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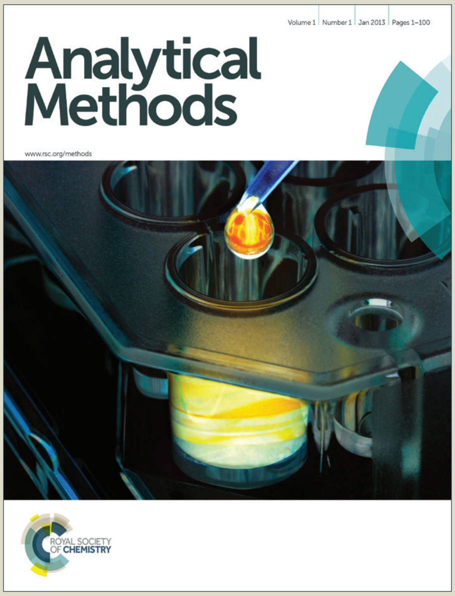

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Determination of hindered phenolic antioxidants in plastic packaging injections by magnetic solid phase extraction followed by high performance liquid chromatography

Wenlong LIAO [a]; Anyi CHEN [a]; Yaling YANG[*a]

Abstract

A simple and effective method based on magnetic solid-phase extraction combined with high performance liquid chromatography was used for the determination of hindered phenolic antioxidants in plastic packaging injections. The extraction and cleanup via Fe_3O_4@CTAB magnetic adsorbent dispersion in injections followed by magnetic isolation and desorption of the analytes using acetonitrile. The cationic surfactant cetyltrimethylammonium bromide (CTAB) coated on the surface of Fe_3O_4 nanoparticles adsorbent was synthesized. Main parameters affecting the adsorption recoveries were evaluated and optimized, including the amount of surfactant and adsorbent, pH, ionic strength, desorption conditions, and sample volume. Under the optimum conditions, the method was successfully applied to the determination of hindered phenolic antioxidants in plastic packaging injections. Low limits of detection (LODs) of 0.14 and 0.15 μg mL^{-1} and limits of quantification (LOQs) of 0.45 and 0.50 μg mL^{-1} were achieved. The mean recoveries were in the range from 85.0 to 93.5% at 5, 10, and 20 μg mL^{-1} spiked levels, and the relative standard deviations (RSDs) were in the range from 1.16 to 2.81%.

Key words: magnetic solid phase extraction; hindered phenolic antioxidants; injections

1. Introduction

Polypropylene (PP), a kind of the plastic materials is widely used for food packaging, lab equipments and automotive parts, due to its excellent mechanical properties, low cost, and superior processibility. However, PP is likely to be degraded when processing at high temperature or for prolonged use under unfavorable conditions, in the case of light, heat, and chemical agents. Thus, a large number of organic and inorganic additives were added to the PP plastic to improve its stability and durability during manufacture, transport and storage. The additives include antioxidants stabilizers, lubricants, softeners and coloring agents [1].

Antioxidants can be classified as primary and secondary based on their action mechanisms. Radical scavengers, one of the well known primary antioxidants, inhibit oxidation by donating a hydrogen atom, thus competing with the polymer in the formation of peroxy radicals. Hindered phenolic antioxidants are another kind of effective primary antioxidants most used in industry, which have good pollution-free and non-discoloring properties compared with the toxicity and discoloration of aromatic amine antioxidants [2-4]. These antioxidants can improve stability of plastics, but they can also migrate from plastics into its content and contaminate it after a long time contact. The related problem of public safety attracts much attention, especially a variety of injections are also stored in this plastic bottles nowadays.

It is obvious that studies on the migration mechanism and migration levels of these additives are very important for the quality control of injections. The sample pretreatment is a significant step because of the trace content of antioxidants in plastic. Recently, some pretreatment techniques were used for sample preparation of additives in plastics, such as liquid−liquid extraction (LLE) [5, 6], solid-phase extraction (SPE) [7-9], solid-phase microextraction (SPME) [10-12], liquid-phase microextraction (LPME) [13], and so on. The aforementioned methods have some limitations to some degree, such as time-consuming, or require large volumes of solvents. Therefore, establishing a rapid, inexpensive, and environmentally friendly method is very necessary. Our team has reported a method to determinate antioxidants based on cloud point extraction (CPE) using tergitol TMN-6 and dodecylpolyoxyethylene ether (AEO9) as the extraction solvent [14, 15].

Recently, the use of iron oxide magnetic nanoparticles (MNPs) has been found extensive applications in a variety of fields [16-20]. Magnetic solid phase extraction (MSPE) based on MNPs is an efficient method for separation and preconcentration of chemical species. Fe_3O_4 nano-particles (Fe_3O_4 NPs) played an important role in our work due to their high surface area and excellent chemical selectivity [21-25]. However, bare Fe_3O_4 NPs can encounter limitations in practical applications, and the hydrophobic surface would mean that the particles cannot disperse well into aqueous solutions [26]. Previous studies have shown that coating the nanomaterials with cationic surfactant cetyltrimethylammonium bromide (CTAB) could greatly inhibit the aggregation of nanoparticles [27].

