

Magnetic Solid-Phase Extraction of Rose Bengal Using Iron Oxide Nanoparticles Modified with Cetyltrimethylammonium Bromide

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Magnetic solid phase extraction (MSPE) based on cetyltrimethylammonium bromide (CTAB)-coated magnetic iron oxide nanoparticles (C-MIONPs) was investigated for the separation, preconcentration and determination of Rose Bengal (RB) in aqueous solutions. The influences of different analytical parameters such as pH, temperature, ionic strength, volume of desorbent solvent, amount of adsorbent and interfering ions in the adsorption of RB on C-MIONPs were investigated. The RB adsorption on C-MIONPs follows Langmuir isotherm. The sizes of C-MIONPs were in the range of 20-80 nm. The method was capable of determining RB concentration in the range of 0.01-1.20 $\mu\text{g ml}^{-1}$. The limit of detection (LOD) of RB based on three times the standard deviation of the blank ($3S_b$) was found to be $5.91 \times 10^{-3} \mu\text{g ml}^{-1}$ ($n = 8$). The relative standard deviation (RSD) for 0.3 $\mu\text{g ml}^{-1}$ and 0.8 $\mu\text{g ml}^{-1}$ of RB were 4.1% and 1.1%, respectively. The proposed method was applied to the determination of RB in Brucella Antigen solution and water samples from the Karoon River.

Keywords: Magnetic solid phase extraction, Cetyltrimethylammonium bromide, Rose Bengal, Preconcentration, Spectrophotometric determination

INTRODUCTION

Most industries use dyes and pigments to color their products. At present, more than 9000 types of dyes have been incorporated in the color index and the biggest consumers of these dyes are textile, tannery, paper and pulp industries, cosmetics, plastics, pharmaceuticals, food processing and electroplating. Perhaps these are the serious polluters of our environment as far as color pollution is concerned [1-2]. The highly colored effluent discharged from these industries into the receiving water body not only causes damage to aquatic life, but also to human beings, by producing carcinogenic and mutagenic effects. There are several treatment technologies like photodegradation, biodegradation, coagulation,

flocculation and electrochemical oxidation available for the treatment of colored waste waters [3].

Magnetic iron oxide nanoparticles (MIONs) have been studied extensively due to their wide range of application in ferrofluids, high-density information storage, magnetic resonance imaging (MRI), biological cell labeling, sorting and separation of biochemicals, targeting, drug delivery and the treatment of waste waters [3-16]. In analytical science MIONs were used as a new adsorbent in solid phase extraction (SPE) method for separation, removal and determination of chemical species [6-20]. At present, considerable attention is being paid to SPE method as a way to isolate and preconcentrate the desired components from a sample matrix. The separation and preconcentration of an analyte from large volumes of solution can take a lot of time using standard column SPE. New SPE techniques based on the use of magnetic or magnetizable

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adsorbents called magnetic solid-phase extraction (MSPE) have been introduced [21-22]. A distinct advantage of this technology is that magnetic materials can be readily isolated from sample solutions by the application of an external magnetic field. Generally, most of the dissolved environmental contaminants are nonmagnetic, and thus do not respond to magnetic field. Magnetic nanoparticles possess large surface areas and have unique magnetic properties. Selective removal of toxic target compounds from complex environmental matrices can be obtained when certain special functional ligands with affinities for target molecules are bound onto these magnetic nanoparticles [11].

Rose Bengal (RB) dye (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein disodium salt, C.I. name: Acid Red 94, Fig. 1) exhibits some unusual spectroscopic and photochemical properties including a large absorption coefficient in the visible region and a high tendency for inter-system crossing to produce a photochemically active triplet excited state due to its photoactivity. It has been used as a photodynamic sensitizer for cancer chemotherapy, as a photosensitizing agent for inactivating biological species such as vaccinia virus, microsomal glucose-6-phosphatase and trypsin [4] as well as a topical ophthalmic diagnostic. RB has been exploited as a promising sensitizer in wastewater treatment due to its water solubility, absorption in the visible region, good quantum yield of singlet oxygen, and inexpensiveness [23]. Although there exist numerous applications of Rose Bengal dye in various areas, it has been explored as a photodynamic sensitizer for cancer chemotherapy and for inactivation of viruses [24]. The toxic character of RB (not as photosensitizer) against melanoma and as a promising chemotherapeutic agent in melanoma treatment has been reported. The direct cytotoxic and proapoptotic effects of RB on MCF-7 cells as a widely-used model system for the study of breast cancer and human fetal skin fibroblasts (HFSF-PI3) as control non-malignant cell lines have also been investigated [25]. Nonetheless, due to the toxicity level of the dye and its discharge into rivers from different laboratories, there is a need to develop effective methods for its removal, recovery and determination in waste water.

