A Sequential Separation and Determination of Some Organic Reagent Dyes in Pollutant Samples by Using Cloud Point Extraction Methodology Coupled with Spectrophotometry

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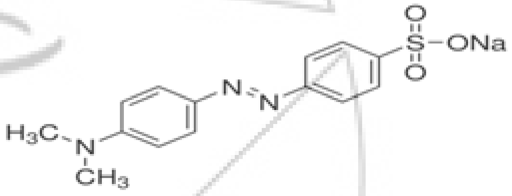
Abstract: *A new spectrophotometric method has been established for the determination of some organic dyes, namely Methyl Orange (MO), Eriochrome Black T (EBT) and Congo Red (CR) after cloud point extraction (CPE). The effects of the pH, surfactant amount, temperature, heating time and salt concentration were investigated. The concentration of the dyes was determined at the optimum conditions using UV-Vis spectectophotometer at each respective* λ_{max}*. It was found that the enrichment factors of 36.7, 75.8 and 19.9 fold for MO, EBT and CR were obtained leading to the limit of detection of 0.059, 0.040 and 0.042* $\mu g\ mL^{-1}$ *respectively. The suggested method gave linear calibration curves in the range of 0.2-1.0, 0.05-1.00 and 0.05-0.90* $\mu g\ mL^{-1}$ *with molar absorpitivity of* $1.12\ x10^5$*,* $5.29x10^4$ *and* $9.85x10^5$ $L\ mol^{-1}\ cm^{-1}$ *for MO, EBT and CR respectively. The developed method was achieved a good accuracy and precision. It was applied successfully for the determination of the dyes in spiked drinking, river waters and in local laboratory waste water samples.*

Keywords: **Organic dyes, Cloud point extraction, Spectrophotometry**

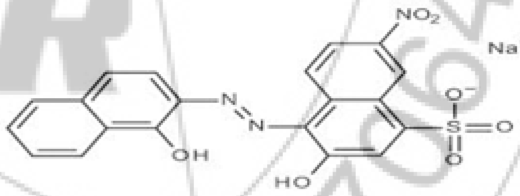
1. Introduction

A substantial amounts of organic dyes as reagents are commonly used in most laboratories especially in the analytical experimental works and biology, in addition to other dyes that employed in different industrial activities which have ultimately been discharged as colored wastes into a sewer system. These dyes are disproportionately toxic and have deleterious effects on the aquatic life and humans even if they are present at low concentration. It was reported that the presence of very small amounts of dyes in water (less than 1 ppm for some dyes) is highly visible and undesirable[1]. Of these dyes, Methyl orange (MO), Eriochrome Black T (EBT) and Congo red (CR) are usually used as indicators in our laboratories and for other industrial purposes. They are classified as a synthetic azo dyes (Figure 1) which may cause serious risks and recognized as potential carcinogenic to humans [2-3] .Thus, the presence of trace quantities of these dyes in environment, particularly in water bodies and wastewater requires finding and designing an analytical method which can be adaptable for the separation and determination of these dyes at trace levels with high degree of reliability to meet the needs of quality control laboratories. This will help in decision-making on how to remove these organic dyes from environmental sources (such as a laboratory waste) in time to prevent them from reaching the aquatic environment.

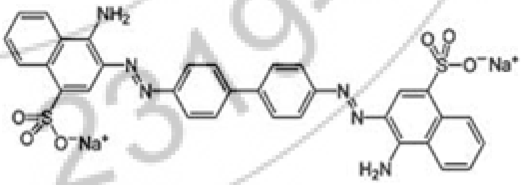
We believe that the so-called the cloud point extraction (CPE) as an alternative to other extraction methods and a prior step can comply with the separation and enrichment of trace concentration of these dyes from different water samples before their determination by analytical techniques. The main reason for our choice the cloud point extraction (CPE) due to its attractive features as a high extraction efficiency , simple, eco-friendly, use relatively non toxic surfactant materials as extractant instead of toxic organic solvents and low cost[4-8]. This has encouraged some authors to employ CPE initially for the extraction and removal of some organic material including dyes from various matrices [8, 9-11]. Also, few reports have been appeared in the chemical