In this paper, Fe_3O_4 NPs were synthesized via a simple chemical co-precipitation method and the surface was modified by CTAB (designated Fe_3O_4@CTAB). Antioxidants were adsorbed onto Fe_3O_4@CTAB which positively charged CTAB ions were adsorbed onto the negatively charged Fe_3O_4 NPs surface. The proposed method was applied to the separation and preconcentration of antioxidants included 2,2'-methylenebis (6-tert-butyl-4-methylphenol) (Cyanox 2246), pentaerythritol tetrakis (3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate) (Irganox 1010), 1,3,5-trimethyl -2,4,6-tris(3,5-di-tert-butyl-4-hydroxybenzyl)benzene (Irganox 1330) in plastic packaging injections prior to be determined by HPLC-UV detection.

2. Materials and method

2.1. Instrumentation and chromatographic conditions

A scanning electron microscopy (SEM) scanning system VEGA3 SBH (Tescan, Czech Republic) with a tungsten electron gun was used to provide electron beam irradiation was used for characterization of Fe_3O_4 NPs. Powder XRD patterns of Fe_3O_4 NPs and Fe_3O_4@CTAB were collected on a Rigaku D/max 2200 powder diffraction meter (Rigaku, Japan). Fourier transformed infrared spectroscopy of Fe_3O_4 NPs and Fe_3O_4@CTAB were obtained from IRTracer-100 (SHIMADZU, Japan) in 400-4000 cm^{-1} wavenumber range. pH-meter Sartorius PB10 (Gttingen, Germany) was used to determine the pH value of solution. Vacuum drying oven BPZ-6033

(Shanghai, China) was used to dry synthesized nanomaterials. The chromatographic experiments were carried out with Agilent 1100 series high-performance liquid chromatography (HPLC) system (Agilent Technologies, U.S.A.) equipped with a reversed phase C18 analytical column of 150×4.6 mm (Agilent TC-C18), maintained at 25 ℃. The conditions of chromatographic method were as follows: the mobile phase was 100% acetonitrile, the flow rate was 1 mL min^{-1}, the injections volume was 20 μL, and the detection wavelength was set at 276 nm.

2.2. Materials

$FeCl_3 \cdot 6H_2O$, ammonium nitrate (Tianjin zhiyuan, China), $FeCl_2 \cdot 4H_2O$ (Shanghai Aladddin, China), cetyltrimethylammonium bromide (Shanghai Bo'ao, China) were all of analytical reagent grade. Acetonitrile of HPLC grade was purchased from Merck (Darmstadt, Germany). Deionized water provided by a Milli-Q system (Millipore, Bedford,MA, U.S.A.). Standards of hindered phenolic antioxidants were supplied by Sigma (St. Louis, Mo., U.S.A.), and the structures were shown in Fig. 1. A stock standard solution containing 0.4 mg mL^{-1}of antioxidants were dissolved in acetonitrile and kept in a freezer (4℃).

2.3. Preparation of modified magnetic iron oxide nanoparticles

The Fe_3O_4 NPs were synthesized by the co-precipitation method with some modification, briefly 5.0 g $FeCl_2 \cdot 4H_2O$ and 6.8 g $FeCl_3 \cdot 6H_2O$ were dissolved in 50 mL of deionized water, the mixture was added dropwise into 50 mL buffer solution of ammonia solution / ammonium nitrate (pH=10) under vigorous stirring and nitrogen gas protection, 30 mL ammonia solution were also added dropwise into the reaction solution at the same time. The obtained Fe_3O_4 NPs were separated by an external supermagnet after 30 min, washed with deionized water for four to five times and vacuum-dried at 60 ℃ for 12 h.

The Fe_3O_4 NPs were functionalized with CTAB according to a similar process presented in literature [28]. Briefly 90 mg of CTAB was dispersed in 10 mL of deionized water, then 0.1g of dried Fe_3O_4 NPs was added into the solution and sonicated (200 W, 40 kHz) for 30 min. The obtained Fe_3O_4@CTAB NPs were collected with an external supermagnet. After washed with deionized water for three times, the Fe_3O_4@CTAB were diluted to 10 mL with deionized water and stored in a freezer (4 ℃) for further use, the concentration of Fe_3O_4@CTAB suspension was estimated to be about 10 mg mL^{-1}.

2.4. MSPE Procedure

Fe_3O_4@CTAB was used in MSPE procedure for separation, precocentration of antioxidants. First, 200 μL stock standard solution was diluted with deionized water to 10 mL in a vial, and 100 μL of Fe_3O_4@CTAB suspension was added into this solution. After completely mixing, the magnetic adsorbents were collected by an external supermagnet. The supernatant was decanted and the adsorbed analytes were eluted with 2 mL acetonitrile for twice, and the eluate was injected into the HPLC system for analysis after filtered with 0.45 μm polyether sulfone filters. The blank tests were carried out under the same conditions with blank solution without adding any analytes. For the real sample analysis, 1 mL of injections was diluted with deionized water to a volume of 10 mL before the quantification of antioxidants. The digital pictures showed the phenomena of Fe_3O_4@CTAB dispersed in sample solution (a) and collected by supermagnet (b).The procedures of adsorption and magnetic separation could be finished within 2 minutes.