In this investigation, cetyltrimethylammonium bromide (CTAB) cationic surfactant was coated on the surface of magnetic iron oxide nanoparticles and the mixture was used

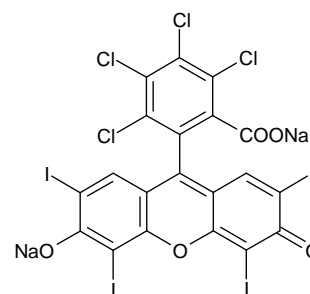


Fig. 1. Molecular structure of Rose Bengal (RB).

for separation, preconcentration and determination of the water soluble anionic dye, RB.

EXPERIMENTAL

Instrumentation

The spectrophotometric measurements were carried out with a Cintra 101 spectrophotometer (GBC SCIENTIFIC EQUIPMENT, Australia) equipped with 1.0 cm matched cells. A transmission electron microscope (TEM, 906E, LEO, Germany), pH-meter (632 Metrohm, Herisau, Switzerland) and a super magnet (1.2 Tesla, $10 \times 5 \times 2$ cm) were used.

Reagents

All chemicals were of analytical reagent grade. Rose Bengal (90% purity), phosphoric acid (85% w/w), hydrochloric acid (37% w/w), methanol (99.9% w/w), ammonia solution (25% w/w), FeCl_3 (96% w/w), $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (99.9% w/w), and cetyltrimethylammonium bromide (>99% w/w) were purchased from Merck (Darmstadt, Germany). Phosphate buffer solution of pH 6 was prepared by mixing appropriate volumes of sodium hydroxide (1.0 M) and phosphoric acid (0.1 M) solutions.

Preparation of Magnetic Iron Oxide Nanoparticles

Magnetic iron oxide nanoparticles (MIONPs) were synthesized according to a procedure published in the literature [26]. The MIONPs were imaged with TEM (Fig. 2).

Preparation of Modified Magnetic Iron Oxide Nanoparticles

In order to coat the iron oxide nanoparticles, 1 ml of

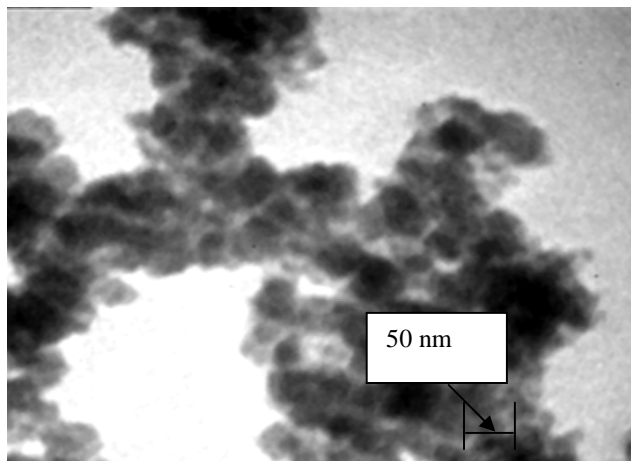


Fig. 2. TEM image of iron oxide nanoparticles (MIONPs).

CTAB solution (0.5% w/v) was added to 0.6 g of dampened nanoparticles and the mixture was diluted to 50 ml with distilled water. The solution mixture was stirred for 2 min with a glass rod. After complete mixing, the beaker was placed on the magnet and C-MIONPs were collected at the beaker bottom. In order to eliminate the excess amount of the surfactant from nanoparticles, the supernatant solution was decanted and the ferrofluid was washed with distilled water several times.

