

Application of a Novel Micro-Cloud Point Extraction for Preconcentration and Spectrophotometric Determination of Azo Dyes

Elham Ghasemi and Massoud Kaykhaii*

*Department of Chemistry, Faculty of Sciences, University of Sistan and Baluchestan,
98135-674 Zahedan, Iran*

A novel room temperature micro-cloud point extraction procedure using triton X-114 surfactant as extracting phase was developed for preconcentration and extraction of three azo dyes (orange G, methyl orange, and acid red 18) from aqueous samples using molecular absorption spectrophotometry in the visible region. The effects of different parameters such as concentration of surfactant and added salting out reagent (Na_2SO_4), pH and type of diluting solvent on microextraction were studied and optimized. Under optimum conditions, calibration curves were linear in the range of 2.0-10.0, 0.2-1.0 and 2-12 mg L^{-1} with the detection limit of 1.6, 0.6 and 111.0 $\mu\text{g L}^{-1}$ for orange G, methyl orange and acid red 18, respectively. The relative standard deviation was better than 13.12%. The method was applied to the determination of the azo dyes in water samples.

Keywords: micro cloud point extraction, orange G, methyl orange, acid red 18, water analysis

Introduction

The importance of synthetic dyes in today industries can't be denied.¹ Every year, thousands tons of such dyes are consumed in food, paper, leather, and textile industries.^{2,3} From these industries, large volumes of dyes are released to environment and find their way to water and soil. Environmental pollution with dyes can have dire effect on animal, plants, and human health.⁴⁻⁷ One of the most important families of dyes is azo dyes that contain $-\text{N}=\text{N}-$ bond in their structures.⁸ So far azo dyes have found many applications. Some azo dyes are quite harmful and poisonous,⁹ and even those that are not toxic, like acid red 18, can be harmful if used in excess.¹⁰ Therefore the importance of their removal/decolorization¹¹⁻¹³ and their determinations¹⁴⁻¹⁷ in environmental samples is obvious. Many analytical methods have been suggested for extraction and determination of azo dyes such as cloud point extraction (CPE),¹⁸⁻²⁰ liquid-liquid extraction (LLE),^{21,22} liquid phase microextraction (LPME),²³ solid phase extraction,²⁴⁻²⁶ solid phase microextraction,²⁷ and molecularly imprinted solid phase extraction.²⁸

Nowadays, based on principals of green chemistry, liquid phase microextraction techniques have become very popular and many new and microextraction

techniques have been developed based on classical liquid-liquid extraction.²⁹ Of these, we can name headspace single drop microextraction,³⁰ direct immersion single drop microextraction,³¹ solidification of floating organic drop microextraction,³² dispersive liquid-liquid microextraction,³³ narrow bore dispersive liquid-liquid microextraction,³⁴ and cold induced dispersive liquid-liquid microextraction.³⁵ In all of these techniques extractant must be an organic solvent. To eliminate the usage of organic solvent which are toxic and inflammable, some researchers tried to replace it with ionic liquid as a new and non-toxic synthetic solvent.^{36,37} But ionic liquids are expensive and are not as green as they are considered.³⁸

Cloud point extraction (CPE), first introduced by Watanabe and Tanaka in 1978,³⁹ is a somewhat green extraction technique that instead of organic solvent uses surfactant as extractant. CPE for determination of metals consists of four simple steps: complex formation of metals with chelating agent, addition of surfactant to sample solution, heating the solution to form cloudy solution (formation of micelles), and phase separation; however the first step is eliminated when CPE is applied on organic analytes.⁴⁰ Although CPE is a very capable technique and can be used for extraction of various analytes,⁴¹ it suffers from some flaws including long extraction time,⁴² necessity of hot water bath and sometimes ice water bath,⁴³ and treatment with large volume of diluting

*e-mail: ghasemi@pgs.usb.ac.ir

solvent (up to 3 mL).⁴⁴ To overcome these problems, in our previous work we introduced a micro-cloud point extraction (MCPE) technique.⁴⁵ While sustaining the merits of CPE, in MCPE water bath step is completely eliminated and the consumption of toxic organic solvents has been reduced from a few mL to a few ten μL . These make MCPE a fast, inexpensive, and green technique.

