, and as an antioxidant.[1]

Several analytical methods have been reported for the determination of ascorbic acid using titrimetry, spectrophotometry, chemiluminescence methods, spectrofluorimetry, chromatography, electrochemical electrophoresis, and enzymatic method. These methods have been reviewed in numerous papers.[1–3] In recent years, a number of solid-phase extraction (SPE) methods have been successfully employed for the indirect determination of AA by ultraviolet-visible (UV-Vis) spectrophotometry and atomic absorption spectrometry (AAS). A number of solid sorbents such as Sephadex QAE A-25, Silica gel L, polyurethane foam, silisic acid-xerogel, SG-SO_3H ion exchanger, and polymeric polyester have been used for the solid-phase UV-Vis spectrophotometric detection of AA.[2] SPE combined with flame atomic absorption spectrometry (FAAS) for the indirect determination of AA has been studied by different research groups. Yebra-Biurrun *et al*.[4] proposed an indirect flow injection (FI) method for the determination of AA based on the reduction of Mn(VII) to Mn(II). The Mn(II) formed was retained online on a poly(aminophosphonic acid) chelating resin and the non-reduced Mn(VII) was determined by FAAS. In another study of the same research group,[5] Fe(III) was reduced by AA to Fe(II), which in turn reacted with 1,10-phenanthroline to form a complex. The coloured complex was adsorbed on a polymeric adsorbent (i.e. Amberlite XAD-4) proportionally to the amount of AA in the sample. The unretained Fe(III) was determined by FAAS. Zhang *et al*.[6] developed an FI system in which Cr(VI) was reduced to Cr(III) by AA. The product Cr(III) was first adsorbed on a cation-exchange resin column, then eluted, and measured by FAAS. Türker *et al*.[7] designed an indirect method for the detection of AA. This method was based on the AA reduction of chromium (VI) to chromium(III), separation of unreacted Cr(VI) as its 1,5-diphenylcarbazide complex on a column filled with Amberlite XAD-16, elution of the complex with methanol/H_2SO_4, and determination by FAAS. As noted above, these techniques require sophisticated, expensive, and non-portable instrumentation, thus conventional SPE is a fixed laboratory method which usually cannot be transported to the field for onsite measurements. The tedious and time-consuming step (elution or desorption) of SPE can be eliminated by colourimetric-SPE (C-SPE) introduced by Dias *et al*.[8] Analyses by C-SPE are very quick, require only the simplest equipment, and can be performed almost anywhere. C-SPE is a novel sorption spectrophotometric technique that combines colourimetric chemistry with SPE to determine target analyte concentration by measuring the colour change of single-use SPE cartridges, membranes and disks.[8] The extracted analyte, which is typically complexed with a colourimetric reagent, is then quantified directly on the solid surface by using a fibre optic reflectance spectrophotometer.

2,2′-Bipyridyl (Bpy) in combination with iron (III) has been reported as a spectrophotometric reagent for AA. Gavrilenko *et al*.[9] proposed the use of Fe(III)-Bpy complex immobilized on optically transparent polymethacrylate for the solid-phase spectrophotometric and visual determination of AA in model

* *Correspondence to: Hayati Filik, Istanbul University, Faculty of Engineering, Department of Chemistry, 34320 Avcılar, Istanbul, Turkey.*
E-mail: filik@istanbul.edu.tr

Istanbul University, Faculty of Engineering, Department of Chemistry, 34320 Avcılar, Istanbul, Turkey

solutions. The goal of this study is to develop a simple method for the determination of AA by reflectance spectroscopy utilizing the classical reaction between Fe(III), Bpy, and AA. The C-SPE method proposed was based on the method reported by Kleszczewski and Kleszczewska.[10] For this task, the ferriin ($[Fe(III)\text{-}(Bpy)_3]^{3+}$) complex was adsorbed on a polymeric adsorbent (i.e. Amberlite XAD-16) and Fe(III) was reduced to Fe(II) on the adsorbent surface with reducing action of analyte. The Fe(II) obtained is then able to react with Bpy in order to form a coloured ferroin complex ($[Fe(Bpy)_3]^{2+}$)[11–13]. In fact, Fe(III) complexed with Bpy is a stronger oxidizing agent than Fe^{3+} (due to selective stabilization of the ferrous state) and Fe(II)-Bpy emerging as the product of the redox reaction has a much more intense colour than the corresponding Fe(III)-complex. The colour formed due to the resulting complex is then directly measured on the sorbent surface using a fibre optic reflectance spectrophotometer.