3. Results and discussion

3.1. Characterization of adsorbents

The SEM-images of synthesized Fe_3O_4 NPs (Fig. 3 ⅰ, ⅱ) showed homogeneous distribution of particles and the determined particle size in the case of Fe_3O_4 NPs were identified in the range of 100-300 nm. Absorption peaks were observed in FT-IR spectra of Fe_3O_4 NPs (Fig. 3 ⅲ) and Fe_3O_4@CTAB (Fig. 3 ⅳ), the band at 550-650 cm^{-1} could reflect the vibration of Fe-O groups [29]. The peaks at 1450, and 2800, 2900 cm^{-1}could be assigned to the stretching vibrations of -C-CH_2 and -C-H groups [30], respectively. These bands are known to be the characteristic bands of CH_2 groups which are presented in CTAB, demonstrated that CTAB had been successfully coated onto the surface of Fe_3O_4 NPs. The crystalline structures of the nanoparticles were identified with XRD. For Fe_3O_4 NPs (Fig. 3ⅴ), diffraction peaks with 2θ of 30.4°, 35.6°, 43.3°, 57.3°, and 62.8°were observed, indicating a cubic spinel structure of the magnetite [31]. The same sets of characteristic peaks were also observed for Fe_3O_4@CTAB (Fig. 3 vi), indicating the stability of the crystalline phase of Fe_3O_4 NPs during CTAB coating.

3.2. Optimization of the MSPE conditions

In order to select the optimum MSPE conditions for the extraction of antioxidants, 10 mL deionized water spiked with 200 μL (0.4mg mL^{-1}) antioxidants was used to study the extraction performance of the MSPE, Fe_3O_4@CTAB was used as adsorbent which containing 0.9 % (w/v) of CTAB in 10 mL aqueous solution (containing 0.1 g of Fe_3O_4 NPs). All the experiments were performed in triplicate and the means of the results were used for optimization.

3.2.1. Effect of the amount of surfactant

The ideal amount of CTAB was determined by coating 0-1.2 % (w/v) of this compound on the surface of Fe_3O_4 NPs. As can be seen (Fig. 4.), in the absence of surfactant, the analytes were hardly adsorbed to the magnetic adsorbent. The adsorption of antioxidants increases remarkably with increasing the amount of CTAB up to 0.6 %, this can be explained by gradual formation of hemimicelles layer, then the recovery increased slightly until 0.9 % of CTAB was added, which show formation of admicelles. A decrease in the recovery after this amount can be attributed to the formation of micelles in the bulk aqueous solution causing re-distribution of the analytes. Thus, the optimum amount of CTAB was 0.9 % in 10 mL Fe_3O_4@CTAB suspension which containing 0.1 g of Fe_3O_4 NPs.

3.2.2. Effect of the amount of adsorbent

The amount of adsorbent was an important parameter, the adsorption behavior of the amount of adsorbent was investigated and the result was shown in Fig. 5. The maximum recoveries was obtained at 100 μL of adsorbent, too much or too little was not suitable for the adsorption efficiency, the analytes could not be completely adsorbed at 50 μL of adsorbent, and the analytes were not easily eluted from the adsorbent when the dosage of adsorbent was too large. According to the results, 100 μL of Fe_3O_4@CTAB suspension was selected in the following experiments.

3.2.3. Effect of solution pH

The pH of the solution was one of the prime factors affecting the adsorption behavior of mixed hemimicelles system. The surface of Fe_3O_4@CTAB were positively charged when the pH value was below the point of zero charge (PZC) which was reported to be 6.4 previously [31]. In this study, the effect of solution pH was investigated by varying the parameter in the range of 3–13. As shown in Fig. 6, the magnetic adsorbents exhibited low adsorption of antioxidants when the pH value was below 5 that because Fe dissolved from Fe_3O_4@CTAB dispersed in the acidic solution, and the solution became dark brown and only partial antioxidants were collected. With the pH value ranged from 9 to 13, the Fe_3O_4@CTAB surface became negatively charged, the bonding interaction between Fe_3O_4@CTAB and antioxidants (which are also negatively charged at this pH range) would be decreased. The pH of sodium chloride injections and glucose injection were determined to be about 5.36 and 4.48, respectively. The pH ranged from 5.43 to 5.66 after the concentration of the samples solution diluted in 10 times, which showed good recoveries. Therefore, there was no need to adjust the pH of test solution.

3.2.4. Effect of salt content of the sample

The extraction efficiency can also be enhanced by the addition of salt. Its effect on the extraction efficiency was investigated by varying the concentration of NaCl in the standard solution. The results (shown as Fig. 7) showed that the recoveries remained above 85% with the concentration of NaCl ranged from 0.00 to 0.02 mol L^{-1} in the test solution, and the optimum recoveries were obtained without the addition of NaCl. The same inhibition trends were also observed by Gao et al.[32] which used graphene oxide as adsorbent, as previously reported, the added NaCl can influence adsorption capacities of tetracycline on graphene oxide by the electrostatics screening effect. We supposed that the adsorption of antioxidants on Fe_3O_4@CTAB would be influenced by cation-π interaction between analytes and Na^+. The electron-rich π system above and below the benzene ring hosts a partial negative charge, the negatively charged region can then interact favorably with positively charged species such as Na^+, so the electrostatic interaction between Fe_3O_4@CTAB and antioxidants would be decreased. As is known to all, the concentration of NaCl in sodium chloride injections is about

0.15 mol L^{-1}, there was no need to add NaCl in sample solution.