Extraction and Determination Procedure

A batch procedure was applied for the extraction process. A beaker containing 100 ml solution of RB ($0.010\text{--}1.20\ \mu\text{g ml}^{-1}$) and 3 ml of 0.1 M phosphate buffer solution of pH 6, was stirred with 0.6 g of damped C-MIONPs (the damped C-MIONPs contains 90% water, so 0.6 g of the damped adsorbent is equivalent to 0.06 g of the dry C-MIONPs) for 2 min. Nanoparticles were collected using a strong magnet (1.2 Tesla) and after the settlement of C-MIONPs, the initial pink colored solution became colorless. After decanting the solution above the nanoparticles, the magnetic solid phase was washed with about 10 ml distilled water and then the adsorbed RB on the C-MIONPs was washed and desorbed with 5 ml of methanol by stirring for 1 min. Then the beaker was placed on the magnet and C-MIONPs were collected. The RB pink color appeared again in methanol solvent as the desorbent. The desorbed solution was transferred into a 1.0 cm cell and its

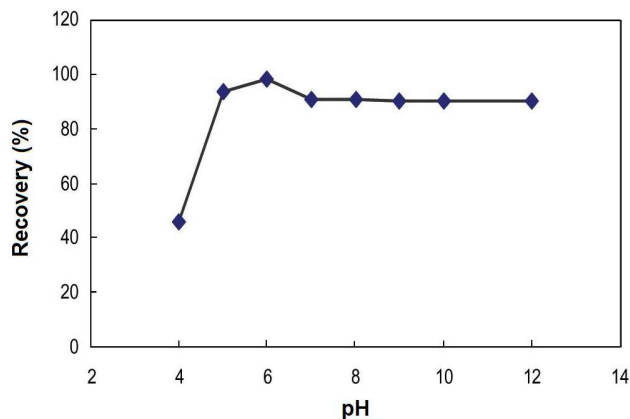


Fig. 3. Effect of pH on the recovery of RB. Conditions: 100 ml solution containing fixed amount ($50\ \mu\text{g}$) of RB; 0.50 g of damped C-MIONs; methanol desorbent solvent, 5 ml.

absorption was measured spectrophotometrically at 554 nm against a blank solution prepared under the same conditions without adding the analyte.

RESULTS AND DISCUSSION

The adsorption and preconcentration of RB by cetyltrimethylammonium bromide (CTAB) surfactant modified MIONPs (C-MIONPs) showed fast separation of this dye from the bulk of water solutions. The adsorption of the anionic dye, RB, by C-MIONPs may be due to electrostatic attraction between the positive charge of the cationic surfactant adsorbed on the iron oxide nanoparticles and the negative charge of RB as an anionic dye. The adsorption was completed within about 3 min. Such a fast adsorption rate could be due to the absence of internal diffusion resistance.

Effect of pH

The pH of the solution plays a significant role in the SPE procedure and must be controlled to improve the adsorption efficiency. The effect of pH on the adsorption of RB ($0.50\ \mu\text{g ml}^{-1}$) is shown in Fig. 3. The pH of the analyte solution was adjusted by using hydrochloric acid (HCl) and sodium hydroxide (NaOH) in the pH range of 4 to 11. As can be seen from Fig. 3, the quantitative recovery was obtained at pH 6. At

low pH values, the magnetic iron oxide nanoparticles dissolve in the acidic solution, the solution becomes dark and no adsorption takes place. At high pH values, ($\text{pH} > 6$) the MIONPs are converted to colloids (due to adsorption of hydroxide ions on the particles surfaces), which decrease the active surface area of the adsorbent resulting in lower sensitivity to the magnetic field, but still acting as a normal solid phase extractor. In further works, the pH of the solutions was adjusted by using phosphate buffer (pH 6) solution. The optimum volume of the buffer solution added to 100 ml of the RB solution was 3 ml; higher volumes decreased the adsorption and the separation of RB.

Effect of Amount of CTAB

The effect of CTAB amount on the adsorption of RB was investigated by coating different amounts of this compound on the surface of nanoparticles. In the case of non-coated MIONPs, only 70% recovery of RB was obtained. Maximum recovery of RB was obtained when 1 ml of 0.5% (w/v) solution of CTAB was coated on 0.60 g of damped MIONPs as shown in Fig. 4. Higher amounts of CTAB did not enhance efficiency.