Apparatus

Experiments were carried out using a commercially available miniature fibre-optic-based spectrometer (Ocean Optics Inc., HR4000CG-UV-NIR) which utilizes a small tungsten halogen lamp (Ocean Optics Inc., Dunedin, Florida, USA) as the light source and a charge-coupled device (CCD)-based detector for reflectance measurements. Light reflected from the sensing layer was transmitted by a bundle of optical fibres to a miniature fibre-optic spectrophotometer (Ocean Optic HR4000CG-UV-NIR) which was connected to a PC (Dell-compatible) and also a printer. For optical isolation, the sensing layer and the detector were kept in a black box to minimize any interference from ambient light. The spectral deconvolution was performed after smoothing the spectra by a 5-point Fourier transform filter using peak fitting module in OriginPro7.0 software (OriginLab Co., Northampton, USA). The pH values of the solutions were measured by a Hanna HI 221 pH-meter using the full range of 0–14. Conical bottom disposable plastic centrifuge tubes (12 ml) were used for phase separations. They are made of clear polypropylene that is fully autoclavable.

Reagents and solutions

All chemicals used were of analytical grade. A solution of ascorbic acid (vitamin C), from Merck Darmstadt, Germany was prepared by dissolving the requisite amount of sample in distilled water. The stock and working solutions of AA were freshly prepared every day and kept in the dark and cold to minimize oxidation. The colourimetric reagent ferriin (Fe(III)-Bpy ($C_5H_4N)_2$) was prepared by mixing Fe(III) and Bpy in the following manner: A weight of 115.7 mg of ammonium iron(III) sulfate dodecahydrate ($NH_4Fe(SO_4)_2 \cdot 12H_2O$) was dissolved in distilled water containing 1.0 ml of 5 M H_2SO_4, and standardized with 0.01 M EDTA (Merck Darmstadt, Germany). Then 125 mg of Bpy was added, and after dissolving by heating at 80 °C and cooling, the mixture solution was diluted to 50 ml with distilled water (Fe(III) : Bpy = 1 : 3). A working solution was prepared by adding a few drops 1% solution of ceric sulfate $Ce(SO_4)_2$ in 5% sulfuric acid to ensure complete oxidation of traces of Fe(II). The colour of the solution was changed from red to yellow. For adjusting the pH of reaction solutions (pH 4.0), an acetic acid/acetate buffer was used. Amberlite XAD-16 Resin (Room and Hass; surface area, 800 m^2/g; wet mesh size, 20–60 mesh) was used after successively washing with ethanol and water, and dried for 2 h at 60 °C. The resins Amberlite XAD-4, XAD-7 and XAD-16 were supplied by Sigma (Madrid, Spain). The resins Amberlite XAD-4, XAD-7 and XAD-16 had the following characteristics: average pore diameters of 50, 90, and 100 A°, respectively; surface areas of 725, 450, and >800 $m^2\ g^{-1}$, respectively; and pore volumes of 0.98, 1.14, and 1.82 ml g^{-1}, respectively. The wet mesh size was 20–60 for every resin.

Data analysis

The reflectance spectrometer was interfaced to a computer by a serial cable. Spectra were then transferred to the computer and downloaded to an MS Excel worksheet to plot reflectance data. The data were then transferred to another worksheet to calculate Kubelka–Munk remission (F) and plot calibration curves. The Kubelka-Munk function (K-M) function, $\Delta F(R)$, is formulated as: $\Delta F(R) = (1 - R)^2/2R = k/s$, where R is the absolute reflectance of the sampled layer, k is the molar absorption coefficient, and s is the scattering coefficient of the sample surface. Within a certain analyte concentration interval, this function varies linearly with concentration. A simplified solution of the differential Kubelka-Munk equations is generally used in literature to relate a chromophore concentration to the intensity of the sample's diffuse reflection:[14]

$$F(R_\infty) = (1 - R_\infty)^2/2\,R_\infty = (k/s)\alpha\ C \qquad (1)$$

where R_∞ is the ratio of the diffuse reflectance of the sample of interest to that of a selected reference sample, and α is the proportionality symbol. If the reflectance signal of the blank sensor at the analytical wavelength is denoted with R_f and of the analyte sensor with R_c, then $\Delta F(R) = (R_f - R_c)$ is linearly correlated to analyte concentration (C) in solution within a resonable concentration range, and therefore, the individual values of k and s were not calculated.

Impregnation procedure

To remove the contaminants, Amberlite XAD-16 resin beads were prepared as described in the literature.[15–18] A weight of 1.0 gram dry resin (Amberlite XAD-16) beads were treated with 2.5 ml of Fe(III)-Bpy solution and 2.5 ml of 3 mol L^{-1} ammonium acetate buffer (pH = 7), and the mixture was shaken at room temperature for 5 min. After this process, the resulting particles were isolated by filtration and washed with water until reagent excess was eliminated from the resin beads. The resulting resin beads (loaded with Fe(III)-Bpy) were filtered off from the supernatant solution, and washed with distilled water to remove the excess Fe(III)-Bpy, then the beads were transferred to a dry filter paper and pressed for easy drying. Finally, the resulting beads were stored in glass bottles with stoppers.