3.2.5. Effect of sorption time

The effect of sorption time was carried out at the optimal condition, the separation and preconcentration of antioxidants was fast and effective, the adsorption was almost completed within 2 min.

3.2.6. Effect of solution volume

Mixed hemimicelles SPE procedure based on magnetic carrier technology avoids many time-consuming steps such as column passing sample loading and filtration and shows a great potential for preconcentration of large volume samples. The volume of test solution was investigated by varying the parameter in the range of 10, 50 and 100 mL, while the dosages of standards were kept in 80 μg. The result was shown as Fig. 8. With the volume of test solution increased, the recoveries showed a declined tendency. Thus, 10 mL was selected as optimum value for the next experiments.

3.2.7. Desorption conditions

Desorption of the analytes from the magnetic adsorbent was studied by using different organic solvents (methanol, acetonitrile, acetone), and the effect of desorption solution volume on desorption efficiency of the analytes was also investigated. As a result (Fig. 9), the recoveries for three desorption solvents were higher than 85%, the eluting power of acetonitrile and methanol were equivalent and stronger than acetone. However, the adsorbent dispersed in methanol was difficult to collect, which would take almost 5 min, and the chromatogram eluted with acetone showed a strong UV absorption peak at 276 nm (the cut-off wavelength of acetone is 330nm) which had an effect on the target analytes. It was also found that all analytes could be quantitatively desorbed from the sorbent by rinsing the sorbent with 2 mL acetonitrile for twice. Thus, 2 mL acetonitrile was selected as the optimum desorption condition.

3.3. Analytical performance

The calibration curves were obtained from peak areas of the reference standards against their concentrations, a series of the standard mixture solutions concentration ranges were selected (regression coefficients (R^2) ≥0.999 for all analytes) to get a good linearity. The LOD of proposed method was in the range from 0.14 to 0.15 μg mL^{-1} based on the ratio of signal-to-noise (S/N = 3), and the LOQ of proposed method was in the range from 0.45 to 0.50 μg mL^{-1} based on the ratio of signal-to-noise (S/N = 10). Detail information regarding the calibration curves, linear ranges, and relative standard deviations (RSD) was shown in Table 1. The typical HPLC chromatograms of separated and preconcentrated antioxidants were shown as Fig. 10.

3.4. Analysis of injections

The validity of the proposed method was examined for the adsorption and desorption of antioxidants migrated from polypropylene bottles to injections. Injections include sodium chloride injections (100mL and 500mL), glucose injection (100mL) were purchased from the university hospital (Kunming, China). The recoveries and repeatability for antioxidants in injections were tested by adding different amounts of standards, the detail data were listed in Table 2. As can be seen, The mean recoveries were in the range from 85.0 to 93.5% at 5, 10, and 20 μg mL^{-1} spiked levels, and the RSDs were in the range from 1.16 to 2.81%.

3. 5. Reusability of adsorbent

In order to investigate the recycling of the adsorbent under optimized conditions, the adsorbent were rinsed sequentially with methanol and acetonitrile alternately, and deionized water (2×5 mL) before application in the next time. No obvious changes were observed in the recoveries for 3 times (Table.3). The results of this study indicate that the adsorbent is reusable without a considerable loss in it adsorption efficiency during extraction procedure.

4. Conclusions

In this study, a fast, simple, stable method for separation and preconcentration of migration levels of Cyanox 2246, Irganox 1010, Irganox 1330 in injections has been developed. This method involved MSPE of antioxidants with Fe_3O_4@CTAB and the determination by HPLC, the as-prepared Fe_3O_4@CTAB had an average diameter about 200 nm. It was notable that the separation and preconcentration of those antioxidants were fast and could be finished within two minutes. Moreover, the consumption of organic solvent was greatly reduced compared with classical methods such as LLE, SPE and so on. The achieved results in the studies showed the potential applications of this method, it is recommended to support drugs security determination of antioxidants in injections.

