



## Spectrophotometric determination of Fe ions using green tea extract

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**Abstract:** A batch spectrophotometric method for determination of Fe (II) and Fe (III) using green tea extract as reagent is proposed. The method is based on complex formation reaction between Fe and polyphenol compounds from green tea in buffered medium (pH = 4.8). Absorbance of Fe-polyphenol complex formed during the reaction was measured at 570 nm wavelength. The linear dynamic ranges are obtained from  $1.0 \times 10^{-5}$  to  $5.0 \times 10^{-4}$  mol/L. The applicability of the proposed method was demonstrated by determination of Fe ions in pharmaceutical dosage forms, orange juice, degassed mineral water and candy.

## INTRODUCTION

This paper describes the possibility of using green tea extract as a natural reagent for spectrophotometric determination of Fe ions in different samples.

The plant for preparation of green tea (*Camellia sinensis*) is widely known. The popularity of this plant is based on its healing properties related to the polyphenol content. Green tea and its extracts are used to enrich energy drinks, juices and other types of foods (Balentine *et al.*, 1997).

One of the interesting properties of polyphenols of green tea is their ability to affect the absorption of iron in physiological system. Polyphenol compounds, particularly catechins, have the ability to react with iron ions and form a Fe-polyphenol complex (Ryan and Hynes, 2007). Various methods of using different natural reagents in order to efficiently develop analytical methods have been described in a literature very often. Plant extracts that can be used as acid-base indicators are the most commonly known. As natural reagents, enzymes can be isolated from tissues and bacteria (Grudpan, *et al.*, 2010, 2011).

One of the examples is a method for spectrophotometric determination of iron ions using a guava leaf extract as a natural reagent that has been +optimized (Settheeworrit *et al.*, 2005). However, Pinyou and co-workers (2010) have developed a new analytical method for the determination of iron ions using flow injections and natural reagents extracted from green tea. Recently, our research

group was optimized the system for sequential analysis by injection based on the same reaction (Martinović Bevanda *et al.*, 2015).

Analytical methods that use natural reagents meet basic principles defined by green chemistry framework. Therefore, we consider it worthwhile to adapt the application of green tea for the batch spectrophotometric determination of Fe ions.

The basic theoretical and practical principles of green chemistry are defined by well-known twelve principles (Anastas and Warner, 1998). The most important among them is the principle in regards to sustainable development concerns as follows: avoid or reduce the use of toxic solvents, reagents, and the production of harmful waste. Furthermore, the benefits of applying green chemistry in industry or education are: faster and cheaper methodology, cleaner and healthier environment. We are witnessing that costs and other issues affecting the sustainability of chemistry education are growing concern nowadays (Kradtap Hartwel, 2012). Therefore, the proposed method can be used in analytical laboratories that do not have the possibility of flow analysis. Also, green principle on which this method is based offers a simple, interesting and innovative approach to modern education for sustainable development. The proposed method was tested in the analysis of real samples. Analytical use and the influence of different matrices were tested on orange juice sample, mineral water, candy and pharmaceuticals.

## EXPERIMENTAL

### Reagents and chemicals

All chemicals were of analytical-reagent grade and all solutions were prepared in deionized (DI) water.

A stock solutions, 50.0 mL each of 0.1 mol/L Fe(II) and Fe(III) were prepared by dissolving 1.3504 g  $\text{FeCl}_3 \times 6\text{H}_2\text{O}$  (Sigma-Aldrich Chemie) and 1.3900 g  $\text{FeSO}_4 \times 7\text{H}_2\text{O}$  (Sigma-Aldrich Chemie) respectively, in DI water containing 1.0 % (v/v) concentrated  $\text{H}_2\text{SO}_4$ . To the stock solution of Fe (II) was added 5.0 mL of  $\text{H}_2\text{O}_2$  due to complete oxidation to Fe (III).

Acetate buffer, pH = 4.8, was prepared by mixing 14.44 g of dissolved sodium acetate in proper volume of deionized water with 5.5 mL of concentrated acetic acid. The obtained mixture was diluted to 1000.0 mL with DI water. If necessary, the pH value of the buffer was adjusted by adding small volumes of NaOH or HCl (4.0 mol/L)

Green tea extract (GTE) suspension was prepared with 8.0 g green tea in 150 mL acetate buffer (0.2 mol/L, pH = 4.8). The suspension was stirred for 10 minutes using magnetic stirrer (300 rpm). Then, the suspension was filtered and diluted with acetate buffer (pH= 4.8) to volume ratio 1:1. The extract was prepared daily. Green tea is purchased from local stores.

### Sample solutions preparation

Twenty tablets of pharmaceutical preparations were accurately weighed and ground. A portion of powder was weighted and dissolved in mixture of 5.0 mL concentrated HCl and DI water. The mixture can be filtered, if necessary. The required aliquot of the filtrate was transferred to 50.0 mL volumetric flask. Hydrogen peroxide (5.0 mL) was added, to ensure the complete oxidation of Fe (II) to Fe (III).