a) Methyl Orange ($C_{14}H_{14}N_3NaO_3S$)
M.wt. 327.33 g mol^{-1}



(b) Eriochrome Black T ($C_{20}H_{12}N_3O_7SNa$)
m.wt. 461.381 g/mol



(c) Congo Red ($C_{32}H_{22}N_6Na_2O_6S_2$); m.wt.696.665

Figure 1: Chemical structure of (a) Methyl Orange (b) Erichrome Black T(c) Congo Red.

literatures, reflecting the use of CPE in combination with selective analytical techniques for the determination of some organic dyes in various matrices. Those included the determination of Eosin B Dye in waster [12], brilliant green in fish farming water [13], Sudan dyes in chilli powder [14], malachite green in fish farmingwater [15], carmoisine and brilliant blue FCF in food samples [16].

In present work, an attempt was made to employ CPE coupled with spectrophotometric technique for the sequential detection of three common organic reagents dyes which used widely in many academic and governmental laboratories. The method based on the separation of each dye from aqueous solution into surfactant-rich phase of Triton X-100 under the selected pH solution and determined by using UV-Vis spectrophotometry at respective λ_{max}. The suggested method was implemented for the determination of three dyes in environmental samples and compared statistically with traditional spectrophotometric method.

2. Material and Methods

2.1 Apparatus

The scanning the absorption spectra and absorbance measurements of the dyes used in this work were recorded by using a Shimadzu double-beam UV–visible spectrophotometer model UV 1800 (Kyoto, Japan) equipped with 10-mm quartz cell. An Inolab pH meter model 7110 (Germany) equipped with a combined glass electrode was employed for the solution pH measurements. The follow-up to the influence of temperature on the extraction process of dyes under study was conducted by using a thermostatic water bath model BS-11(JEIO TECH, Korea).

2.2 Materials and Reagents

Distilled water and chemicals used in this work are of analytical reagent grade. The organic dyes used in this work are; methyl orange (MO), Eriochrome Black T (EBT) and Congo Red (CR) with purity of 99% and supplied by Riedel-de Häen® (Germany). The nonionic surfactant (Triton X-100) whose chemical formula is $C_8H_{17}C_6H_4\ (OC_2H_4)_n$ at purity of > 99.6% was purchased from Sigma (Sigma Ultra, UK) and used without further purification. A stock standard solution of 1000 µg mL^{-1} of each dye was prepared by dissolving 0.1 g of the reagent in water and diluting to 100 mL in a volumetric flask. A 0.1 M hydrochloric Acid solution was prepared from concentrated solution of HCl (37%) by pippting 8.33 mol of 12M HCl (BDH, UK) in 1000 mL volumetric flask and diluted to mark with water. A stock Solution of 0.1 M ammonia solution was prepared by taking 0.2 mL of 25 % NH_3(BDH, UK) in 25 mL water.

2.3 General CPE Procedure

To an aliquot of 1 mL of a solution containing known amount of each dye; MO, EBT and CR standard or sample solution at (pH =7.5 for MO, 6.0 for EBT, and 5.0 for CR), 0.3 mL (for MO and CR), 0.2 mL (for EBT) of Triton X-100 (100%) were mixed in a 10-mL standard flask and diluted to mark with distilled water. The contents of the flask were stimulated by heating the contents in a water bath at 80 ^{0}C for (MO and EBT) and, 85 $^{\circ}$C (for CR) at 20 min (for MO and CR) and 30 min for EBT. The separation of the two phases of each dye was occurred immediately where the surfactant-rich phase became a highly viscous (without need of centrifugation) and settled down at the bottom of the flask making the aqueous phase easily discarded inverting the flask. Later, the surfactant-rich phase in the tube was dissolved in 2 mL of ethanol and the absorbance of the resulting solution of MO, EBT and CR was measured at λ_{max} of 434, 536 and 506 nm in a 10 mm quartz cell respectively, against a reagent blank. The residual quantity of each dye in aqueous phase was determined by using spectrophotometric method in order to determine the distribution ratio (D).