Notes and references

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Figures

Fig. 1. Structures of three kinds of hindered phenolic antioxidants

Fig. 2. Digital pictures of Fe_3O_4@CTAB dispersed in sample solution (a) and collected by supermagnet (b)

Fig. 3. SEM images of Fe_3O_4 NPs (ⅰ , ⅱ), FT-IR spectra of Fe_3O_4 NPs (ⅲ) and Fe_3O_4@CTAB (ⅳ), XRD patterns of Fe_3O_4 NPs (ⅴ) and Fe_3O_4@CTAB (ⅵ)

Fig. 4. Effect of the amount of CTAB

Fig. 5. Effect of the amount of sorbent

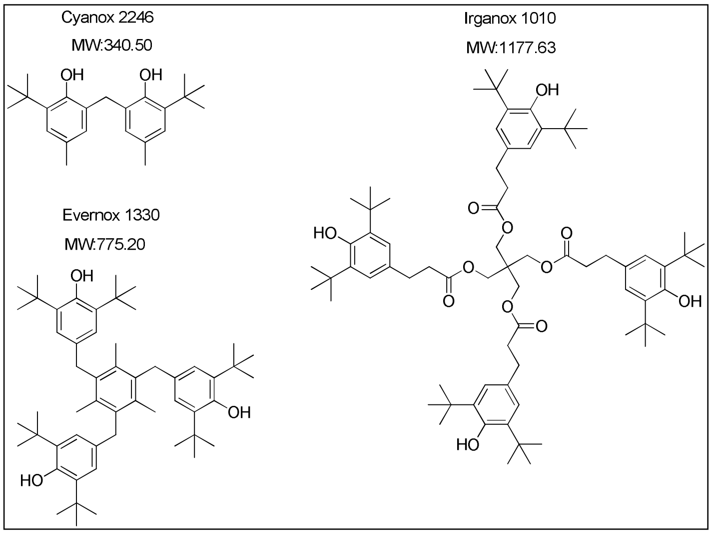
