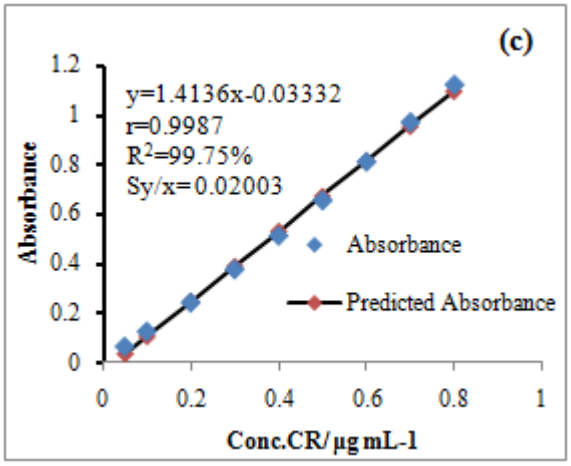

Figure 7: Calibration line fit plots for (a) MO (b) EBT (c) CR

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 spectrophometrically 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}(α0.05,ϕ=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	X_d= 0.03725 S_d=0.051616 t_{cal}(n=4)= 1.44 t_{crit}(α0.05,ϕ=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 $t=x_d\sqrt{n}/s$
1	0.456	0.558	$\overline{X_d}$= -0.02875 S_d=0.049982 t_{cal}(n=4)= 1.15 t_{crit}(α0.05,ϕ=3) =3.182 ρ-value=0.333
2	0.696	0.699	
3	0.639	0.631	
4	0.220	0.238	

References

[1] S. Abedi, F. Nekouei, ," Removal of Direct Yellow 12 from Water Samples by Cloud Point Extraction Using Triton X-100 as Nonionic Surfactant ", E J.Chem., 8: 1588-1595,2011.

[2] K. Belay, A. Hayelom," Removal of Methyl Orange from Aqueous Solutions Using Thermally Treated Egg Shell (Locally Available and Low Cost Biosorbent)" Int. J. Innov. and Sci. Res., 8: 43-49 ,2014.

[3] H.Kusic, N. Koprivanac, L. Srsan," Azo Dye Degradation Using Fenton Type Processes Assisted by UV Irradiation: A Kinetic Study", J. Photochem. Photobio. A, 181:195-202, 2006.

[4] H.Watanabe, T. Saitoh, T. Kamidate, H. Haraguchi, "Distribution of Metal Chelates Between Aqueous and Surfactant Phases Separated From a Micellar Solution of a Nonionic Surfactant", Mikrochim. Acta, 106:83-90,1992.

[5] A.E. Fernandez, Z.S. Ferrera, J.J.S. Rodrinuez, " Application of Cloud-point Methodology to the Determination of Polychlorinated Dibenzofurans in Sea Water by High-performance Liquid Chromatography ", Analyst, 124: 487-491, 1999.

[6] P. Krystyna, "Chemical Speciation and Fractionation of Metals in Wine", Chemical Speciation and Bioavailability, 19:1-8, 2007.

[7] E.Kilinc, V. Lepane, A. Viitak, B. Gumgum, "Off-line Determination of Trace Silver in Water Samples and Standard Reference Materials by Cloud Point Extraction–Atomic Absorption Spectrometry",