Application to pharmaceutical dosage form

An average weight of three tablets was determined. The tablets were finely powdered and an accurate weight of this material or the content of capsules, equivalent to 1×10^{-3} mol L^{-1} of AA in final solution was transferred into 100-ml volumetric flasks. Then, 50 ml of distilled water was added, and the solution was mechanically shaken for 10 min. This solution was filtered to remove any insoluble matter. After filtration, working solutions were prepared by transferring suitable aliquots of the clear filtrate and diluting with distilled water. An aliquot of this solution was analyzed for ascorbic acid content by the proposed procedure.

Reflectance measurement

Plastic tubes were used in test studies. An adsorbent-loaded plastic column (12-ml plastic tube, with graduations in millilitres) was used for preconcentration of AA. A plastic tube was filled to the 0.7 ml (i. e. 0.7 cm) mark line with the adsorbent (XAD-16-Fe(III)-Bpy). The filling mass of the Amberlite XAD-16- Fe(III)-Bpy resin was 300.64 ± 1.2 mg (N = 5). One millilitre of AA solution at a concentration ranging from 0.352 to 8.8 mg L^{-1} was taken into the tube. Two millilitres of the acetate buffer solution were added to adjust the pH of the mixture to 4.0. After shaking for 5 min, the supernatant solution was decanted (or pipetted) and the resin beads remained in the tube. After this process, the fibre-optic probe was dipped into the test tube and the reflection of the adsorbent surface was measured at (555.8 ± 0.1) nm. For practical analytical applications, the resin beads were not washed but instead directly measured after decantation. The measurements were expressed as relative reflectance, which is defined as the difference between the reflectance of the Fe(II)-Bpy complex resulting from AA reaction (R_c) and that of the immobilized reagent alone (R_f), both recorded at the same wavelength (555.8 nm). Amberlite-XAD-16/Fe(III)-Bpy (λ_{max} = 505.1 nm) was used as the reference. Percentage reflectance is defined as the difference in the reflectances of the reference and the sample, divided by that of the reference, i.e. R,% = 100 ($R_f - R_c$)/R_f. The arbitrary unit (sensing layer) and the detector were kept in a black box to minimize any interference from ambient light.

Results and discussion

Reflectance spectra

The C-SPE procedure for the determination of AA was based on its reducing reaction on Fe(III)-bpy and following the reflectometric determination of Fe(II)-Bpy. Figure 1 shows the reflectance spectra of Fe(III)-Bpy before and after reaction with AA. Reaction with AA caused an increase in the reflectance intensity due to the change in colour of the reagent phase. The reflectance spectra measured against reagent blank shows its maximum reflectance at 555.8 nm. All reflectance measurements in this study were therefore carried out at this wavelength.

Effect of reagent pH on adsorption by the resin

The effect of Fe(III)-Bpy solution pH on the adsorption efficiency was investigated by varying the pH of the Fe(III)-Bpy solution between 2.0 and 8.0 with the aid of a suitable buffer system. Better results were obtained with a pH value higher than 6.0 (Figure 2). A pH of 7.0 was selected, and the reagent pH was adjusted with an ammonium acetate buffer solution. The use of 2.5 ml of 3.0 M ammonium acetate solution (i.e. neutral solution) was recommended. As a precaution, pH >7 was not used due to possible hydrolysis and ferric hydroxide precipitation from the Fe(III)-Bpy reagent.

Effect of pH on AA measurement

One of the effective variables on the C-SPE response is pH of AA solution. For this means, the influence of pH over the range 3.0–7.0 on the response of C-SPE sensing layer was studied. The pH was adjusted by addition of appropriate acetate buffer solutions. As seen from Figure 3, the maximum response was obtained in the

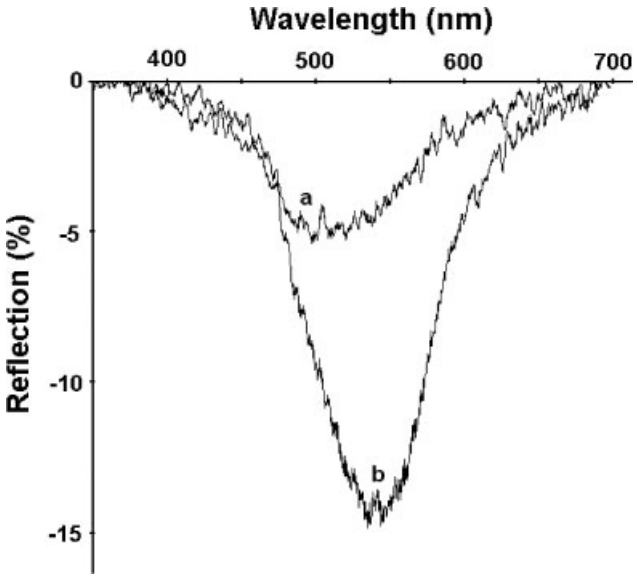

Figure 1. Reflectance spectra of immobilized Fe(III)-Bpy before, curve (a), and after, curve (b), reaction with 5.28 mg L^{-1} AA. Response time: 7 min.