2.4 Statistical Analysis

The Minitab version 14 (Minitab Inc., State College, PA, USA) and Excel 2010 (Microsoft Office®) were used to execute all statistical calculations such as regression and correlation analysis, ANOVA and significance tests.

3. Results and Discussion

3.1 Absorption Spectra

The absorption spectra of MO, EBT and CR were recorded between 330-800 nm by taking 0.5 µg mL^{-1} of each dye followed the general CPE procedure against blank solution prepared under similar conditions without those dyes. Figure 2 shows the spectrum of each dye, indicating that the absorption maxima (λ_{max}) occur at 434, 536 and 506 nm with molar absorpitivity of 1.12x10^{5}, 5.29x10^{4} and 9.85x10^{5} L mol^{-1} cm^{-1} for MO, EBT and CR, respectively, thereby these wavelengths maxima were adopted during this study.

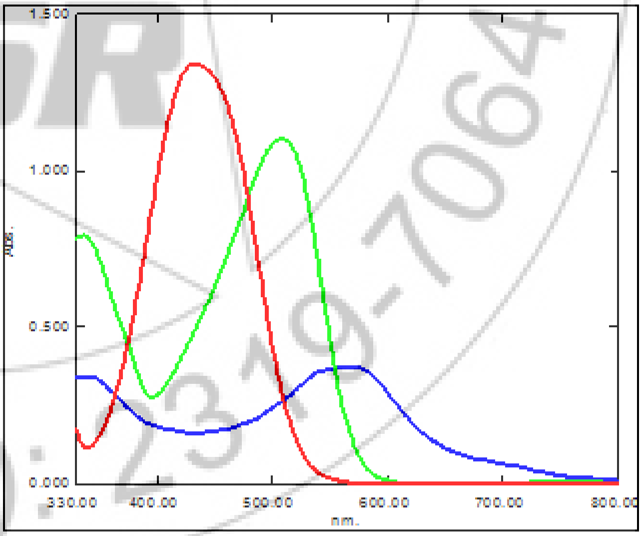


Figure 2: Absorption spectra of (a) MO (b) CR and (c) EBT

3.2 Optimization of CPE method

All important parameters that affect the extraction of each dye, such as the solution pH, Triton X-100 amount, and equilibration temperature and incubation time were optimized to establish the optimum parameters which can be used for the determination of these reagents in the real samples. The absorbance of each dye was measured spectrophotometrically at respective λ_{max} for all experiments and the distribution ratios (D) were calculated from which the percent extraction efficiencies were computed.

3.3 Effect of pH

In this study, the influence of pH on the cloud point extraction of 0.5 μg mL^{-1} of each dye (MO, EBT, and CR) was studied in the range of 1–10, by the addition 0.1 M hydrochloric or ammonium solution. The results are shown in Figure 3. It was observed that the extraction efficiency (%E) of all dyes increases with increasing in the solution pH up to 4, 5 and 6 for CR, EBT and MO respectively and thereafter reaches to plateau. At low pH , the extraction efficiency of each dye was very poor due to the protonation for sites consistency in the dye molecules which make these protonated dyes (more hydrophilic) unsuitable to interact strongly with the hydrophobic micelles of Triton X-100, and a smaller amunt of the analyte is therefore extracted [17]. Obviously, the extraction of each dye was maximum at nearly neutral pH value as shown in Figure 3. Thus the pH of 7.5, 6.0 and 5.0 for MO, EBT and CR were chosen as the optimum pH for complete entrapping of the dye molecules in the surfactant-rich phase.