Fig. 6. Effect of pH value

Fig. 7. Effect of NaCl concentration

Fig. 8. Effect of volume of test solution

Fig. 9. Effect of eluent type

Fig. 10. HPLC-UV chromatograms: (a) 2 mL of sodium chloride injection spiked with antioxidants (b) 2 mL of sodium chloride injection preconcentrated by Fe_3O_4@CTAB, (c) whitout preconcentrated



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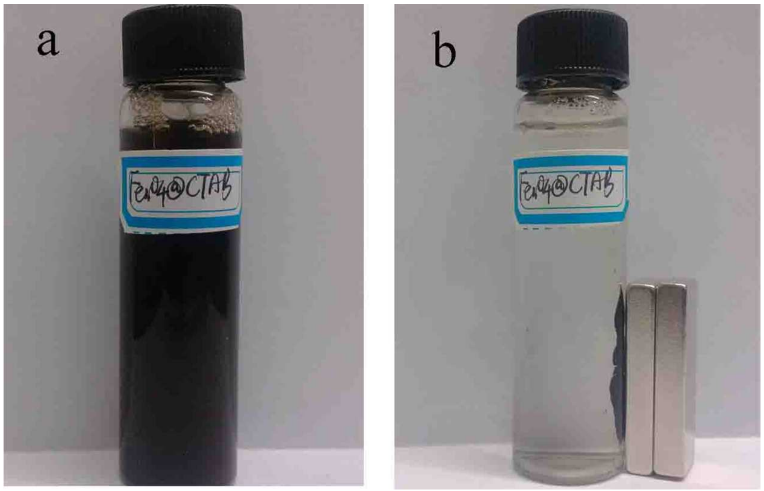

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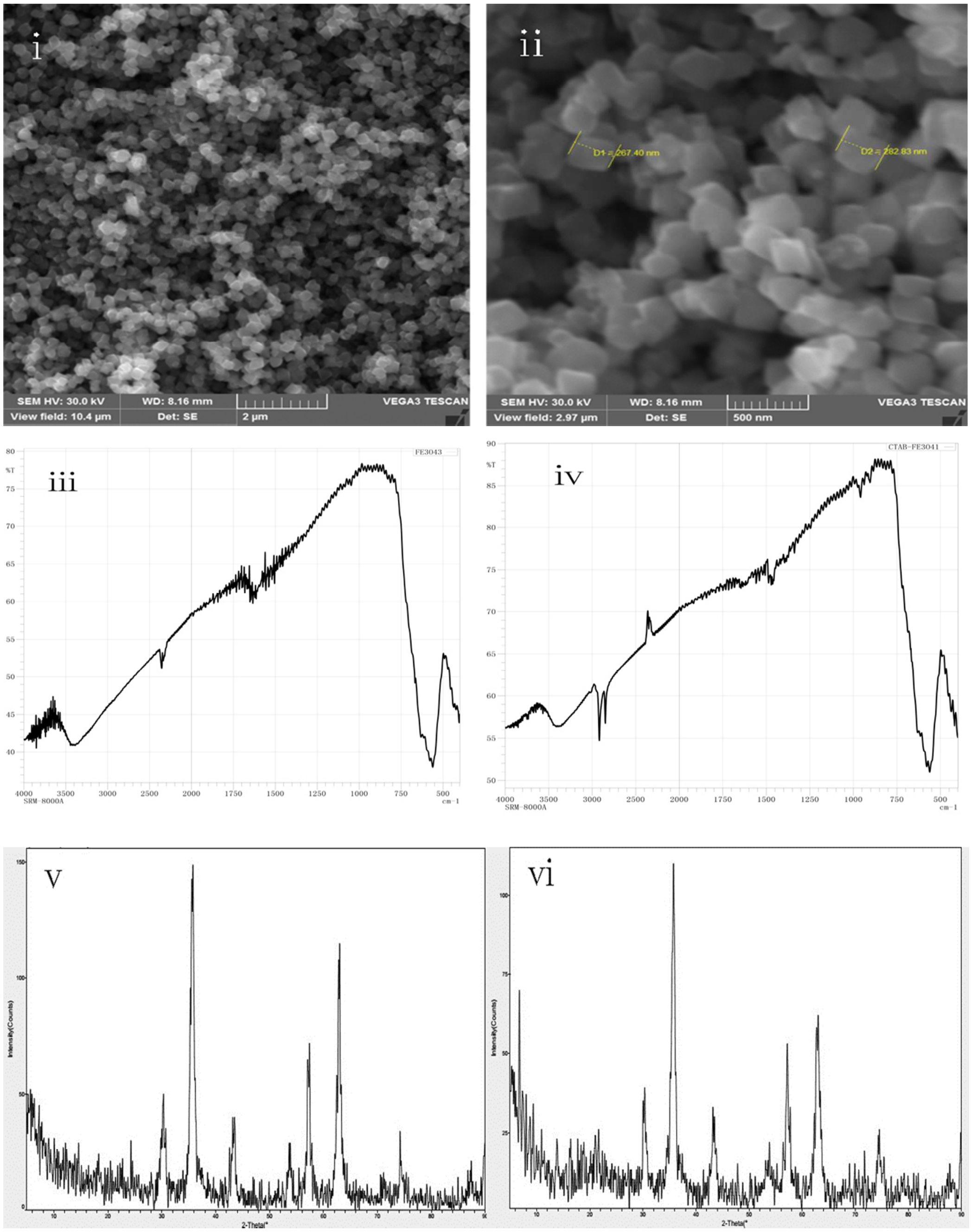