In this paper MCPE was successfully applied for the determination of three azo dyes, orange G, methyl orange, and acid red 18 (Figure 1) in tap and wastewater samples. Acid red 18 (Panceau 4R) is a popular food color⁴⁶ and many methods have been proposed for its determination.^{47,48} Methyl orange (acid orange 52 or gold orange) and orange G (orange gelb or wool orange) have many applications as textile dyeing stuff and staining agents in laboratories. These three dyes are of the most abundant used dyeing agents throughout the world and therefore can find their way to environmental sources as hazardous pollutions.^{49,50} To our best knowledge this is the first report of determination of these dyes with cloud point procedure. Non-ionic surfactant, triton X-114, was used as the extractant phase.

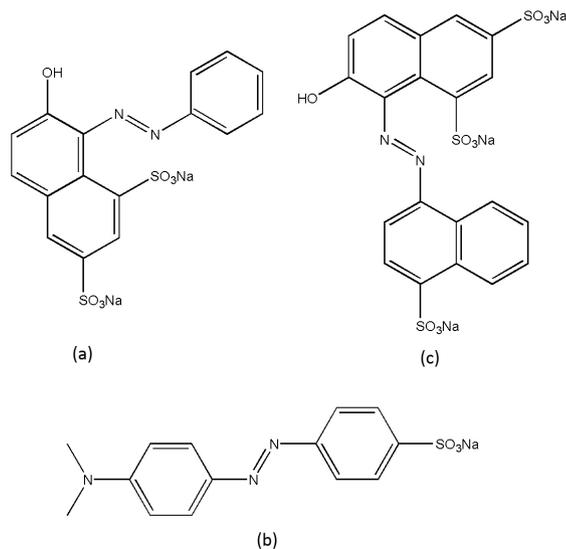


Figure 1. Structures of azo dye studied in this research. (a) Orange G; (b) methyl orange; (c) acid red 18.

Experimental

Instrument

A Shimadzu UV-Vis spectrophotometer, UV-160 (Kyoto, Japan), equipped with two microcells (10 μL capacity, Starna, UK, catalog No. 16.10-Q-10/Z15) was used for measuring the absorbance and recording the spectra. A Metrohm (Herisau, Switzerland) model EasySeven pH meter was used

for pH measurements and a Behdad centrifuge (Tehran, Iran) was applied for centrifugation.

Reagents and chemicals

All chemicals were of analytical grade and were purchased from Merck KGaA (Darmstadt, Germany). They were used as received. Triton X-114 (2%, v/v) and Na_2SO_4 (5%, m/v) solutions were prepared in doubly distilled water. Stock solutions of each dye containing 500 mg L^{-1} dye, were prepared by dissolving 0.050 g of dye in 100 mL doubly distilled water, individually. Working solutions were obtained by daily dilution of the stock solutions.

Micro cloud point extraction procedure

An aliquot of the sample solution containing appropriate amounts of dyes was transferred into a centrifuge test tube with conical bottom and proper volume of 2% (v/v) triton X-114 and 0.5 mL phosphate buffer was added to it. To form a cloudy solution, 0.5 mL of Na_2SO_4 solution (5%, m/v) was added to the mixture. Immediately after the addition of salt, the solution became cloudy. Then it was made up to 10 mL with double distilled water and went under centrifugation for a few minutes at 4000 rpm. The enriched micellar phase, settled at the bottom of the test tube, was around 30-35 μL , of which 20 μL was transferred to a vial and dissolved in 40 μL of the diluting solvent. 10 μL of this mixture was transferred to a microcell for spectrophotometric determination in the desired wavelength.

Results and Discussion

Absorption spectra

The absorption spectra of three analytes in the solution obtained after application of the MCPE under optimized condition were recorded at the wavelength range of 400 to 800 nm against the reagent blanks as shown in Figure 2. The results indicate that the maximum absorption wavelength were 488, 448 and 510 nm for orange G, methyl orange and acid red 18, respectively. Therefore, these wavelengths were selected as the chosen absorption wavelengths for further determinations of dyes. During all of the following experiments, the blank absorbance of all reagents was corrected.