Effect of Amount of C-MIONPs

The optimum amount of the adsorbent required for the quantitative recovery of RB, was obtained by investigating the effect of different quantities of pretreated MIONPs (C-MIONPs) ranging from 0.25 to 0.60 g. Maximum recovery percentage was obtained when 0.50 g of C-MIONPs was used, as shown in Fig. 5.

Desorbing Solvent

Desorption of the adsorbed RB took place in the presence of pure methanol as the desorbing solvent. The amount of the desorbed RB was determined spectrophotometrically at 554 nm. It was observed that the amount of the desorbed RB increased with increasing the volume of methanol from 2 to 5 ml, and the dye could be completely desorbed by 5 ml of pure methanol. This study revealed that more than 98% of the adsorbed RB could be desorbed and recovered from C-MIONPs by pure methanol.

Effect of Electrolyte

The effect of electrolyte concentration (adjusted by KCl)

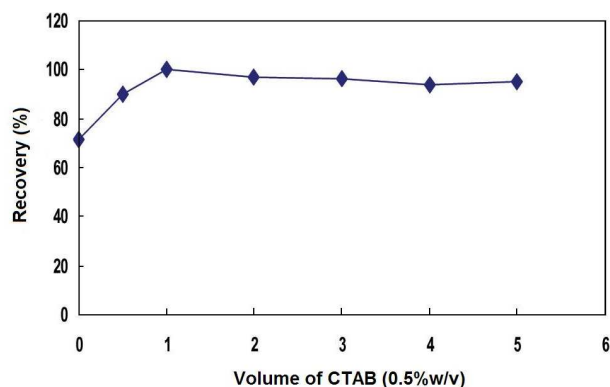


Fig. 4. Effect of CTAB volume (0.5% w/v) on the recovery of RB. Conditions: 100 ml solution containing fixed amount (50 μg) of RB; 0.60 g of damped C-MIONPs; pH 6; volume of buffer solution, 3.0 ml; methanol desorbent solvent, 5 ml.

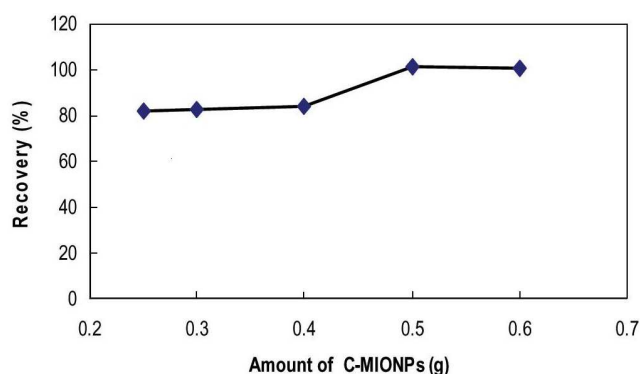


Fig. 5. Effect of C-MIONPs adsorbent amount on the recovery of RB. Conditions: 100 ml solution containing fixed amount (50 μg) of RB; pH 6; volume of buffer solution, 3.0 ml; methanol desorbent solvent, 5 ml.

on the adsorption and desorption of RB was determined. It was observed that the adsorption and desorption of RB remained almost constant within the concentration range of 0.02-0.10 M of KCl in the test solution. This implied that the electrostatic attraction between the positive charge of cetyltrimethylammonium ion on the MIONPs and the negative charge of RB ion was not affected significantly by KCl

concentration under the experimental conditions.

Effect of Solution Temperature

The effect of temperature on the adsorption of RB was examined in the range of 10-40 °C. The results show that the adsorption of RB (0.50 µg ml⁻¹) in a 50 ml solution using 0.5 g of damped C-MIONPs at pH 6 increased with increasing the temperature of the test solution by 5 degree intervals. The recovery percent of RB changed from 84% for 10 °C to 98% for 40 °C. A temperature of 25 °C (recovery 97%) was used for further experiments.