Fig. 3 . SEM images of Fe_3O_4 NPs (i , ii), FT-IR spectra of Fe_3O_4 NPs (iii) and Fe_3O_4@CTAB (iv), XRD patterns of Fe_3O_4 NPs (v) and Fe_3O_4@CTAB (vi)

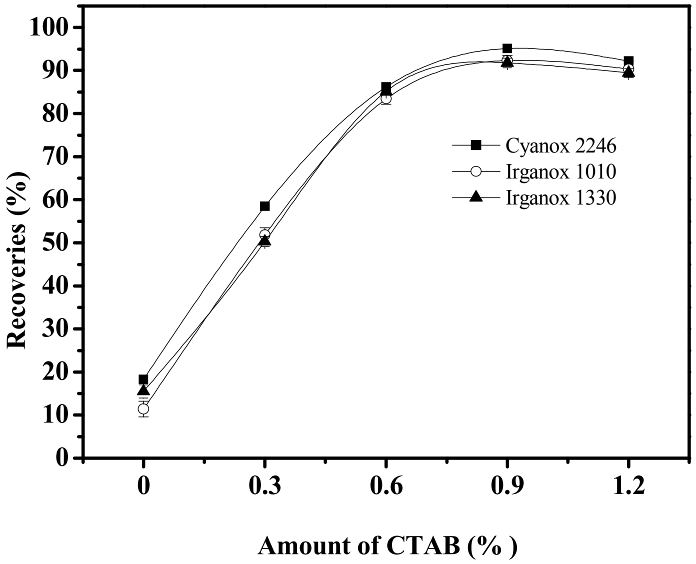


Fig. 4. Effect of the amount of CTAB

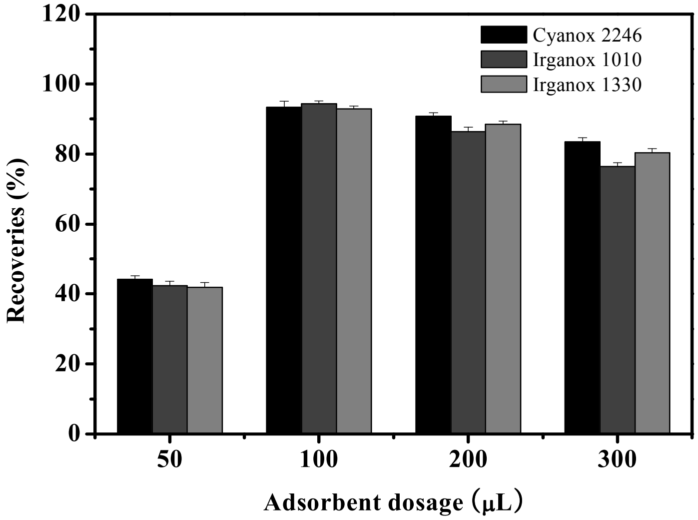


Fig. 5. Effect of the amount of sorbent

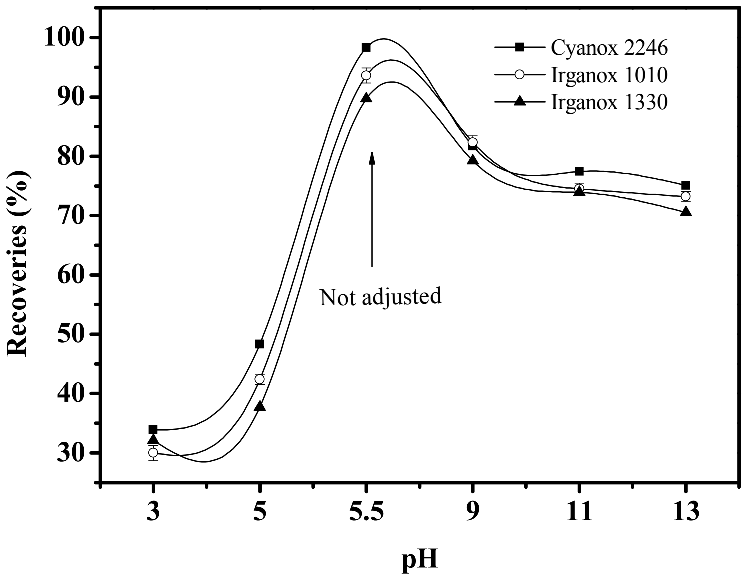
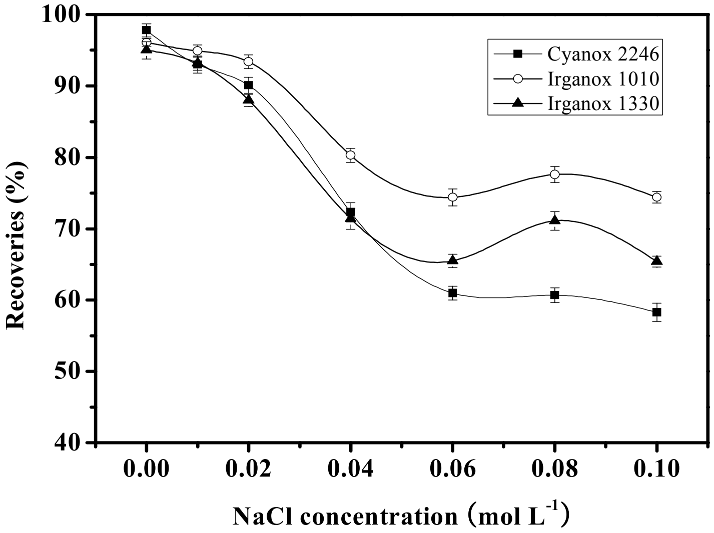


Fig. 6. Effect of pH value of test solution



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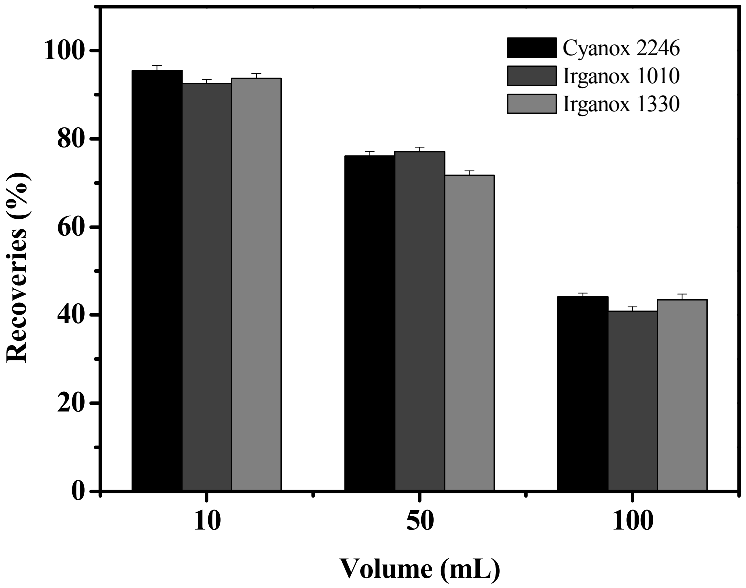
39 **Fig. 7.** Effect of NaCl concentration



