Dispersive Solid Phase Extraction Combined by Dispersive Liquid-Liquid Microextraction for Preconcentration of Brilliant Green from Fish and Seawater Samples and Its Determination by Spectrophotometry

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ABSTRACT: *A highly sensitive, simple, and speed technique was employed for the determination of brilliant green in fish (Sphyraena jello) and seawater samples by visible spectrophotometry after its extraction and enrichment with chitosan- zinc oxide nanoparticle coupled with dispersive liquid-liquid microextraction. Ultra-trace concentrations of brilliant green were dispersed to the organic phase in DLLME method after adding dispersive solvent and chitosan- zinc oxide nanoparticles. The experimental factors such as the amount of chitosan- zinc oxide nanoparticles, the concentration of Triton X- 114, type of volume of extraction and dispersive solvents, extraction time, rate and time of centrifugation, the volume of sample, and pH were investigated to order to enhance of the extraction efficiency. Under optimum extraction conditions, the volume of chloroform (as extraction solvent) and methanol (as dispersive solvent) were 100.0 µL and 550.0 µL, respectively; the amount of chitosan- zinc oxide nanoparticles was 15.0 mg; the time of extraction was 4.0 min; rate and time of centrifugation were 3000.0 rpm and 8.0 min, respectively, the volume of sample was 8.0 mL, and pH of the sample solution was 4.0. After optimizing the microextraction conditions and instrumental factors, an enrichment factor of 169.0 was achieved. The analytical curve (absorbance vs. concentration) was linear over the range 1.0-200.0 µg/L of brilliant green. The limit of detection and relative standard deviation were 0.3 µg/L and < 6.1 %, respectively. The protocol was successfully employed for the determination of brilliant green in the seawater of Chabahar Bay and fish (Sphyraena jello) samples.*

KEYWORDS: *Brilliant green, Chitosan- zinc oxide nanoparticle, Dispersive liquid-liquid microextraction, Chabahar Bay, Spectrophotometry, Seawater, Fish sample.*

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INTRODUCTION

Dyes are toxic and harmful to human beings, microorganisms, and the ecosystem. Also, they are mutagenic and carcinogenic. Up to day, these compounds are generally applied in many fields of technology such as paper, textile, leather, food, cosmetic and pharmaceutical industries. Large amounts of these materials are continuously entering the environment such as seawater from these industries [1-3].

Brilliant green (BG, Fig. 1) is a triphenylmethane dye that has been utilized in the fish farming industry for many decades due to its broad anti-microbial, anti-fungal spectrum, and anti-parasitic, its high efficiency in the prevention and treatment of certain fish diseases and its low cost. BG similar mentioned properties of dyes is harmful and toxic and has mutagenic and carcinogenic effects which affect aquatic biota and humans. It causes generally eye burns in humans and animals. BG is applied in textile dying and covers paper in the paper industry [4,5]. The minimum required performance limit of the dyes is 2.0 ng/g concentration which is used in the fish industry according to the European Union. Also, a minimum sensitivity of 1.0 ng/g is needed for the regulatory testing based on the US Food and Drug Administration (FDA) [6]. Therefore, the development of a new method for ultra-trace determination of dyes such as BG is required.

Various analytical methods have been reported for the determination of dyes such as BG in different real samples including conductometry [4], electrochemistry [7], electrochemiluminescence [8], high-performance liquid chromatography [9], liquid chromatography-mass spectrometry [10], capillary electrophoresis [10], and spectrophotometry [4,11].

In addition, in water environmental samples, the target analyte is often present at trace concentrations. So, the sample preparation, pre-concentration, and extraction step are required [2,3].

Different methods of extraction such as molecularly imprinted polymer [1,2], molecularly imprinted polymer pipette-tip solid phase extraction [1,2], graphite-based magnetic $NiFe_2O_4$ decorated exfoliated in pipette-tip [3], ionic liquid assisted dispersive liquid-liquid microextraction [6], hollow fiber solid/liquid phase microextraction (HF- SLPME) [11], carbon nanotube assisted pseudo- stir bar solid/liquid microextraction [11], have been used for pre-concentration of dyes including BG in various real samples.