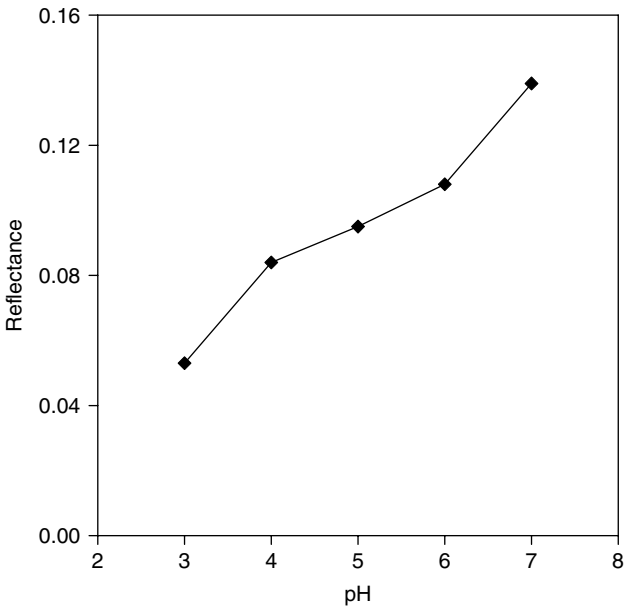

Figure 2. Effect of pH on the adsorption of Fe(III)-Bpy reagent. Fe(III)-Bpy reagent: 2.5 ml, [AA] = 5.28 mg L^{-1}, response time = 7 min.

presence of acetate buffer at pH 4. The decrease of reflectance at higher pH may be attributed to the autoxidation of AA (Figure 3). The presented results were similar to reported studies on formation of Fe(II)-Bpy complex in solution.[9,10]

Effect of reagent (Fe(III)-Bpy) concentration

The XAD-16 resin was chosen as support material because it has been customarily employed for the immobilization of different types of reagents,[15–18] providing satisfactory results, usually better than those obtained with XAD-2 and XAD-7 resins. The effect of reagent concentration on reflectance was studied by varying the volume of Fe(III)-Bpy reagent (Fe(III):Bpy = 1 : 3) in the range of 0.5–3.5 ml. Complex formation was complete and reflectance was

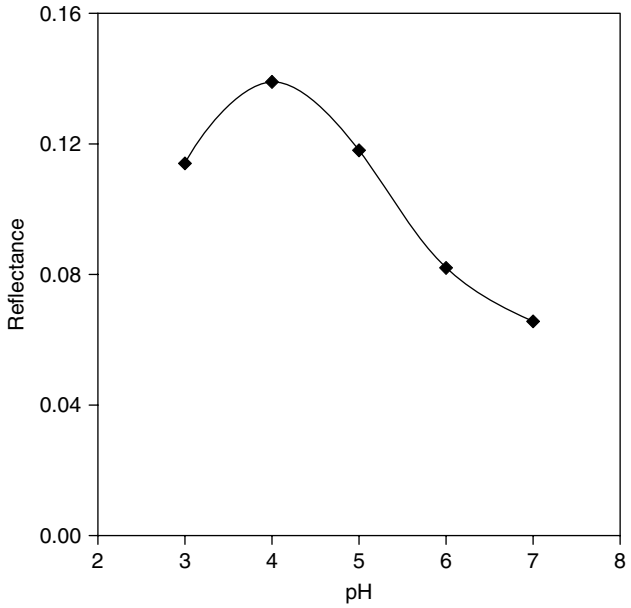

Figure 3. Effect of the pH on the reflectance of Fe(II)-Bpy complex. [AA] = 5.28 mg L^{-1}, response time = 7 min.

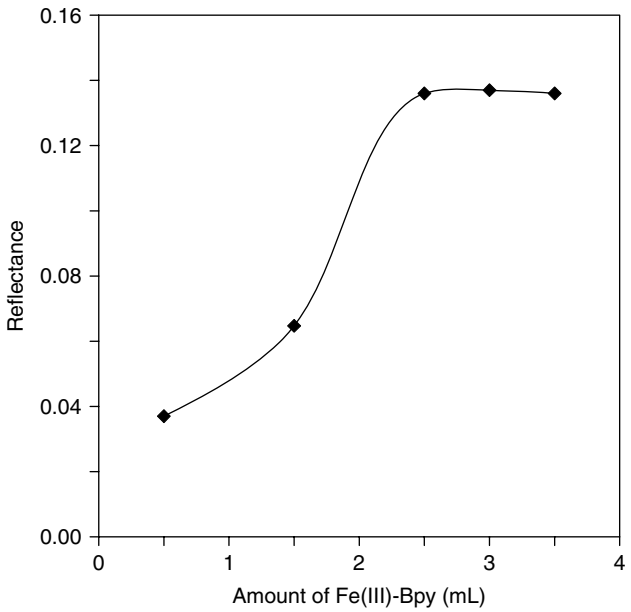

Figure 4. Effect of amount Fe(III)-Bpy on the reflectance. [AA] = 5.28 mg L^{-1}, response time = 7 min.

maximum when the amount exceeded 2.5 ml, and no change in reflectance was observed above this range (Figure 4). Therefore, 2.5 ml of Fe(III)-Bpy (1 : 3) solution was sufficient to obtain a maximum and reproducible reflectance for 5.28 mg L^{-1} of AA, and this volume was fixed for further studies (as in 'Impregnation procedure').