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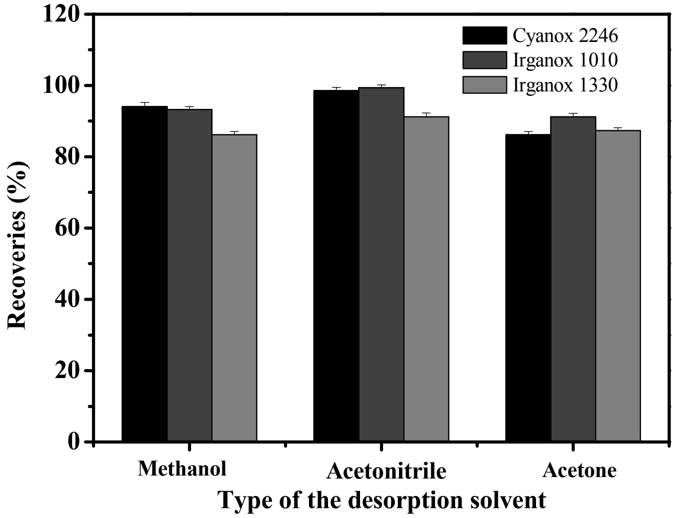
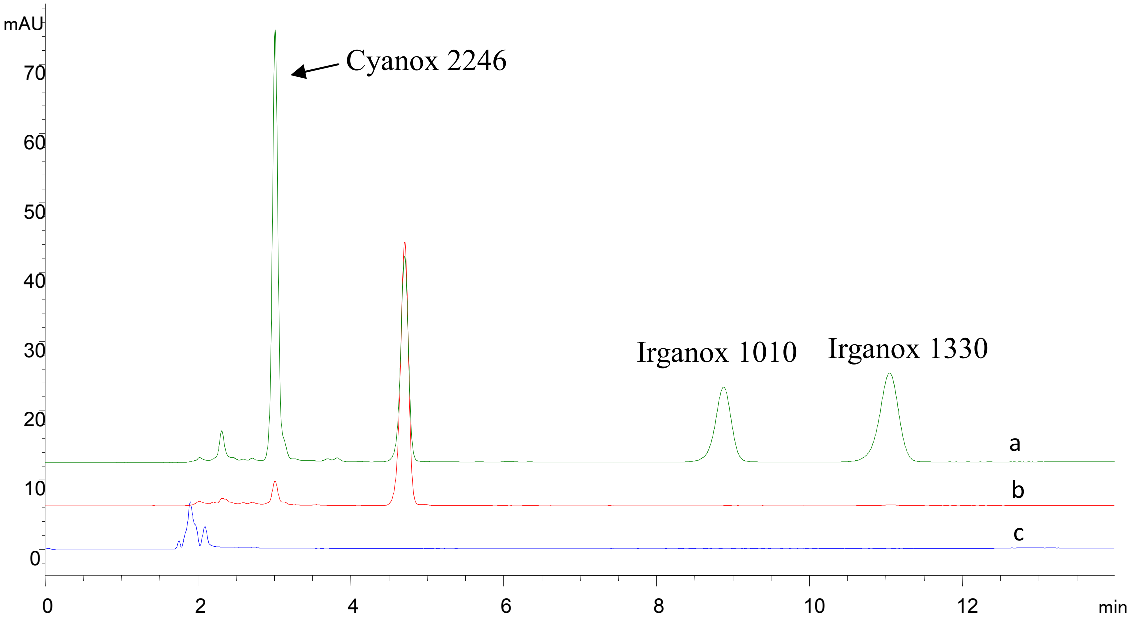


Fig. 9. Effect of eluent type



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Tables

Table 1. Calibration curves, linearity, LODs, LOQs for the analytes.

Table 2. Results of determination and recoveries of injection samples spiked with three target analytes

Table 3. Reusability tests for adsorption-desorption of antioxidants by using the same Fe_3O_4@CTAB NPs

Table 1. Calibration Curves, Linearity, LODs, LOQs for the analytes.

Compounds	Regression (Y=aX+b)	R^2	Linear range (μg mL^{-1})	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	RSD （n=6）
Cyanox 2246	y = 12.31x - 8.841	0.9999	0.78—50	0.14	0.46	2.27%
Irganox 1010	y = 6.952x + 0.918	0.9999	0.78—100	0.15	0.50	1.16%
Irganox 1330	y = 9.571x + 12.07	0.9999	1.56—100	0.14	0.45	2.81%

Table 2. Results of determination and recoveries of injection samples spiked with three target analytes

Samples	Spiked (μg mL^{-1})	Detected (μg mL^{-1})			Recovery (%) [a]		
		Cyanox 2246	Irganox 1010	Irganox 1330	Cyanox 2246	Irganox 1010	Irganox 1330
Sodium chloride injection (Size: 100 mL)	0	5.87±0.05	2.55±0.12	nd [b]			
	5	9.84±0.41	6.45±0.24	4.41±0.08	90.5	85.4	88.2
	10	13.9±0.12	10.85±0.04	8.5±0.32	87.6	86.4	85.0
	20	23.1±0.33	20.82±0.16	17.85±0.34	89.3	92.3	89.2
Glucose injection (Size: 100 mL)	0	3.98±0.19	nd	nd			
	5	8.04±0.21	4.43±0.16	4.55±0.12	89.5	88.6	91.0
	10	12.72±0.24	9.02±0.11	8.91±0.25	91.0	90.2	89.1
	20	22.31±0.13	18.7±0.38	18.21±0.16	93.0	93.5	91.0
Sodium chloride injection (Size: 500 mL)	0	4.98±0.07	nd	nd			
	5	8.84±0.5	4.47±0.43	4.33±0.14	88.6	89.4	86.6
	10	13.46±0.31	9.02±0.13	8.83±0.32	89.8	90.2	88.3
	20	22.41±0.25	18.42±0.32	17.24±0.28	89.7	92.1	86.2

[a] Mean of six determinations

[b] Not detected.

Table 3. Reusability tests for adsorption-desorption of antioxidants by using the same Fe_3O_4@CTAB NPs

Reuse times.		1	2	3	4	5
Recoveries (%) (40 μg mL^{-1})	Cyanox 2246	98.1	97.4	95.5	91.3	86.5
	Irganox 1010	97.3	95.1	93.7	90.5	85.2
	Irganox 1330	98.2	96.3	95.8	91.4	85.9