Samples of degassed mineral water, juices and candy are spiked with Fe (III) and Fe (II) to verify the applicability of the method on samples with different matrices. An aliquot of 20.0 mL of liquid samples was mixed with required volume of standard solution of Fe ions.

Ten pieces of candies were accurately weighed and ground. A portion of powder was weighted and dissolved in deionized water. The working solution of this sample was also spiked with required volume of standard solution of Fe ions.

### Apparatus

The absorbance was measured with a double-beam UV-Vis spectrophotometer, UV-1800 Shimadzu, with 1.0 cm width paired quartz cuvettes.

Measurements of pH were performed using a pH meter (Mettler Toledo, Schwerzenbach, Switzerland) equipped with a combined glass electrode.

### Procedure

To determine both, Fe (III) and Fe (II), reaction solutions were prepared as follows. The volume of 3.0 mL of working solution of green tea extract (GTE) and 5.0 mL of standard solution of Fe ions or sample was precisely pipetted in laboratory tube with stopper. The formation of stable complex between polyphenols from green tea and the Fe ion is practically instantaneous. Depending on the concentration of the Fe ion, the color of the reaction

solution is in different shades of deep blue. The absorbances of these obtained solutions against reagent blank were measured at 570 nm.

## RESULTS AND DISCUSSION

### Optimization of process of preparing a natural reagent and reaction condition

The effect of water, acetate buffer and temperature on the stability of the tea extract was tested. Also, both Fe (II) and Fe (III) could form color complexes with green tea extract and Fe (III)-green tea complex has higher molar absorptivity (Pinyou *et al.* 2010) and therefore for the optimization experiment, Fe (III) was used.

The optimization of the preparation of the green tea reagent was made as follows. Volumes of 2.0, 4.0 and 8.0 g of green tea were added to the 150.0 mL of acetate buffer (pH 4.8) or water heated to 40 ° C. The extraction time was 10, 20 and 30 minute at mixing conditions (300 rpm). Mixture with water is thermostated at 40°C during extraction. Then the plant material was separated by filtration and the extracts were diluted with acetic buffer (pH= 4.8) or ultra-pure water in a 1:1 ratio.

Obtained reagents were used in optimization experiments for reaction condition for determination Fe ion. A volume of 5.0 mL green tea reagent and 5.0 mL working solution of Fe (III) were mixed. In resulting solutions concentration of Fe (III) was  $1.0 \times 10^{-3}$  mol/L and  $1.0 \times 10^{-4}$  mol/L. The absorbance was measured at 570 nm.

The obtained results show that absorbance was increased with increasing green tea weight mass, but it also slight increase of absorbance with higher extraction time, in both acetic buffer and DI water was observed. In order to obtain method with good sensitivity, stability and reasonable time of reagents preparation, 8.0 g of green tea and 10 minute extraction time in acetate buffer (pH = 4.8) were chosen as optimal for green tea reagents.

It has been observed that the absorbance of the reaction solution made with older reagents (24 h after preparation) is decreased by 33%, which indicate that tea extracts should be made daily.

In the next experiment, it was necessary to determine the optimal method for preparing the reaction solution, the volume of extract and the standard / sample solution which were mixed.

A green tea extract was prepared as described above. Different volumes of green tea reagents were mixed with 5.0 mL of standard Fe (III) solution,  $c = 1.0 \times 10^{-4}$  mol/L in the following ratios: 1:1 (5 mL GTE + 5 mL Fe (III) ); 3:5 (3 mL GTE + 5 mL Fe (III) ) and 1:5 (1 mL GTE + 5 mL Fe (III) ).

In this experiment one concentration of Fe (III)  $1.0 \times 10^{-4}$  mol/L was used and the highest absorbance was obtained for the reaction solution prepared by mixing 1.0 mL GTE with 5.0 mL of Fe (III) ion standard solution.

Also, reaction solutions were prepared at the same ratios, but this time with all five concentrations in the tested concentrations range. Measured and compared absorbance indicate that volume ratio (GTE:Fe ) 3:5 give better regression of calibration line.

**Table 1.** Equations of calibration lines for Fe (III) and Fe (II), linear regression coefficient, R<sup>2</sup> and detection limits

Concentration range, mol/L	Equation	R <sup>2</sup>	Detection limit, mol/L
1.0×10 <sup>-5</sup> - 5.0×10 <sup>-4</sup>			
Fe (III)	A = 1.105·c + 0.0188	0.9997	8.08×10 <sup>-6</sup>
Fe (II) + H <sub>2</sub> O <sub>2</sub>	A = 1.524·c + 0.0195	0.9980	9.52×10 <sup>-6</sup>

### Analytical application

Under the optimized conditions given above, rectilinear calibration graphs were obtained in the concentration ranges from 1.0×10<sup>-5</sup> to 5.0×10<sup>-4</sup> mol/L. The limits of detection (3s<sub>b</sub>/m, three of the standard deviation blank divided by slope of the calibration curve) and regression equations are given in Table 1.