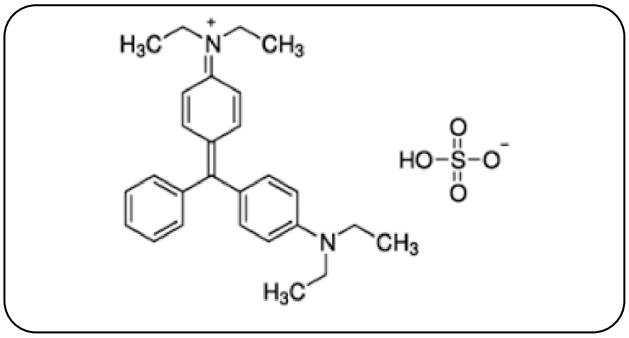

Fig. 1: Structure of BG.

In recent years, various methods of microextraction (the techniques applied to a minimal volume of organic solvent) have been used for enrichment goals of different analytes. Among the various microextraction, Dispersive Liquid-Liquid MicroExtraction (DLLME) indicates excellent enrichment characteristics, and the technique is very fast (DLLME for the first time reported with *Rezaee et al*.) [12-15]. In DLLME, target compound extraction is practically instantaneous due to the enormous contact surface between the receptor and donating solvents. To obtain dispersion, a third solvent can be applied to both solvents. In DLLME, after adding a proper volume of extraction and dispersive solvent to the aquatic sample, a cloudy solution consisting of three solvents (water solution, extraction, and dispersive solvent) is formed. After extraction of the target analyte of the water sample in fine droplets of extraction solvent, the method is followed by centrifugation, the organic phase is taken and injected into an analytical instrument for analysis [16-19].

Chitosan (a high molecular weight cationic polysaceharide) has attracted considerable interest due to its non-toxicity, good mechanical properties, antibacterial activity, and biocompatibility ability. Thus, it has been employed in environmental, medicinal, biomaterial, and other industrial fields [20]. Hybrid materials based on chitosan have been reported [20].

Zinc oxide (ZnO) semiconductor has different favorable properties, consisting of high electron mobility, good transparency, wide bandgap, and room-temperature luminescence. Loading of zinc oxide nanoparticles on the chitosan can be used as a new sorbent and assisted in dispersing of analytes in the organic phase [20-23].

The aim of the research is to utilize chitosan- zinc oxide hybrid for better performance of dispersing of the analyte in the organic phase in dispersive liquid-liquid microextraction

for pre-concentration and extraction of BG from seawater of Chabahar Bay and fish samples. Finally, the extracted BG was determined by Vis spectrophotometry. To the best of our knowledge, this is the first report on using chitosan- zinc oxide nanoparticles coupled with dispersive liquid-liquid microextraction for such application and this is also the first report on the determination of brilliant green in seawater of Chabahar Bay (Oman Sea). Another novelty of the research is the simultaneous use of dispersive solvent and chitosan-zinc oxide nanoparticles in DLLME procedure for the better performance of extraction of brilliant green.

EXPERIMENTAL SECTION

Materials

Zinc sulphate was provided by Fluka AG (Switzerland). All other chemicals were obtained from Merck (Darmstadt, Germany) and were of analytical grade. BG stock solution (500.0 mg/L) was achieved by dissolving 50.0 mg of the analyte in methanol and diluting it to 100.0 mL in a volumetric flask. The stock solution was stored at 4.0 ^{0}C in dark. Working solutions of BG were obtained daily with a suitable dilution of the stock solution by ultrapure water.

Instrumental

Spectrophotometry was carried out by a UNICO S2100 Vis spectrophotometer (China) equipped using 100 µL quartz microcells (model Q- 01701, Stara Company, UK) at the wavelength of 625 nm for BG. Model 630 Metrohm pH meter of Switzerland was utilized for measurement of pHs.