Optimization of MCPE

To improve the extraction efficiency, important experimental parameters which can potentially affect

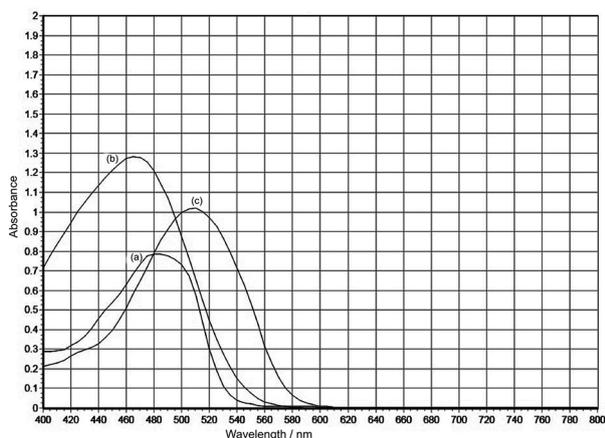


Figure 2. Absorption spectra of (a) 7.0 mg L⁻¹ orange G; (b) 0.6 mg L⁻¹ methyl orange; (c) 10.0 mg L⁻¹ acid red 18 after extraction by MCPE under optimized condition.

the enrichment performance, such as pH of sample solution, concentration of salt, kind of diluting solvents, concentration of the surfactant, and centrifugation time have been investigated and optimized for proposed MCPE. The univariant method was used to simplify the optimization procedure. A series of experiments were designed for this goal as discussed below. Number of replicates of analysis was at least three for each experiment.

Effect of pH

The effect of pH on extraction efficiency of dyes was investigated closely. pH of the sample solutions were adjusted either by 0.1 mol L⁻¹ HCl or 0.1 mol L⁻¹ NaOH solutions in the range of 3.0 to 9.5. After then, 0.5 mL Na₂SO₄ (5%, m/v) and 0.5 mL triton X-114 (2%, v/v) was added to the solution and the extraction was carried out as described earlier. Based on the obtained results shown in Figure 3, the best pH for orange G, methyl orange, and acid red 18 is 8.0, 7.0, and 6.0, respectively. Therefore before extraction procedure, the pH of each sample solution was

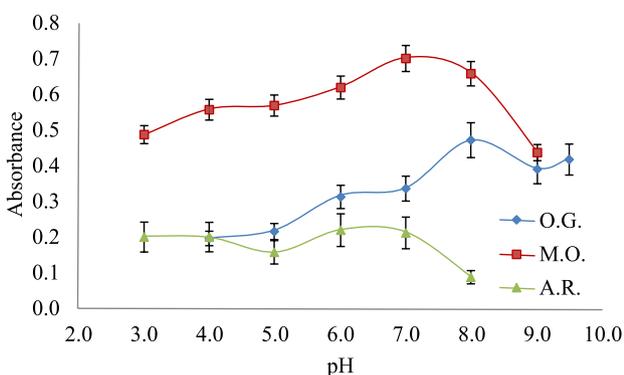


Figure 3. Effect of pH of aqueous solution on MCPE of 4.0 mg L⁻¹ orange G (O.G.); 0.5 mg L⁻¹ methyl orange (M.O.); 2.0 mg L⁻¹ acid red 18 (A.R.); extraction condition: Na₂SO₄, 0.25%, m/v; triton X-114, 0.1%, v/v; time of centrifuge, 5 min at 4000 rpm.

adjusted with addition of 0.5 mL phosphate buffer with desired pH value.

Effect of salt concentration

In the proposed MCPE, the formation of micelles which are necessary for extraction of analytes, takes place in brine solution. Therefore the concentration of salt can have great effect on extraction of analytes. Among the salts tested for this purpose (NaCl, KCl, K₂SO₄ and Na₂SO₄), Na₂SO₄ showed the best effect on forming the turbid solution as was expected since it can imply more ionic strength on aqueous solution; therefore it was chosen for further experiments.