Proceedings of the Estonian Academy of Sciences, 58:190–196 ,2009.
[8] Z. A-A. Khammas, A. A. Ghali, K.H. Kadhim, " Cloud Point Extraction Procedure for the Determination of Mercury by Spectrophotometry Using a New Synthesized Ligand", Iraqi Nat. J.Chem. 49: 25-37, 2013.
[9] W. Ling, J. Gui-bin, C. Ya-qi, H. Bin, W. Ya-Wei, S. Da-zhong," Cloud Point Extraction Coupled with HPLC-UV for the Determination of Phthalate Esters in Environmental Water Samples" J.Environ. Sci., 19: 874–878, 2007.
[10] R. Mosaic, F. Nekouei, " Cloud Point Extraction of Toxic Reactive Black 5 Dye from Water Samples Using Triton X-100 as Nonionic Surfactant", E. J. Chem., 8:1606-1613, 2011.
[11] A. Arunagiri, P. Kalaichelvi, S. Cherukuri, A. Vijayan, " Studies on Removal of Reactive Blue Dye Using Cloud Point Extraction", Int. J. Chem. and Environ.Eng.., 3:16-23,2012.
[12] A. Shokrollah, Z. Malekhosseini, " Spectrophotometric Determination of Trace Amounts of Eosin B dye After Cloud Point Extraction", Chem. Sci. Trans., 3:37-44, 2014.
[13] H. Tavallali, M.Ostovar," Trace Spectrophotometric Determination of Brilliant Green in Fish Farming Water Samples", Inte. J.ChemTech Res., 1: 199-203, 2009.
[14]
[15] W. Liu, W.J. Zhao, J.B.Chen, M.M. Yang, " A Cloud Point Extraction Approach Using Triton X-100 for the Separation and Preconcentration of Sudan Dyes in Chilli Powder", Anal. Chim. Acta, 605: 41–45, 2007.
[16] N. Pourreza, S. Elhami, "Spectrophtometric Determination of Malachite Green in Fish Farming Water Samples After Cloud Point Extraction Using Nonionic Surfactant Triton X-100", Anal. Chim. Acta, 596: 62–65, 2007.
[17] N. Pourreza, M. Ghomi," Simultaneous Cloud Point Extraction and Spectrophotometric Determination of Carmoisine and Brilliant Blue FCF in Food Samples", Talanta, 84: 240–243, 2011.
[18] T. Saitoh, W.L. Hinze," Concentration of Hydrophobic Organic Compounds and Extraction of Protein Using Alkylammoniosulfate Zwitterionic Surfactant Mediated Phase Separations (Cloud Point Extractions)", Anal. Chem., 63: 2520-2525, 1991.
[19] M.A. Mesquita da Silva, V.L. Azzolin Frescura, A.J. Curtius," Determination of Trace Elements in Water Samples by Ultrasonic Neubilization Inductively Coupled Plasma Mass Spectrometry After Cloud Point Extraction", Spectrochim. Acta Part B: At. Spec., 55:803-813, 2000.
[20] J.L. Manzoori, G. Karim-Nezhad, " Development of a Cloud Point Extraction and Preconcentration Method for Cd and Ni Prior to Flame Atomic Absorption Spectrometric Determination", Anal. Chim. Acta, 521:173–177, 2004.
[21] I.R. Ali, "PhD thesis" University of Baghdad, College of Science for Women, 2013.
[22] E.K. Paleologos, D.L.Giokas, M.I. Karayannis," Micelle-mediated Separation and Cloud-point Extraction"Trends Anal. Chem., 24: 426–443, 2005.
[23] H. Filik, D. Giray, " Cloud Point Extraction for Speciation of Iron in Beer Samples by Spectrophotometry", Food Chemistry,130: 209–213, 2012.

Author Profile

Zuhair Khammas, Asst. Prof. Dr. of Analytical Chemistry in Department of Chemistry, College of Science for Women, University of Baghdad. .He received the B.Sc in Chemistry in 1974, M.Sc in instrumental Methods of analysis from Strathclyde University/UK in 1984, and Ph, D in Analytical Chemistry from University of Baghdad in 1996. Head of Chemistry Department from 2002-2006. He is teaching statistics and chemometrics in analytical chemistry for postgraduate students. He is supervised number of MSc and PhD students and published over 35 research papers in national and international journals. He has a co-authored two books in spectrochemical analysis published in University of Mosul. He is a member of scientific committee at chemistry department.

Hala Metaeb, She graduated with B.Sc. degree in chemistry from college of Science, University of Diyala, Iraq in 2011. She is now preparing her M.Sc in analytical chemistry at College of Science for Women, University of Baghdad.