Procedure

15.0 mg of chitosan- zinc oxide nanoparticles were placed in a plastic tube and water sample (8.0 mL, pH 4.0) including 200.0 µg/L of BG and a suitable concentration of Triton X-114 (0.2 % v/v). Then, 100.0 µL of organic solvent (chloroform) and 550.0 µL of dispersive solvent (methanol) were added to the mixture. The mixture was ultrasound for 4.0 min to get a cloudy solution. Next, the solution for phase separation was centrifuged at 3000.0 rpm for 8.0 min. Finally, the organic phase was taken out with a micro syringe and determined by Vis spectrophotometry at a wavelength of 625.0 nm.

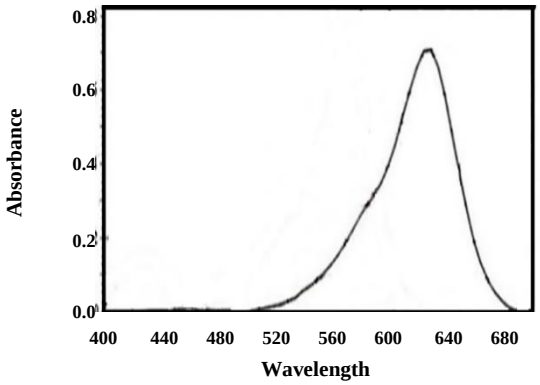

Fig. 2: Absorption spectra of 175.0 µg/L of BG. Conditions: Sample solution, 8.0 mL; disperser solvent (methanol), 550.0 µL; extracting solvent (chloroform), 100.0 µL; amount of chitosan-zinc oxide nanoparticles, 15.0 mg; concentration of Triton X- 114, 0.2 % (v/v); time of extraction, 4.0 min; rate of centrifugation, 3000.0 rpm; time of centrifugation, 8.0 min; and pH=4.0.

RESULTS AND DISCUSSION

Absorption spectra

After enrichment of BG by modified DLLME with the application of the suggested protocol, its absorption spectra were investigated at the wavelength range of 400.0 to 700.0 nm against the reagent blanks including distilled water (Fig. 2). The observations indicate that the maximum absorption wavelengths were 625.0 nm for BG.

Optimization of modified DLLME

Different factors for the modified DLLME (MDLLME) procedure such as type and volume of dispersive solvent, type, and volume of extraction solvent, the mass of chitosan-zinc oxide nanoparticles, the concentration of Triton X- 114, time of extraction, the rate and time of centrifugation, volume of sample and pH were investigated and optimized.

Selection of type of extraction and dispersive solvent

DLLME method is a three-phase system and the nature of dispersive and extraction solvents has a significant role in this technique. In the case of extraction solvent, it must be denser than water, have the ability to the enrichment of target analytes, and have good extraction behavior. In this case, the ability of n-hexane, toluene, chloroform, and tetrachloride carbon, as extraction solvents were optimized. Observation expressed in Fig. 3 explained chloroform for BG as extraction solvent provided maximum extraction efficiency.

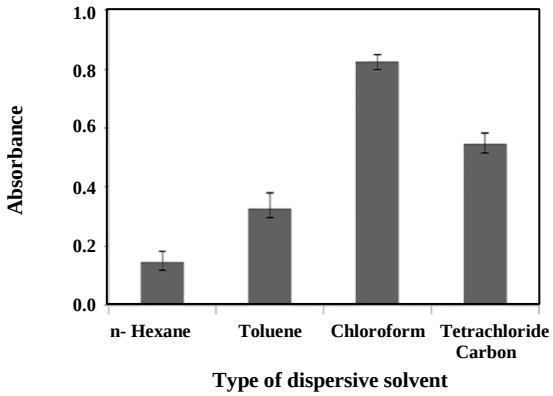

Fig. 3: Effect of type of extraction solvent on extraction efficiency of brilliant green with modified DLLME. Conditions: Sample solution, 8.0 mL; disperser solvent (methanol), 550.0 µL; extracting solvent, 100.0 µL; amount of chitosan- zinc oxide nanoparticles, 15.0 mg; concentration of Triton X- 114, 0.2 % (v/v); time of extraction, 4.0 min; rate of centrifugation, 3000.0 rpm; time of centrifugation, 8.0 min; and pH=4.0.