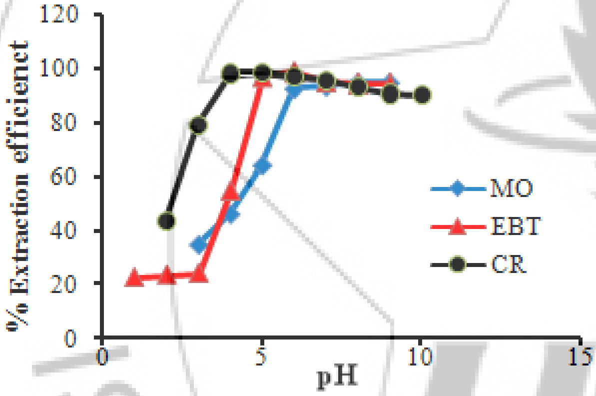


Figure 3: The effect of pH on the percent extraction efficiency of three dyes by CPE.

3.4 Effect of Triton X-100 amount

The amount of the surfactant plays a considerable role in determining the efficiency of the extraction of an analyte which lead to the formation a small volume and high density of cloud point extraction layer (i.e. surfactant-rich phase) compared to the bulk aqueous phase, thus maximize the extraction efficiency[18-19]. Thus, the experiments were conducted by taking a concentration of 0.5 μg mL^{-1} of each dye and varying volume from 0.050 to 1.00 mL of Triton X-100, keeping other parameters constant and follow the general CPE procedure. The findings were presented in Figure 4. As can be seen from Figure 4 that the extraction efficiency (%E) of MO and CR was highest(93.60 and 98.59%) when the volume of Triton X-100 reached to 0.3 mL, while a maximum extraction of EBT dye was 99.00% at 0.2 mL of Triton X-100. This means that the equilibrium state of the extraction process is achieved as well as the smallest volume and viscous layer of cloud point are obtained. The increased volume of Triton X-100 means departing from the critical concentration under optimal conditions and this process will lead to the disperse and prevent the ideal conglomerate and therefore reduced extraction efficiency of the dyes under study[20]. Therefore, the volume of Triton X-100 of 0.3, 0.2, and 0.3 mL were selected for MO, EBT and CR respectively, as optimum in this study.

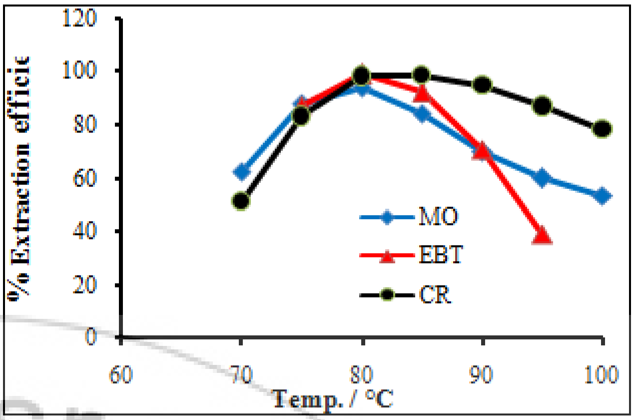


Figure 4: The effect of Triton X-100 on the percent extraction efficiency of three dyes by CPE.

3.5 Effects of temperature and time

The effect of equilibration temperature and incubation time is very important in CPE method so that to achieve easy phase separation and complete extraction [21-22]. Using optimum pH, Triton X-100 amount and 0.5 μg mL^{-1} of each dye, individual experiments were carried out to study the effect of these two parameters by varying the time from 5 to 40 min at 80 °C and temperature from 70 to 95 °C at 30 min for EBT and 20 min for both MO and CR, followed the general CPE procedure. Preliminary experiments indicated that at temperature below 70 °C, no concrete phase separation (i.e. two phases weren't formed) was observed due to very low number of micelles formed with the stability dyes at this temperature which led to decrease the distinguish organic phase. However, it was noticed (Figure 5) that the extraction efficiency increased with increasing temperature and reached maximum at 80 °C for MO and EBT at 20 min achieving %E of 94.10 and 99.00% respectively, while the maximum cloud point temperature of CR dye was of 85 °C at 30 min giving an extraction efficiency of 98.80%. At higher temperature, a poor in extraction efficiency was observed due to thermal decomposition of dyes- micelles phase.