Different brine sample solutions containing different concentration of sodium sulfate in the range of 0.125-1.00% (m/v) were prepared and to these solutions 0.5 mL triton X-114 (5%, m/v) and 0.5 mL suitable buffer was added. Higher concentrations of salt were not applied because it was observed that in high concentrations, dilution of the enriched phase takes place with difficulty. This is because the micelles in such media do not tend to dissolve in organic solvent (Supplementary Information section, Figure S1). Therefore the best chosen concentration of Na₂SO₄ was 0.25% (m/v) for all analytes.

Selection of diluting solvent

Before determination of analytes by spectrophotometer, it is necessary to decrease the viscosity of the surfactant rich-phase in a polar organic solvent to make easier to manipulate it. For this purpose, some conventional organic solvents including acetone, ethanol, methanol, and acetonitrile were investigated and 20 μL of enriched micellar phase was diluted with 40 μL of solvent before spectrophotometric determination. The obtained data showed that methanol, acetonitrile, and acetone were the best solvents for orange G, methyl orange, and acid red 18, respectively (Supplementary Information section, Figure S2).

Effect of concentration of triton X-114

The extractant phase in cloud point extraction is a surfactant. Because of its availability, low cost, non-toxic, and non-flammable properties, triton X-114 as the non-ionic surfactant, is the mostly used surfactant in cloud point extraction.⁵¹ Therefore we also selected this surfactant as a green reagent for MCPE. The concentration of triton X-114 can affect the extraction efficiency and enrichment factor. In order to optimize its concentration, different amounts of triton X-114 (0.05-0.2%, v/v) were subjected to the same MCPE procedure on sample solutions containing 0.5 buffer and 0.5 mL Na₂SO₄ 5%. At concentrations more than 0.1% v/v triton X-114, we had a decrease in the absorbance

of orange G and methyl orange which was probably due to the dilution of the analyte in larger volume of enriched micellar phase, and for acid red 18, the absorbance remained relatively the same. Accordingly, 0.1% (v/v) of triton X-114 was chosen as the best concentration of surfactant for all analytes (Figure 4). It must be noted that in quantities less than 0.1% of triton X-114, the volume of enriched micellar phase, sedimented at the bottom of the centrifuge tube was so tiny that taking 20 μL of it for further determinations are not possible.

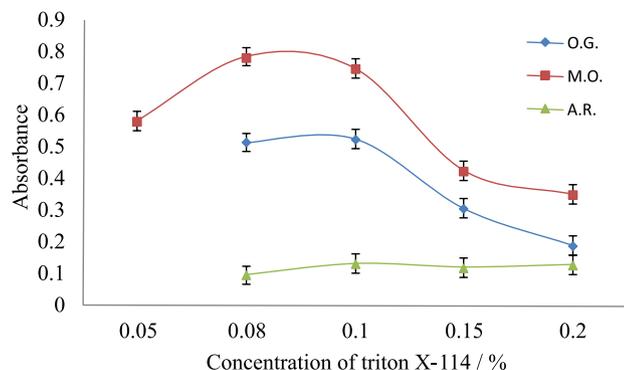


Figure 4. Effect of concentration of triton X-114 on MCPE of 4.0 mg L⁻¹ orange G (O.G.), 0.5 mg L⁻¹ methyl orange (M.O.), and 2.0 mg L⁻¹ acid red 18 (A.R.). Extraction condition: Na₂SO₄, 0.25%, m/v, 0.5 mL buffer, time of centrifuge, 5 min at 4000 rpm.

Effect of the centrifugation time

Since separation of micellar enriched phase and aqueous phase takes place very slowly, similar to CPE, we utilized centrifugation for this purpose. For this, similar sample solutions (0.25% triton X-114, 0.5 mL buffer, 0.25% Na₂SO₄) were prepared and after formation of cloudy solution, they were subjected to centrifugation under different times (3 to 9 min). Aside from maximum absorbance of analytes, the volume and purity of the sedimented phase were also considered as important parameters. The best time of centrifugation was found to be 8 min for orange G, 6 min for methyl orange, and 5 min for acid red 18 at 4000 rpm (Supplementary Information section, Figure S3).