Response time and colour stability

The sensing time of the C-SPE is a very important parameter for rapid measurement, and there have been reports in the literature on the possibility of selective AA determination in the presence

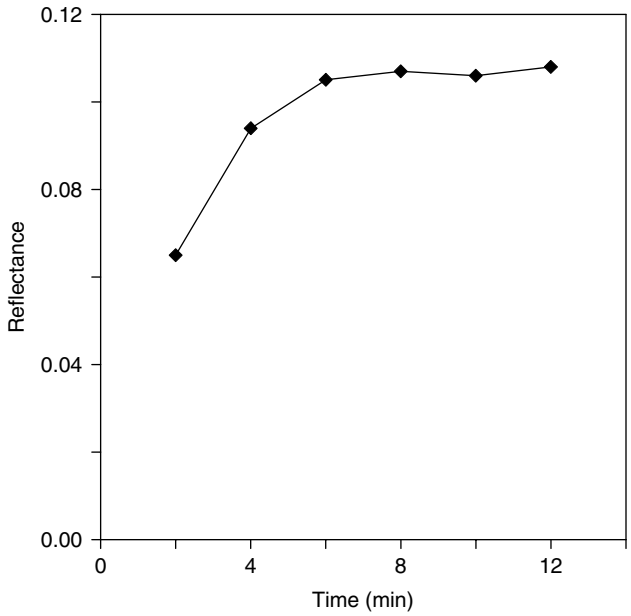

Figure 5. Response time of AA at room temperature to impregrated Fe(III)-Bpy. [AA] = 1.76 mg L^{-1}.

of flavonoids if the redox reaction resulting in coloured complex formation was carried out rapidly. Figure 5 shows reflectance response of the C-SPE at 555.8 nm as a function of time when the C-SPE layer was exposed to 5.28 mg L^{-1} AA. The effect of the extraction time was examined in the range of 1–10 min. The reflectance of C-SPE layer initially increased with time, and remained constant after a measurement time of 5 min. To remain on the safe side, a 7-min system response time was chosen in this study since it yielded a more stable response for a wider AA concentration range. The effects of temperature and heating time on the formation of the coloured complex were studied. The reaction of AA with the reagent proceeded relatively slowly at room temperature. Higher temperature may be used to accelerate the reaction. Maximum reflectance due to Fe(II)-Bpy complex formation was obtained after heating for about 1 min on a water bath at 80 °C. Further heating caused no appreciable change in the colour. The obtained complex was very stable for a minimum period of 30 min.

UV-Vis spectra of $Fe^{II,III}$(Bpy) complexes and of the supernatant solution after AA measurement with C-SPE

The UV-Vis spectra of aqueous phases related to ascorbic acid (AA) measurement are shown in Figure 6. The spectra of the aqueous supernate after reaction of AA solution (2×10^{-5} M) with the (Fe(III)-Bpy)-loaded Amberlite XAD-16 sorbent (Figure 6A), of the aqueous phase containing Fe(III)-Bpy (1 : 3) at pH = 4 (Figure 6B), and of the aqueous phase after Fe(III)-Bpy reaction with AA (2×10^{-5} M) at pH = 4 (Figure 6C), when evaluated together, clearly show that the intensely coloured Fe(II)-Bpy complex, once formed on the Amberlite sorbent, did not desorb into solution under the working conditions to yield a negative error in AA calculation.

Calibration curve, detection and quantification limits, precision, and statistics

Reflection values of several concentrations of AA were measured at pH 4 (reflectance, %) and converted to plots of Kubelka-Munk