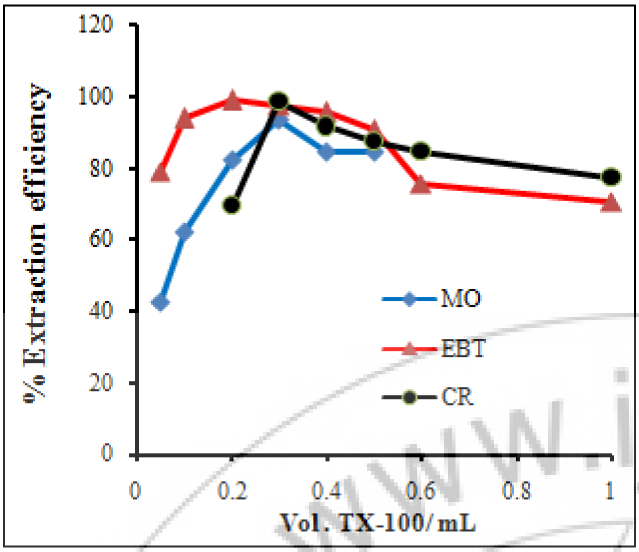


Figure 5 : Effect of temperature on the percent extraction efficiency of three dyes by CPE

Using the optimum conditions established in the previous sections, the incubation time was varied from 5 to 40 min as shown in Figure 6. The results revealed that maximum extraction efficiency was obtained at 20 min for the two dyes MO and CR achieving extraction efficiency of 93.60 and 99.66 % respectively, whilst a maximum extraction efficiency of 99.00% at 30 min for EBT was attained. At longer heating time than optimum, the extraction efficiency of the dyes was observed to decrease due to the dispersion of micelles (i.e. no agglomeration) in aqueous solution which are unable to completely entrap the dye molecules.