Table 1. Analytical figures of merit for MCPE extraction of dyes

Parameter	Orange G	Methyl orange	Acid red 18
Equation of calibration curve	$A = 0.161C_{OG} - 0.2195$	$A = 2.3432C_{MO} - 0.3014$	$A = 0.1319C_{AR} - 0.1066$
Dynamic range / (mg L ⁻¹)	2.0-10.0	0.2-1.0	2.0-12.0
R ² (determination coefficient)	R ² = 0.9547	R ² = 0.9903	R ² = 0.9847
Repeatability (RSD, n = 5) / %	2.46	13.12	9.93
Detection limit / ($\mu\text{g L}^{-1}$)	1.6	0.6	111.0
Limit of quantification / ($\mu\text{g L}^{-1}$)	5.3	2.1	370.6
Enrichment factor / fold	3.83	36.12	3.35

RSD: relative standard deviation.

Linear range, limit of detection and precision

Analytical figures of merit for the proposed MCPE method, obtained under optimum conditions for the target analytes are shown in Table 1. The calibration curves for each analyte are presented in Supplementary Information section, Figures S4-S6. Limits of detection (LODs) and limit of quantifications (LOQs) were calculated based on 3S_b/m and 10S_b/m criterion respectively, where S_b is the standard deviation of 10 blank measurements and m is the slope of calibration graph. The repeatability of the method, expressed as relative standard deviation (RSD), was calculated for five replicates of the standard at an intermediate concentration of the calibration curve of each dye.

The enrichment factor (EF) was calculated as the ratio between the analyte concentration in the extracting phase (C_{micelle}) and the initial concentration of the analyte (C₀) within the sample (equation 1):³⁴

$$EF = C_{micelle}/C_0 \quad (1)$$

Analysis of real samples

The MCPE procedure was applied on tap water and wastewater, as for standard solutions. Wastewater samples (from the recycling waste water system of The University of Sistan and Baluchestan) were filtered through 0.45 μm nylon membranes prior to analysis. Since no dye pollution was observed in both samples, the samples were spiked with 3 different concentrations of each dye to investigate the matrix effect on their determination. The results are shown in Table 2. As can be seen, recoveries of all three azo dyes are adequate; therefore, we can justify this assumption that matrix effect is negligible for the analysis of these three dyes.

Conclusions

In this study, a fast, economical and easy to operate

Table 2. Analytical results for determination of three dyes in tap water and wastewater (n = 3)

	Tap water		Wastewater	
	Added / (mg L ⁻¹)	Recovery / %	Added / (mg L ⁻¹)	Recovery / %
Orange G	3.00	119.17	3.00	111.70
	7.00	100.92	7.00	91.42
	10.00	106.37	10.00	107.40
Methyl orange	0.30	90.55	0.30	107.74
	0.60	77.34	0.60	71.79
	0.90	90.58	0.90	80.77
Acid red 18	4.00	120.65	4.00	117.24
	7.00	109.93	7.00	120.61
	12.00	102.83	12.00	105.98

method based on micro-cloud point extraction for preconcentration and determination of traces of three azo dyes (orange G, methyl red and acid red 18) is presented. This is the first report of determination of these dyes with cloud point procedure. Triton X-114 was used as a non-ionic and green extractant solvent. In comparison to the similar methods of extraction, MCPE is less expensive, more environmental friendly and faster. In this paper we coupled our MCPE method with spectrophotometry equipped with microcells, as an inexpensive, fast and available instrument; therefore, we successfully minimized toxic organic solvents consumption and increased the sensitivity for the determination of the target analytes. Since spectrophotometric instrumentations are simple, inexpensive and mostly available in common laboratories, the proposed MCPE method is applicable in ordinary laboratories without need of expert personnel. Despite the fact that due to the power of carrying out the separation, organic dyes are mostly determined by HPLC; however, it is an expensive instrument which uses pure, toxic organic solvent. On the other hand, the main drawback of the developed MCPE is relatively high RSD values due to the use of microcells.

Supplementary Information

Supplementary data, including figures of effect of concentration of salt concentration, diluting solvent, and time of centrifuge on MCPE, alongside with calibration curves (Figures S1-S6) are available free of charge at <http://jbcbs.sbg.org.br> as PDF file.

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