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Polymethacrylate Colorimetric Sensor for Evaluation of Total Antioxidant Capacity

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Abstract

A new colorimetric sensor using an immobilized chromogenic redox reagent was devised for measuring the total antioxidant level in a liquid sample without requiring sample pretreatment. The reagent, Fe(III) – 1,10-phenanthroline (Fe(III)-phen), was immobilized into a polymethacrylate matrix (PMM), and the absorbance changes associated with the formation of the highly colored Fe(II)-phen chelate as a result of reaction with antioxidants was measured at 510 nm. The developed optical sensor was used to screen total antioxidant capacity of some black and green teas, red and white wines.

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Keywords: Antioxidant capacity; immobilized reagents; antioxidants; colorimetric sensor; transparent polymeric matrix

1. Introduction

The fact of harmful effect of reactive oxygen species on human health is well-known. The accumulation of reactive oxygen species in the organism, unless counterbalanced by antioxidants taken in through diet, may cause oxidative damage to DNA and cellular membranes under "oxidative stress" conditions, eventually giving rise to certain human diseases, especially cardiovascular disease and some types of cancer. In this context, the measurement of antioxidant capacity of food and biological samples through development of selective and sensitive

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new techniques has recently gained importance[1,2] . Optical sensors based on immobilized reagents in a solid matrix are particularly suited to rapid and low-cost screening applications. Optical chemical sensors play an important part in industrial, environmental and clinical monitoring thanks to their low cost, possibility for miniaturization, great flexibility, and inexpensive use outside the laboratory[3,4]. Among different types of optical chemical sensors, colorimetric sensors are especially attractive because they recognize analytes through color change that allows obtaining the visually observed and easily measurable analytical signal[5]. The analytical signal measurement can be carried out using not only standard spectrophotometric equipment, but also some modern engineering solutions like portable fiber optic spectrometer connected to a mobile computer or smart (or even cell) phone and also the naked eye without the use of an expensive equipment. Although a great many colorimetric sensors have been developed over the years for a wide range of analyte samples, there is very limited study about the usage of optical sensors for molecular spectroscopic total antioxidant capacity assays. Bener, M. et al. adapted the cupric reducing antioxidant capacity assays to an optical version[6,7].

In the present work, we have studied the feasibility of transforming the solution-based Fe(III)-based antioxidant assay[8,9] to the colorimetric sensing by using polymethacrylate matrix (PMM) with an immobilized Fe (III) – 1,10-phenanthroline system. The usage of PMM allows to combine both the solid phase capability to immobilize reagents without losing the matrix transparence and the reagents capability to participate in the analytical reaction with analytes accompanied by an optical effect[10,11].

2. Experimental

2.1. Materials and chemicals

The PMM is a specially created material containing functional groups which provide ability to extract both the reagent and determined substance. Transparent 10 × 10 cm polymethacrylate plates, thickness (0.60 ± 0.04) mm, were prepared by the radical block polymerization of methacrylate and (alkyl)acrylates of alkaline (or alkaline earth) metals at the temperature 60-70 °C for 3-4 hours[10]. Then these plates were cut as 6.0 × 8.0 mm working platelets (weight ca. 0.05 g) for analyses.

All reagents were of analytical grade and used as purchased without further purification. Deionized and distilled water was used in all experiments. Solution of Fe(III) with a concentration of 1 mg/mL was prepared by the dissolution of precise weighed portions of iron(III) ammonium sulfate in sulfuric acid solutions according to the procedures reported in standard[12]. Solution of 1, 10-phenanthroline (0.5%) was prepared by dissolving 0.05 g of reagent in water with 1-2 drops of HCl and diluting it to 100 mL. The antioxidants stock solutions were freshly prepared in water or ethanol: solutions of 1 mg/mL ascorbic acid, tannin, cysteine, catechin and solution of 0.1 mg/mL gallic acid in water, solutions of 0.1 mg/mL quercetin, rutin, luteolin and dihydroquercetin in ethanol. Citrate buffer solutions were prepared from 0.03 M sodium citrate with 0.05 M hydrochloric acid, pH 3. Solution of aluminum chloride ($AlCl_3$) was prepared by dissolving 0.5 g of reagent n ethanol and and diluting it to 25 mL.