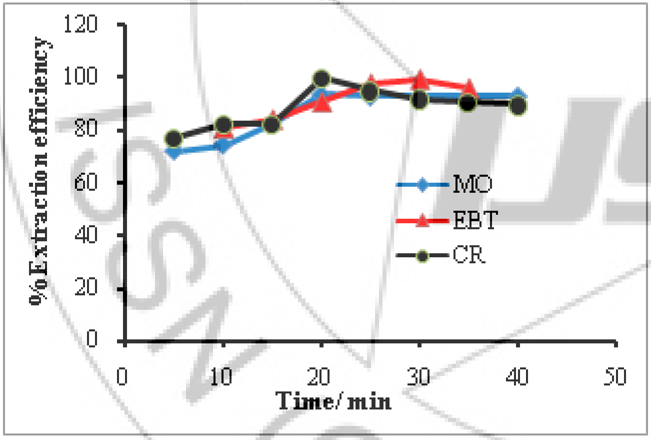


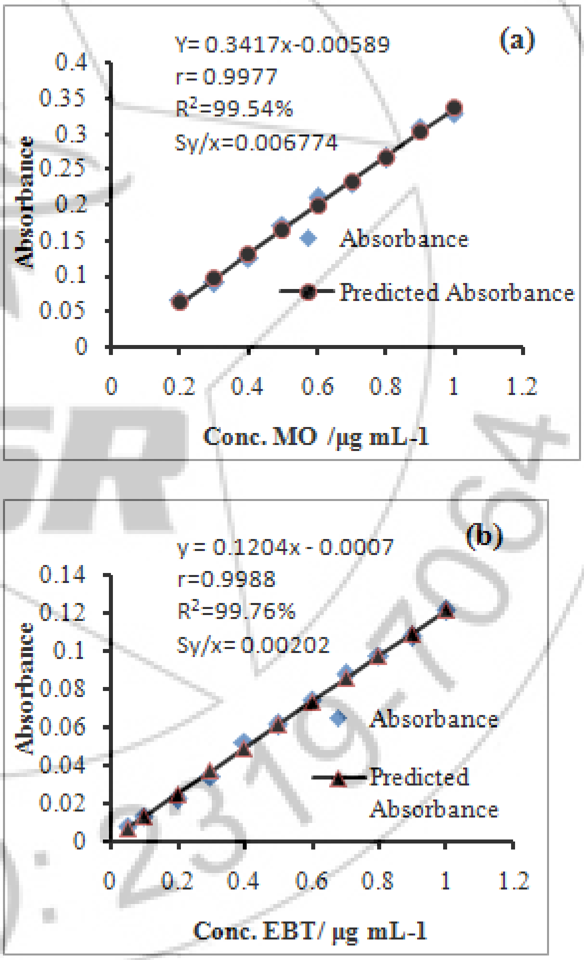
Figure 6: Effect of time on the percent extraction efficiency of three dyes by CPE.

3.6 Effect of Electrolytic Salts

The effect of divers electrolytic salts such as NaCl, KCl, NH_4Cl, $NaNO_3$, KNO_3, and NH_4NO_3 at constant concentration of 0.4 M were investigated upon the extraction of dyes according to general CPE procedure. It was noted that most of added salts at concentration of 0.4 M have no appreciable effect on the extraction efficiency of these dyes except NH_4NO_3 which reduced the extraction efficiency of EBT dye down to 77.50%. However, most of these salts at the selected concentration has less contribution in promotion of dehydration of the cloud point layer and therefore, it is not necessary to add any of these electrolytes to the extraction system in the study of these dyes.

3.7 Calibration graphs

A series of MO, EBT and CR standard solutions ranging from 0.05-5.00 µg mL^{-1} were prepared ,followed the general CPE and the absorbance signals of each dye solutions were measured spectrophotometrically at respective λ_{max}, so that to prepare the scatter plots from which the best fitted lines were selected for each dye at the optimum established conditions. The calibration line fitted plots of each dye are presented in Figure 7 and the statistical analytical results for the calibration data of each dye are summarized in Table 1. As can be seen from Figure 1, the working calibration lines were found to be of 0.2-1.0, 0.05-1.00 and 0.05-0.90 µg mL^{-1} for MO, EBT and CR respectively, from which the concentration of each dye was determined in the real samples. The proposed method can be achieved an enrichment factors of 36.6, 75.8 and 19.9 fold , leading to obtain the limits of detection (LOD) of 0.059, 0.04 and 0.042 µg mL^{-1} for MO, EBT and CR respectively. The molar absorpitiviy and Sandell's sensitivity were also calculated as shown in Table 1, indicating the suggested method has highly sensitive.



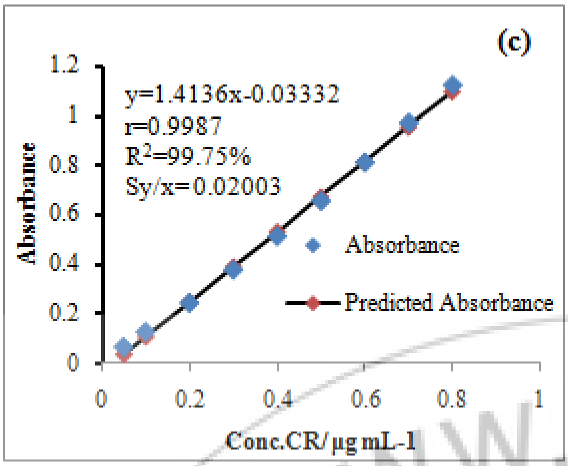


Figure 7: Calibration line fit plots for (a) MO (b) EBT (c) CR

Table 1: Analytical figures of merit of the determination of dyes by CPE-Spectrophotometry