Adsorption Isotherm

The capacities of C-MIONPs to adsorb RB were examined by measuring the initial and final concentrations of RB in 0.1 M of phosphate buffer solution at pH 6 and 25 °C in batch system (Fig. 6). The Langmuir isotherm equation was used to describe the relationship between the amount of RB adsorbed and its equilibrium concentration in solutions.

$$\frac{C}{q} = \frac{1}{Kq_m} + \frac{C}{q_m}$$

where C (mg l⁻¹) is the equilibrium concentration of the RB in the solution, q (mg RB/mg adsorbent) is the equilibrium adsorption amount of RB, q_m is the maximum adsorption amount of RB per milligram of the adsorbent (mg/mg) and K is the Langmuir adsorption equilibrium constant in liter per mg of the adsorbent (l mg⁻¹). Figure 7 shows a linear relationship between C/q and C using the experimental data obtained, suggesting the applicability of the Langmuir model ($R^2 = 0.992$). Values of q_m and K calculated from the least square methods were 0.371 (mg mg⁻¹) and 8.639 (l mg⁻¹), respectively.

Effect of the Sample Volume

Due to the low concentrations of the dye in real samples, a high preconcentration factor was required. Therefore, maximum applicable sample volume was determined by increasing the dilution of the RB dye solution, while keeping its total amount fixed at 50 µg. Different feed volumes between 100 and 500 ml were tested. The obtained results showed that the recoveries of RB were quantitative up to 350

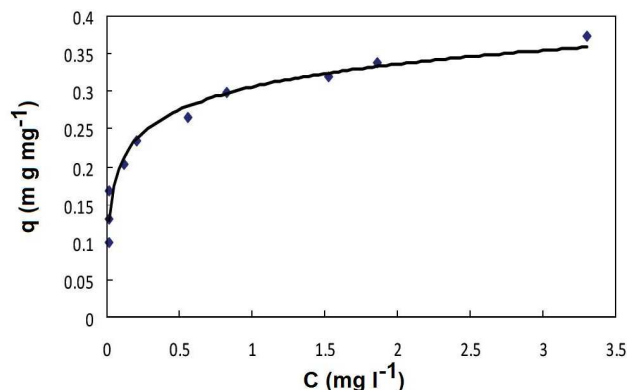


Fig. 6. Adsorption isotherm of RB on C-MIONPs. Conditions: same as those in Fig. 5.

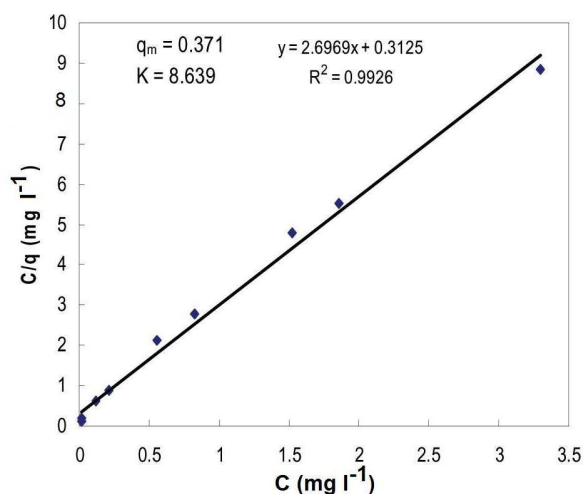


Fig. 7. Plot of C/q against C for the adsorption of RB on C-MIONPs. Conditions: 0.60 g of damped C-MIONPs; 50 ml of 5-27 µg ml⁻¹ solution of RB containing 3 ml of phosphate buffer (pH 6); temperature, 25 °C.

ml of sample volume (recovery > 95%). At volumes higher than 350 ml, the analyte was not adsorbed effectively probably due to lower magnetic field strength at high dilutions. As it was mentioned previously, the final solution volume after eluting was 5 ml, therefore, the preconcentration factors of 70 was obtained for the analyte (RB).

Effect of Interfering Ions

The optimum experimental conditions which have been described were used to study the effect of some ions and three dyes (orange G, amaranth and methylene blue) on the determination process of RB. To this end, separation and determination of RB were carried out in the presence of co-existing ions or dyes. The maximum acceptable relative error was $\pm 5\%$. The obtained results are shown in Table 1. The table shows that Fe^{3+} , orange G, amaranth and methylene blue strongly interfered even at the same concentration as that of RB, whereas most of the investigated ions did not interfere

during the separation, preconcentration, and determination. The interferences of Ca^{2+} , Mg^{2+} , Cr^{3+} , Pb^{2+} and Fe^{3+} can be eliminated by adding appropriate amounts of EDTA. The interferences of dyes can not be eliminated.