In order to obtain good sensitivity and the same condition in reaction solution with sample and standard, calibration lines were prepared for both, Fe (III) and Fe (II). For determination of Fe (II) in standard or in real samples H<sub>2</sub>O<sub>2</sub> was added because of oxidation of Fe (II) to Fe (III).

In order to evaluate the potential of the proposed method to analysis of real sample, the method was applied for analysis of commercially available pharmaceutical samples, Table 2. The measurements with spiked samples

of degasses mineral water, juice and candy, (Table 3.) were assessed to verify the possible interference of different matrices. As shown in Table 2. the proposed method was successfully applied for the determination of Fe (II) in pharmaceutical preparation.

As it could be assumed, measurements obtained for spiked samples of juice, candy and mineral water showed poor recovery.

In the candy sample, there was very high concentration of sugars (w/w 95 %) that was more than 1000 –fold sugars excess than in spiked samples. The sugars in candy can be interference, and main reason for poor recovery.

It was found that the system could tolerate some cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cr<sup>3+</sup>, Cu<sup>2+</sup> and Mn<sup>2+</sup>) at least up to 1:1 concentration ratio (Pinyou *et al.* 2010). In the spiked sample of mineral water concentration ratio was much higher. Tested aliquot (20 mL) of spiked sample of mineral water contains 0.1972 mg/L (from labeled value) of cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup>). The amount of Fe added is 0.038 mg/L, which significantly exceeds the ratio of 1:1. We can conclude that cations present in the sample interfere determination of Fe ion, which is the main reason for poor recovery.

**Table 2.** Results for the determination of Fe (II) in pharmaceuticals

Sample	Taken, mol/L	Found ± SD, mol/L (n = 3)	Recovery
Heferol, Alkaloid AD Skopje	1.0×10 <sup>-4</sup>	9.7×10 <sup>-5</sup> ± 0.029	97.0 %
Retafer, Krka Farma doo	1.0×10 <sup>-4</sup>	9.6×10 <sup>-5</sup> ± 0.003	96.0 %

**Table 3.** Results for the determination of Fe (III) and Fe (II) ions in spiked samples

Sample	Fe (III), mol/L			Fe(II), mol/L		
	Added	Found* ± SD	Recovery	Added	Found* ± SD	Recovery
Orange juice	5.0×10 <sup>-4</sup>	3.6×10 <sup>-4</sup> ± 0.024	72.0 %	5.0×10 <sup>-4</sup>	3.8×10 <sup>-4</sup> ± 0.029	76.0 %
Degasses mineral water	7.0×10 <sup>-5</sup>	2.4×10 <sup>-5</sup> ± 0.014	34.2 %	7.0×10 <sup>-5</sup>	3.3×10 <sup>-5</sup> ± 0.008	47.0 %
Candy	1.0×10 <sup>-4</sup>	3.2×10 <sup>-5</sup> ± 0.011	32.0 %	1.0×10 <sup>-4</sup>	3.6×10 <sup>-5</sup> ± 0.005	36.0 %

\*Values are mean of three replicates (n=3)

### CONCLUSIONS

The proposed spectrophotometric method can be a good “green” alternative for routine analysis of the Fe ions in pharmaceuticals. The main advantages of the proposed methodology are the simple “green reagents” approach with detection limit of Fe ions at 8.08×10<sup>-6</sup> mol/L and low cost.

Good linearity between measured signal and concentration was achieved in the range from 1.0 × 10<sup>-5</sup> to 5.0 × 10<sup>-4</sup> mol/L for both, Fe (II) and Fe (III). Considering that the proposed method meets some principles of green analytical chemistry it is very suitable as “Green Chemistry for life” (PhosAgro /UNESCO/ IUPAC Partnership) approach in undergraduate and graduate analytical chemistry laboratory.

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## Summary/Sažetak

U ovom radu je opisana mogućnost primjene ekstrakta zelenog čaja kao prirodnog reagensa za spektrofotometrijsko određivanje iona Fe u realnim i laboratorijskim uzorcima. Reakcijski sustav se temelji na formiranju kompleksa između iona Fe i polifenola iz ekstrakta zelenog čaja u puferiranom mediju pri pH = 4,8. Absorbancija formiranog tamnoplavog kompleksa mjeri se pri valnoj duljini od 570 nm. Linearna ovisnost postignuta je u području koncentracija od  $1,0 \times 10^{-5}$  do  $5,0 \times 10^{-4}$  mol/L. Utjecaj različitih matrica testiran je na uzorcima voćnog napitka, negazirane mineralne vode, i bombone u koje je dodana određena koncentracija iona Fe (III) i Fe (II), a mogućnost analitičke primjene testirana je na farmaceutskim pripravcima. Predložena metoda je jednostavna, jeftina i primjenjiva u analitičkim laboratorijima.