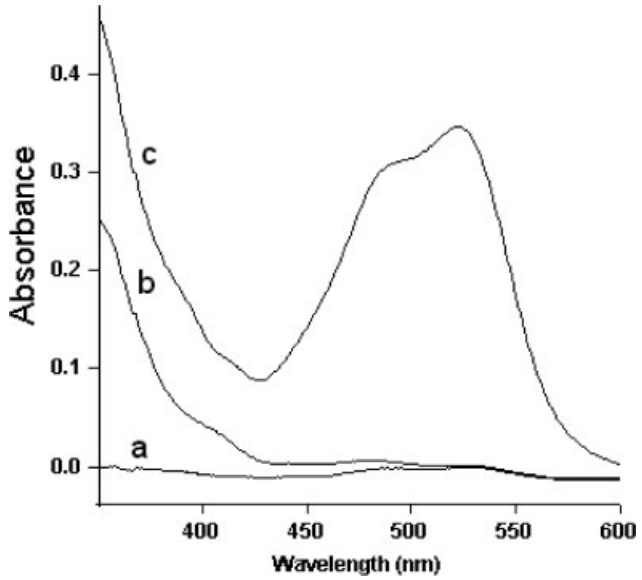

Figure 6. The UV-Vis spectra of aqueous phases related to ascorbic acid (AA) measurement: (a) Spectrum of the aqueous supernate after reaction of AA solution (2×10^{-5} M) with the (Fe(III)-Bpy)-loaded Amberlite XAD-16 sorbent; (b) Spectrum of the aqueous phase containing Fe(III)-Bpy (1 : 3) at pH = 4; (c) Spectrum of the aqueous phase after Fe(III)-Bpy reaction with AA (2×10^{-5} M) at pH = 4.

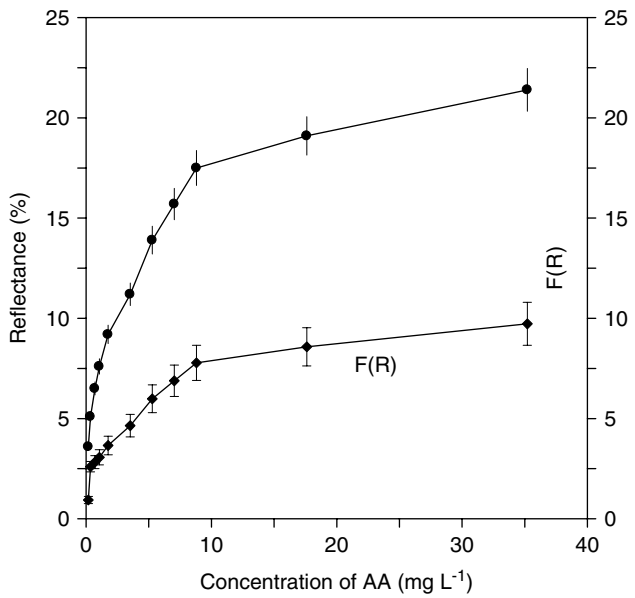

Figure 7. Effect of AA concentration on the C-SPE response.

function (F(R)) against concentration as shown in Figure 7. Under the chosen optimal conditions, the calibration curve of F(R) was plotted as a function of AA concentration (Figure 8). The equation of the analytical calibration curve obtained was $F(R) = (0.655 \pm 0.020)$ C (mg L^{-1}) + (2.27 ± 0.23) (r = 0.9903). The optimal linearity of plots of $F(R)$ against C falled in the concentration range from 0.35 to 8.8 mg L^{-1} at 555.8 nm using 10 ml samples (N = 11 samples). The linear dynamic concentration range of most molecular spectroscopic sensors is about an order of magnitude or slightly wider, in accordance with the findings of this work. For example, a multi-enzyme biosensor

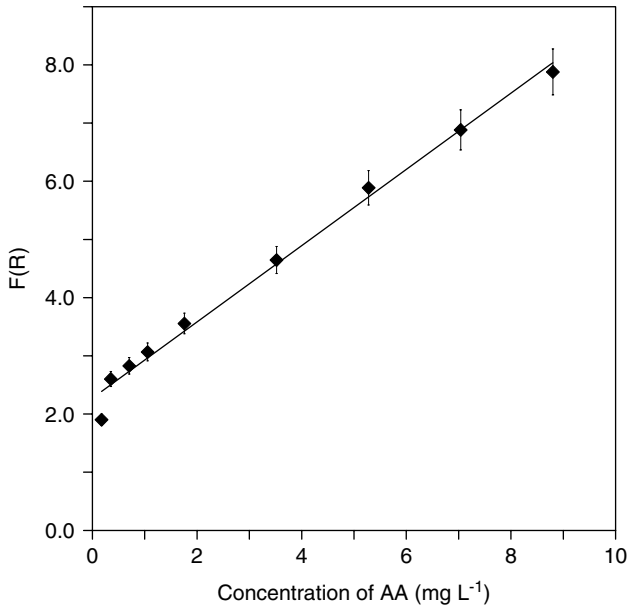

Figure 8. Ascorbic acid calibration curve showing the linear concentration range (0.35–8.8 mg L^{-1}).

Table 1. Recovery of ascorbic acid (1.76 mg L^{-1}) in the presence of pharmaceutical excipients

Excipients	Amount (mg L^{-1})	Recovery (%)
Citrate	35.716	97
Oxalate[a]	142.11	101
Salicylate	16.011	100
Tartrate	230.08	100
Glutamic acid	147.13	101
Glucose	198.17	102
Saccarose	342.30	101

[a] Oxalate masked with 1 ml of 1% $CaCl_2$ (w/v).

for ascorbic acid (AA), less sensitive than the current method, yielded a linear concentration range of 44–352 mg L^{-1}.[19] The reference method of AA determination used in this work involving a modified CUPRAC spectrophotometric method[3] has a linear range: 1.4–14 mg L^{-1}, confirming that the linear range of the proposed method is useful for direct applications to suitably diluted solutions.