Reusability of the C-MIONPs

The reusability of the adsorbents in several successive adsorption and desorption processes was tested. The obtained results showed that the modified magnetic nanoparticles could be reused for three times without any considerable loss in their adsorption efficiency.

Table 1. Effect of Interfering Ions on Adsorption-Desorption and Recovery of RB by C-MIONPs (Conditions: 100 ml RB of $0.2 \mu\text{g ml}^{-1}$; pH = 6; C-MIONPs, 0.6 g; Volume of Methanol Desorbent Solvent, 5 ml)

Ions ^a	Tolerance limit (mg l^{-1})
Na^+ , K^+ , NH_4^+ , Co^{2+} , Mn^{2+} , Cl^- , F^- , I^- , HCO_3^- , EDTA, NO_3^- , CO_3^{2-} , SO_4^{2-} , PO_4^{3-}	1000
Ni^{2+}	800
Cu^{2+}	500
Ca^{2+} , Mg^{2+}	100
Cr^{2+}	50
Pb^{2+}	10
Fe^{3+}	1
Orange G, Amaranth, Methylene blue	0.5

^aAll cations were prepared from nitrate salts and anions were prepared from sodium or potassium salts.

Table 2. Recovery Test for Adsorption-Desorption of RB by C-MIONPs in Brucella Antigen Solution and Spiked RB in Karoon River Water

Sample	Added RB ($\mu\text{g ml}^{-1}$)	Found RB ($\mu\text{g ml}^{-1}$) ^a	Recovery (%)
Brucella antigen ^b	-	0.34 ± 0.01	-
Brucella antigen	0.200	0.53 ± 0.02	96.50
Brucella antigen	0.400	0.72 ± 0.03	96.25
Karoon river water ^c	-	ND	-
Karoon river water	0.200	0.19 ± 0.01	95.70
Karoon river water	0.400	0.39 ± 0.02	98.90

^a(n = 3). ^bBrucella antigen: pH = 6.5. ^cKaroon river water main components: $\text{Ca}^{2+} = 82$; $\text{Mg}^{2+} = 49$; $\text{Na}^+ = 68$; $\text{CO}_3^{2-} = 91$; $\text{Cl}^- = 44$; $\text{SO}_4^{2-} = 35$; $\text{NO}_3^- = 9 \mu\text{g ml}^{-1}$; pH = 7.1; TDS = 387; EC = 1340.

Analytical Performance

The analytical features of the presented method such as linear range of the calibration curve, limit of detection and precision were also examined. The calibration graphs were linear in the range of 0.01-1.20 $\mu\text{g ml}^{-1}$ of RB. The equation for this calibration graph was $A = 1.129 C_{\text{RB}} + 0.003$ ($r = 0.9996$). The limit of detection (LOD) based on three times the standard deviation of the blank ($3s_b$), under optimal experimental conditions, was $5.91 \times 10^{-3} \mu\text{g ml}^{-1}$ of RB ($n = 8$). The relative standard deviations for 0.3 and 0.8 $\mu\text{g ml}^{-1}$ of RB ($n = 8$) were 4.1% and 1.1%, respectively.

Analytical Applications

To determine the efficiency of the proposed method for the adsorption and desorption of RB in a real sample, it was applied to the determination of RB in Brucella Antigen sample solution. Different recovery tests were carried out to evaluate the reliability and accuracy of the method. The developed method was also applied to the analysis of water from the Karoon River. The analytical results, along with the recoveries for the spiked samples, are given in Table 2. The results showed the practicality of the proposed method to real sample analysis. Excellent recoveries indicated that the complex matrix of Brucella Antigen and river water samples do not interfere with the analysis of RB.

CONCLUSIONS

It was found that RB could be removed from an aqueous solution by (CTAB)-coated magnetic iron oxide nanoparticles (C-MIONPs). The proposed methodology is simple, sensitive and cheap, especially if sophisticated techniques such as spectrofluorometry were not available. In addition, it is notable that both the adsorption and desorption of RB are fast and could be completed within 5 min. The proposed method is recommended for the removal and/or determination of RB in different samples.

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