2.2. Procedure

Sensing PMM preparation. The immobilization of Fe(III)-phen into PMM was done in two steps. At the first step, PMM were treated in solutions of Fe(III) for 5 min, and then in solutions of 1,10-phenanthroline for 5 min. The polymer plate after the adsorption of the reagents remained colorless and transparent.

Antioxidant activity sensor procedure. PMM with an immobilized Fe(III)-phen (PMM–Fe(III)-phen) was put into 50.0 mL of antioxidant sample solution of different concentrations at pH 3 and shaken with a laboratory shaker for 45 min at room temperature, then the matrix was removed and dried with a piece of filter paper. Sample absorbance was measured in the maxima of absorption bands of the formed Fe(II)-phen in PMM at 510 nm.

Determination of total content of flavonoids and tannins in tea samples. Total flavonoid and tannin contents were measured with $AlCl_3$ according to a known method[13] using rutin and tannin as a standard for determination of tea flavonoids and tannins, respectively. The tea extract (1 mL) or 0.25 – 1.00 mL of standard rutin solution (1 $g \cdot L^{-1}$) was added to 25 mL volumetric flask containing 5 mL of water. To the above mixture, 1 mL of 2 % $AlCl_3$ was added and the total volume was made up to 25 mL with water. The solution was mixed well and after 40 minutes, the absorbance was measured at 410 nm against blank solutions, containing all the same components as analyzed

solution except $AlCl_3$. The flavonoid content was calculated from corresponding equation of the rutin calibration curve and was expressed as g of rutin per 100 g tea leaves. Total tannins were determined by measuring the absorbance at 320 nm; their content was calculated as described above.

Determination of total content of phenols in wine samples. For the relationship between antioxidant power and levels of wine polyphenols, total phenolics content was measured by the Folin-Ciocalteu method using tannin as a standard. Folin-Ciocalteu's reagent , 0.5 mL, was added to 0.1 – 0.2 mL of wine sample in 25 mL volumetric flask. To the above mixture, 0.5 mL of 30 % NaOH was added and the total volume was made up to 25 mL with water. The solution was mixed well and after 30 minutes, the absorbance was measured at 630 nm against blank solutions, containing all the same components as analyzed solution except Folin-Ciocalteu's reagent. A calibration curve was obtained using various concentrations of tannin. Results were expressed as g of tannin per 100 mL of wine.

Sample Preparation. Freshly prepared, filtered, and cooled tea infusions (2.5 g of dry tea leaves/200 mL of boiling distilled water with incubatiion in water bath for 45 min) were diluted as appropriate with distilled water.

2.3. Apparatus

Absorption spectra and absorbance of PMM matrix and solutions were recorded on Evolution 600 spectrophotometer (Thermo Fisher Scientific Inc., USA) against a polymer plate prepared under the same conditions, without reagents.

The pH values were measured by I-160 ionometer (NPO "Izmeritelnaya tekhnika", Russia) with a glass pH-selective electrode. The ionometer had absolute error ± 0.020 pH and was calibrated at 25 °C using buffer solutions with pH 1.00 and 9.18.

3. Results and discussion

As an antioxidant for the study we selected the most frequently encountered in real objects antioxidant phenolic nature such as quercetin, tannin, rutin, gallic acid, catechin, luteolin, and non-phenolic nature - ascorbic acid and cysteine. PMM–Fe(III)-phen upon exposure to a solution of antioxidants turns orange because of the reduction of Fe(III) to Fe(II) and the formation of the complex of Fe(II) with 1,10-phenanthroline (Fe(II)-phen) in PMM. Figure 1 presents absorption spectra of Fe(II)-phen in PMM produced after contact of PMM–Fe(III)-phen with antioxidant sample solution (pH 3.0–3.5). The absorbance of the Fe(II)-chelate formed as a result of redox reaction with reducing antioxidants was measured at 510 nm. Table 1 summarizes the linear equations, correlation coefficients (r), and linear concentration ranges of pure antioxidants. As can be seen from Table 1, all antioxidants could be assayed with the colorimetric sensor-based PMM–Fe(III)-phen.