The limit of detection (LOD), defined as the lowest concentration level that can be detected to be 'statistically different' from an analytical blank,[20] and given by $c_L = ks_b/m$ (where c_L, s_b and m are limit of detection, standard deviation of the blank, and slope of the calibration curve, respectively, and k is a numerical factor usually taken as 2 or 3) was 0.18 mg L^{-1}. The limit of quantification (LOQ), taken as (10/3) LOD with the recommendation of IUPAC,[21] was 0.6 mg L^{-1}. The LOQ was greater than the lower value of the linear concentration range (i.e. 0.35 mg L^{-1}) because of the high blank value of measurements (i.e. c_L increases with s_b). The reproducibility of the used C-SPE sensing layer was checked by five replicate determinations (N = 5) at 5.28 mg L^{-1} level of AA. The reflectance measurements were highly reproducible; the coefficients of variation (CV) for 5.28 mg L^{-1} AA solution were 2.9% (intra-day reproducibility) and 3.8% (inter-day reproducibility). The

Table 2. Determination of ascorbic acid in commercial pharmaceuticals (n = 3)

Sample (Supplier)	Declared concentration mg unit^{-1}	Proposed C-SPE Found mg unit^{-1} ± SD	Reference method[a] Found mg unit^{-1} ± SD	*F* value (19.00)[b]	*t* value (2.776)[c]
Calcium Sandoz (Novartis)	1000	998 ± 5.0	990 ± 7.6	2.31	1.52
Bemiks-C (Eczacıbaşı)	100	94 ± 3.5	99 ± 5.5	2.47	1.33
Redoxon (Bayer)	500	501 ± 4.0	493 ± 3.0	1.77	2.77

[a] Reference method[3].
[a,b] Values of *t* and *F* at 95% confidence level.
[c] Table values for N-2 = 4 degrees of freedom: $t_{.95} = 2.776$, $F_{2,2} = 19.0$.

intra- and inter-day CVs rose to 5.1 and 6.7%, respectively, for measurements around LOQ.

Effect of excipients

In order to evaluate the selectivity of the developed method for the analysis of pharmaceutical formulations, the effect of the presence of several species, which can occur in real samples with ascorbic acid, was investigated. To test the efficiency and selectivity of the proposed analytical method to tablets, a systematic study was carried out for additives and excipients (e.g. glutamic acid, lactose, glucose, citric acid, acetysalicylic acid, dextrose, talc, calcium hydrogen phosphate, magnesium stearate, and starch) that are usually present in dosage forms. Experimental findings showed that there was no interference from additives or excipients for the examined method, as shown in Table 1.

Applications

The proposed methods were successfully applied to determine AA in pharmaceutical tablets. The data are presented in Table 2. The results obtained by the proposed method have been compared with those from the existing method.[3] For N = 3 repetitive determinations of AA by the proposed and reference (modified CUPRAC[3]) methods for validation, the statistical Student's *t*- and F-tests carried out on Calcium Sandoz (Novartis), Bemiks-C (Eczacıbaşı), and Redoxon (Bayer) tablets containing 1000, 100, and 500 mg AA per unit, respectively, revealed that there were no significant differences between the means and the variances of the two populations at 95% confidence level. Thus, the proposed method was as accurate and precise as the reference method.[3]

Conclusions

The proposed method is simpler, less time-consuming and more sensitive than the published indirect SPE method. The C-SPE method for determination of vitamin C is fast and facile. $Fe(Bpy)^{3+}$ easily oxidizes ascorbic acid with great electromotive force, because there is a large difference between the standard reduction potentials of the oxidant and reductant, i.e. E^0 of the $\{Fe(Bpy)^{3+}/Fe(Bpy)^{2+}\}$ couple is 1.023 V (in 1 M H_2SO_4) and of the $\{ascorbate^{\bullet}, H^+/ascorbate^-\}$ couple (at pH = 7) is 0.282 V.[22] The coloured complex responsible for reflectance measurement has a high stability constant, i.e. Log β_3 for $Fe(Bpy)_3^{2+} = 17.5$.[23] The method is quite useful for pharmaceutical analysis and allows the determination of ascorbic acid in the presence of common excipients usually encountered in commercial tablet formulations.

Acknowledgement

The authors gratefully acknowledge the financial support from the Scientific and Technical Research Council of Turkey (TUBİTAK Grand No: 109T856) and Istanbul University Scientific Research Fund (BYP Grant No: 3489 and 3490).