Parameter	MO	EBT	CR
λ_{max} (nm)	434	536	506
Regression equation with CPE procedure	y = 0.3417x- 0.00589	y = 0.1204x -	y = 1.4136x - 0.0333
Standard error for regression line(sy/x)	0.006774	0.00202	0.020037
Correlation coefficient(r)	0.9977	0.9988	0.9987
Coefficient of determination (R^2)	99.54%	99.76%	99.75%
C.L. for the slope (b± tsb) at 95%	0.3417±0.0207	0.1204±0.00445	1.4136±0.0631
C.L. for the intercept (a± tsa) at 95%	-0.0059±0.0135	0.0007±0.00263	- 0.0333± 0.0301
Concentration range (µg mL^{-1})	0.2-1.2	0.05-1.00	0.05 – 0.90
Limit of Detection (µg mL^{-1})	0.059	0.040	0.042
Limit of Quantitation (µg mL^{-1})	0.198	0.167	0.140
Sandell's sensitivity (mg cm^{-2}/0.001A.U)	0.0029	0.0083	0.00071
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	$1.12\ 10^5$	$5.29\ 10^4$	9.85×10^5
RSD% (n=3) at 0.3 µg mL^{-1}	1.67%	0.81%	0.52
Preconcentration factor	33.0	50.0	33.0
Enrichment factor	36.7	75.8	19.9

3.8 Precision and Accuracy

For the purpose of evaluating the proposed method in terms of the presence of systematic and random errors or not, samples of drinking water were collected from the campus and river water from Diyala river. The river water samples were filtered immediately to avoid any suspended materials which certainly affect the determination. Each sample was spiked with 0.2, 0.4 and 0.6 µg mL^{-1} of each analyte and subjected to the general CPE procedure for three replicate measurements. The results were summed up in the Table 2. The results revealed that there are no highly significant determinate errors, indicating no contribution of other constituents present in the selected water samples affect the determination of dyes by using the proposed method. Also, the suggested method has shown an acceptable precision in term of repeatability (RSD %), through the analysis of three replicate for each sample which ranged between 0.47 and 6.83% as shown in Table 2.

Table 2: Representative recovery percentages and precision for analysis of M.O, E.B.T and C.R in tap and river water by the proposed CPE-Spectrophotometry.

Analyte	Sample	Amount added µg mL^{-1}	Amount found µg $mL^{-1)}$	Rec (%)	Mean Rec±t($\alpha_{0.05}$)s/√n	E_{rel} (%)	RSD (%) (n=3)
M.O	Tap water	0.2	0.196	98.0	99.1± 3.508	-2.0	2.94
		0.4	0.384	96.0	97.2± 4.317	-4.0	2.62
		0.6	0.619	103.1	102.4±2.934	3.1	0.74
	River water	0.2	0.208	104.0	103± 3.042	4.0	2.22
		0.4	0.379	94.9	96.6± 5.172	-5.1	0.97
		0.6	0.610	102.2	104± 5.476	2.2	0.47
E.B.T	Tap water	0.2	0.209	104.9	103.2±4.603	4.9	4.68
		0.4	0.401	100.2	99.3± 2.738	0.2	4.10
		0.6	0.620	104.1	103.1±3.042	4.1	3.92
	River water	0.2	0.193	96.6	97.5± 3.365	-3.4	6.83
		0.4	0.409	102.2	101.1±3.346	2.2	4.10
		0.6	0.616	102.7	102± 2.129	2.7	3.24

C.R	Tap water	0.2	0188	94.1	95.4± 3.955	-5.9	0.79
		0.4	0.374	93.7	95.2± 4.564	-6.3	0.62
		0.6	0.590	98.4	99.01±1.825	-1.6	0.12
	River water	0.2	0.186	93.3	94.7± 4.259	-6.7	0.63
		0.4	0.377	94.4	96.4± 6.085	-5.6	0.59
		0.6	0.596	99.3	98.1± 3.804	-0.7	0.47