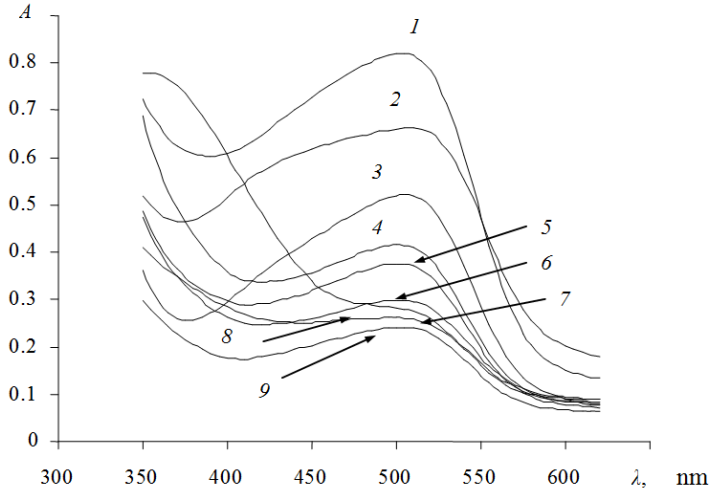

Fig. 1. Absorption spectra of Fe(II)-phen chelate produced as a result of contact of PMM–Fe(III)-phen with antioxidant sample solution: *1* - gallic acid, *2* - quercetin, *3* - ascorbic acid, *4* - catechin, *5* - dihydroquercetin, *6* - tannin, *7* - luteolin, *8* - rutin, *9* – cysteine

The slope values of linear calibration curves of the studied antioxidants ranges from 0.98 for cysteine to 95.38 for gallic acid that is explained by different antioxidant capacity of the studied antioxidants. The order of antioxidant capacity is gallic acid>quercetin>ascorbic acid>catechin>dihydroquercetin>tannin>luteolin>rutin>cysteine. Generally, this order is in accordance with that of other widely used electron transfer-based antioxidant assays.

Table1. Linear calibration equations, correlation coefficients (r), and linear concentration range of the tested antioxidants with respect to the colorimetric sensor based on PMM–Fe(III)-phen

Antioxidants	Linear equation	r	Linear range, g·L^{-1}
Gallic acid (*GA*)	A_{510}=0.13+95.38·c_{GA}	0.999	0.001-0.01
Quercetin (*QR*)	A_{510}=0.15+50.17·c_{QR}	0.993	0.001– 0.01
Ascorbic acid (*AA*)	A_{510}=0.12+19.61·c_{AA}	0.999	0.005 – 0.03
Catechine (*CT*)	A_{510}=0.13+12.22·c_{CT}	0.995	0.005 – 0.05
Dihydroquercetin (*DQR*)	A_{510}=0.13+10.38·c_{DQR}	0.999	0.005 - 0.03
Tannin (*T*)	A_{510}=0.11+8.11·c_{T}	0.995	0.01 – 0.05
Luteolin (*L*)	A_{510}=0.15+7.35·c_{L}	0.993	0.005 – 0.03
Rutin (*R*)	A_{510}=0.16+2.24·c_{R}	0.994	0.005 – 0.03
Cysteine (*CYS*)	A_{510}=0.14+0.98·c_{CYS}	0.997	0.005 – 0.03

Ascorbic acid (AA) was chosen as a standard for the antioxidant activity due to its reducing properties and availability. The dependence of PMM–Fe(III)-phen absorption on ascorbic acid concentration and the scanned images of samples are presented in Figure 2. The total antioxidant capacity (gAA·L^{-1}) was determined by measuring the absorbance at 510 nm of PMM–Fe(III)-phen after its contact with antioxidants and using the corresponding equation of the calibration graph of ascorbic acid (Table 1) to the following:

$$total\ \ antioxidant\ \ capacity = \left(A_{510} - 0.12\right)/19.61\left(gAA \cdot L^{-1}\right)$$

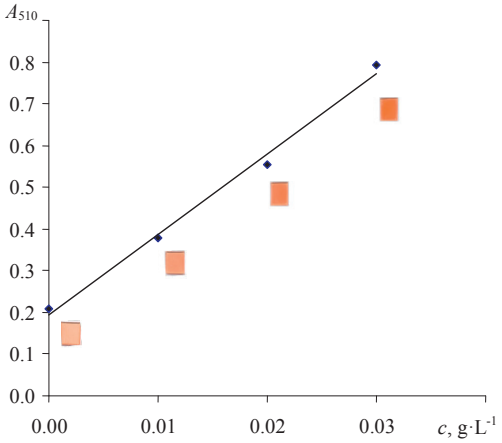

Fig.2 - The dependence of PMM–Fe(III)-phen absorption on ascorbic acid concentration and the scanned images of samples

The sensor was used to assess the antioxidant activity of black and green teas (Table 2), red and white wines (Table 3). The obtained results were compared with the content of total flavonoids and tannins of teas and total phenols of wines.