References

[1] S. P. Arya. Photometric methods for the determination of vitamin C. *Anal. Sci.* **1998**, *14*, 889.
[2] O. A. Zaporozhets, E. A. Krushinskaya. Determination of ascorbic acid by molecular spectroscopic techniques. *J. Anal. Chem.* **2002**, *57*, 343.
[3] M. Özyürek, K. Güçlü, B. Bektaşoğlu, R. Apak. Spectrophotometric determination of ascorbic acid by the modified CUPRAC method with extractive separation of flavonoids-La(III) complexes. *Anal. Chim. Acta* **2007**, *588*, 88.
[4] M. C. Yebra-Biurrun, R. M. Cespon-Romero, A. Moreno-Cid. Flow injection flame AAS determination of ascorbic acid based on permanganate reduction. *Atom. Spectr.* **2001**, *2*, 346.
[5] M. C. Yebra-Biurrun, R. M. Cespo-Romero, P. Bermejo-Barrera. Indirect flow-injection determination of ascorbic acid by flame atomic absorption spectrometry. *Microchim. Acta* **1997**, *126*, 53.
[6] Z. Q. Zhang, Y. C. Jiang. Flame atomic absorption spectrometry for the automatic indirect determination of ascorbic acid based on flow injection and reduction of chromate. *Atom. Spectr.* **2000**, *21*, 100.
[7] A. R. Türker, Ö. Yalçınkaya, A. Tunçeli. Application of column solid phase extraction of chromium for indirect determination of ascorbic acid by flame atomic absorption spectrometry. *J. Food Drug Anal.* **2008**, *16*, 83.
[8] N. C. Dias, M. D. Porter, J. S. Fritz. Principles and applications of colourimetric solid-phase extraction with negligible depletion. *Anal. Chim. Acta* **2006**, *558*, 230.
[9] N. A. Gavrilenko, G. M. Mokrousov, O. V. Dzhiganskaya. An Optical Sensor for the Determination of Ascorbic Acid. *J. Anal. Chem.* **2004**, *59*, 871.
[10] T. Kleszczewski, E. Kleszczewska. Flow injection spectrophotometric determination of L-ascorbic acid in biological matters. *J. Pharm. Biomed. Anal.* **2002**, *29*, 755.
[11] C. Nakanishi, S. Ikeda, T. Isobe, M. Senna. Silica $[Fe(bpy)_3]^{2+}$ composite particles with photo responsive change of colour and magnetic property. *Mater. Res. Bull.* **2002**, *37*, 647.
[12] C. Nakanishi, S. Ikeda, T. Isobe, M. Senna. UV-Induced Formation of [Fe(bpy)3]2+ Complex and its Confinement into Silica Fine Particles. *J. Sol-Gel Sci. Tech.* **2003**, *26*, 103.
[13] J.–J. Jin, Y. Haramoto, M. Nanasawa. Synthesis and photoinduced colouration of ferric bipyridine complex linked viologen units in a polymer matrix. *Macromol. Rapid Commun.* **1999**, *20*, 135.
[14] F. Boroumand, H. van den Bergh, Quantitative diffuse reflectance and diffuse transmittance infrared spectroscopy of surface - derivatized silica powders, *Anal. Chem.* **1994**, *66*, 2260.
[15] H. Filik, D. Aksu, R. Apak, I. Boz. Rapid sensing of molybdenum by combined colourimetric solid-phase extraction – Reflectance spectroscopy. *Sens. Actuators B* **2009**, *141*, 491.
[16] H. Filik, Z. Yanaz. A sensitive method for determining total vanadium in water samples using colourimetric-solid-phase extraction-fibre optic reflectance spectroscopy. *J. Hazard. Mater.* **2009**, *I72*, 1297.

[17] H. Filik, M. Hayvalı, E. Kılıç, R. Apak, D. Aksu, Z. Yanaz, *et al*. Development of an optical fibre reflectance sensor for *p*-aminophenol detection based on immobilised bis-8-hydroxyquinoline. *Talanta* **2008**, *77*, 103.
[18] H. Filik, D. Aksu, R. Apak, I. Şener, E. Kılıç, An optical fibre reflectance sensor for *p*-aminophenol determination based on tetrahydroxycalix[4]arene as sensing reagent, *Sens. Actuators B* **2009**, *136*, 105.
[19] G. L. Long, J. D. Winefordner, Limit of detection: A closer look at the IUPAC definition. *Anal. Chem.* **1983**, *55*, 712A.
[20] *IUPAC Compendium of Chemical Terminology* 2nd Edn. **1997**, Available at: http://www.chemsoc.org/chembytes/goldbook/. Access date: Jan. 2011.
[21] V. Volotovsky, N. Kim, Determination of glucose, ascorbic and citric acids by two-ISFET multienzyme sensor, *Sensors & Actuators B* **1998**, *49*, 253.
[22] P.-G. Pietta, Flavonoids as antioxidants, *J. Nat. Prod.* **2000**, *63*, 1035.
[23] P. C. C. Oliveira, J. C. Masini, Sequential injection determination of iron (II) in anti-anemic pharmaceutical formulations with spectrophotometric detection, *Anal. Lett.* **2001**, *4*, 389.