3.9 Determination of dyes in laboratory wastewater

Four wastewater samples were withdrawn randomly from the collected wastewater containers before the chemical treatment in each laboratory, classified according to the type of experiments (i.e. containers related to each indicator used; MO and Congo Red for acid-base titration and EBT for titration of calcium) which have been carried out by the first stage students in the analytical chemistry of Department of Chemistry /College of Science /University of Diyala. Each sample was subjected to the general CPE procedure and the dyes were determined spectrophotometrically at their respective λ_{max} for triplicate measurements. All samples, on the other hand, have been analyzed by direct conventional UV-Vis spectrophotometric method for the purpose of comparison with the proposed method. The results are summed up in Tables 3, 4 and 5.

The statistical analysis of the results shown in Table 3,4 and 5 showed that the calculated experimental values $|t|$ were of 0.27, 1.44 and 1.15 .These t values are less than the critical value of 3.182 (α=0.05. dof=3, two-tailed) and supported by p values [P(T<t) which was 0.803, 0.245 and 0.333 for MO, EBT and CR respectively, indicating acceptance of null hypothesis (Ho) which specified that there appears insufficient evidence to suggest the accuracy of the established CPE-Spectrophtometry differs from that of traditional UV-Vis method (i.e. there is a good agreement between the results obtained by the two methods).

Table 3: Determination of MO in laboratory wastewater samples by proposed method.

Sample No.	*Proposed method ($\mu g\ mL^{-1}$)*	*Traditional UV-Vis Spectropho-tometry ($\mu g\ mL^{-1}$)*	*Paired t-Test $t=\overline{x}_d\sqrt{n}/s$*
1	**0.639**	0.623	$\overline{X}_d$= 0.0028 S_d=0.023531 $t_{cal}(n=4)$= 0.27 $t_{crit}(\alpha 0.05,\phi=3)$= 3.182 ρ-value=0.803
2	**0.575**	0.564	
3	0.901	0.940	
4	0.510	0.500	

4. Conclusions

In this work, the combined CPE-Spectrophotometry was established for the sequential determination of dyes such MO, EBT and CR in drinking and polluted water samples for the first time. The proposed method was compared statistically with traditional UV-Vis spetrophotometric technique, showing no significant difference at 0.05 significance level. This method proved to be a high sensitive, low detection limit with good precision and accuracy. The addition of electrolytic solutions was unnecessary to the extraction system of these dyes and gave high extraction efficiency in one step extraction.

Table 4: Determination of EBT in laboratory wastewater samples by proposed method.

Sample No.	*Proposed method ($\mu g\ mL^{-1}$)*	*Traditional UV-Vis Spectropho-tometry ($\mu g\ mL^{-1}$)*	*Paired t-Test $t=\overline{x}_d\sqrt{n}/s$*
1	0.591	0.620	$\overline{X}_d$= 0.03725 S_d=0.051616 $t_{cal}(n=4)$= 1.44 $t_{crit}(\alpha 0.05,\phi=3)$=3.182 ρ-value=0.245
2	0.840	0.743	
3	0.483	0.440	
4	0.226	0.188	

Table 5: Determination of EBT in laboratory wastewater samples by proposed method.

Sample No.	Proposed method ($\mu g\ mL^{-1}$)	Traditional UV-Vis Spectropho-tometry ($\mu g\ mL^{-1}$)	Paired t-Test $t=x_d\overline{\sqrt{n}/s}$
1	0.456	0.558	$\overline{X}_d$= -0.02875 S_d=0.049982 $t_{cal}(n=4)$= 1.15 $t_{crit}(\alpha 0.05,\phi=3)$ =3.182 ρ-value=0.333
2	0.696	0.699	
3	0.639	0.631	
4	0.220	0.238	

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