The antioxidant power of green teas was found to be mainly higher than that of black teas; this is in accordance with the results obtained by the ferric reducing/antioxidant power assay[8]. Furthermore, the antioxidant power of teas correlated with the total flavonoid and tannin contents (Figure 3).

Table 2. Total flavonoids, total tannins, and total antioxidant capacityof green and black teas (n = 3. P = 0.95)

Tea trade mark and name	Total antioxidant capacity (g of AA/100 g of tea leaves)	Total flavonoids (g of rutin/100 g of tea leaves)	Total tannins (g of tannin/100 g of tea leaves)	Total amount of flavonoids and tannins
		Green teas		
Jaf	11.2±1.5	1.38±0.06	6.5±0.1	7.38
Tess	5.9±0.4	1.97±0.06	4.4±0.3	6.37
Princess Java	4.4±0.5	2.47±0,07	3.3±0.1	5.77
Lipton	4.3±0.8	1.65±0.06	4.3±0.5	5.95
		Black teas		
Riston	4.7±0.5	1.51±0.07	6.6±0.4	8.11
Ahmad	4.0±0.4	1.69±0.04	5.8±0.4	7.49
Beseda	3.5 ±0.7	1.48±0.02	5.8±0.4	7.28

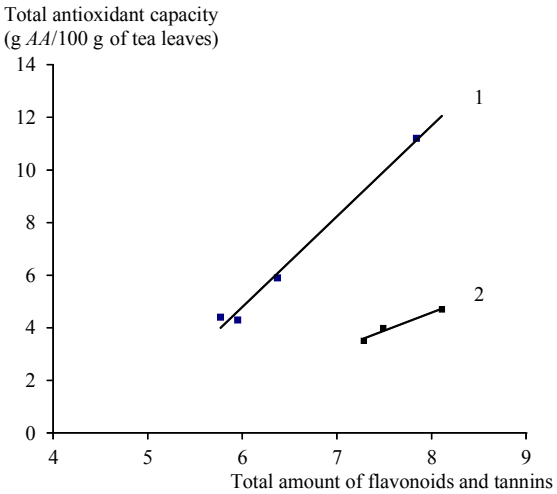

Fig.3 - The relationship between the total antioxidant capacity and total amounts of flavonoids and tannins of green (1) and black (2) teas: correlation coefficients 0.995 (1) and 0.983 (2)

The results of this study demonstrate that red wine has better antioxidant properties than white wine. The higher total antioxidant capacity of red wines is in agreement with the results of studies in which the antioxidant potential of wines was measured with the ferric reducing/antioxidant power assay[14]. The antioxidant properties of wines are attributed to the phenolic compounds contained in it. From the results (Table 3) it is seen that the antioxidant power of wines correlated with the total phenolic content.

Table 3. Total phenols and total antioxidant capacity of red and white wines (n = 3, P = 0.95)

Sample	Total antioxidant capacity (g of AA/100 mL of wine)	Total phenols, (g of tannin/100 mL of wine)
	Red wines	
Isabella, semi-sweet	0.030±0.002	0.095±0.011
Merlo, dry	0.025±0.005	0.095±0.002
Cabernet, dry	0.070±0.006	0.26±0.04
	White wine	
Chardonnay, semi-sweet	0.012±0.003	0.036±0.003
Muscat, semi-sweet	0.010±0.001	0.0281±0.0001

4. Conclusion

This work describes the design of a colorimetric sensor on basis of PMM–Fe(III)-phen that is reasonably and sensitive for the determination of total antioxidant capacity in teas and wines. The Fe(III)-phen chromogenic reagent was immobilized in polymethacrylate matrix, and the absorbance change associated with the formation of the highly colored Fe(II)-phen chelate as a result of reaction with antioxidants was measured at 510 nm. The colorimetric PMM–Fe(III)-phen sensor can be used for quantitative assessment of total antioxidant capacity of complex matrices such as teas and wines in ascorbic acid equivalent units. The sensor is small and cheap, suitable to fit in a portable instrument for in situ antioxidant analysis. The proposed solid-state method has some advantages over solution-based assays including the potential for use samples without pretreatment. PMM–Fe(III)-phen is suitable for use with coloured or turbid samples which are not possible with the solution-based by the ferric reducing.

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