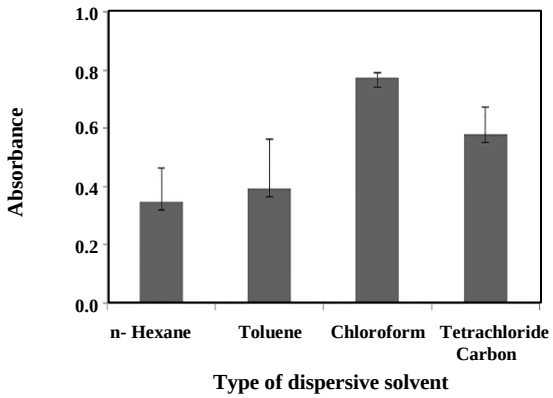

Fig. 4: Effect of type of dispersive solvent on extraction efficiency of brilliant green with modified DLLME. Conditions: Sample solution, 8.0 mL; disperser solvent, 550.0 µL; extracting solvent (chloroform), 100.0 µL; amount of chitosan- zinc oxide nanoparticles, 15.0 mg; concentration of Triton X- 114, 0.2 % (v/v); time of extraction, 4.0 min; rate of centrifugation, 3000.0 rpm; time of centrifugation, 8.0 min; and pH=4.0.

In dispersive solvent, the main point chosen for dispersive solvent is its miscibility in both the organic phase (extraction solvent) and the aqueous phase (sample solution). The modified DLLME technique coupled with Solid-Phase Extraction (SPE) generally is not employed dispersive solvent but in the work for the better extraction efficiency, enrichment, and pre-concentration of BG, it is used. This decreases the interfacial tension between the organic and aquatic phases and accelerates the formation of droplets of organic solvent in an aqueous sample. In this case, methanol, ethanol, acetonitrile, and acetone were examined. The result indicated that methanol is the best absorbance among these dispersive solvents and was chosen for subsequent runs (Fig. 4).

The volume of extraction solvent

To study the effect of the volume of extraction solvent, mixtures consisting of different volumes (50.0- 200.0 µL) of extraction solvent were introduced to the modified DLLME procedure. When the volume of extraction solvent was increased up to 100.0 µL, the extraction efficiency also increased. At higher volumes of chloroform because of the increasing sedimented phase volume and dilution of the BG, the absorbance of the analyte was decreased (Fig. 5). So, in the next runs, the optimum volume of 100.0 µL was selected as the volume of extraction solvent.

The volume of dispersive solvent

To investigate the effect of the volume of dispersive solvent, extraction of BG was carried out by utilizing 400.0 to 600.0 µL of dispersive solvent. The achieved observations are indicated in Fig. 6. Based on the result, 550.0 µL of the volume of dispersive solvent was chosen for subsequent experiments.

Effect of amount of chitosan- zinc oxide nanoparticles

The goal of using chitosan- zinc oxide nanoparticles hybrid only is not the role of its sorbent. The goal is the application of material (chitosan- zinc oxide nanoparticles) that can enter into both phases including the organic phase (extraction phase) and water phase (sample). Enter and exit chitosan- zinc oxide nanoparticles to the two phases by stirring in an ultrasonic bath, it can transfer more target compounds into the organic phase (extraction phase).

In order to optimize the amount of chitosan- zinc oxide nanoparticles on absorbance, different masses (0.0, 5.0, 10.0, 15.0, and 20.0 mg) of it, were investigated. A higher response was described for BG analyte when the mass of chitosan- zinc oxide nanoparticles was increased up to 15.0 mg. Further increasing the amount of chitosan- zinc oxide nanoparticles provided no significant increase in absorbance. Then, a mass of 15.0 mg was used for all following experiments.

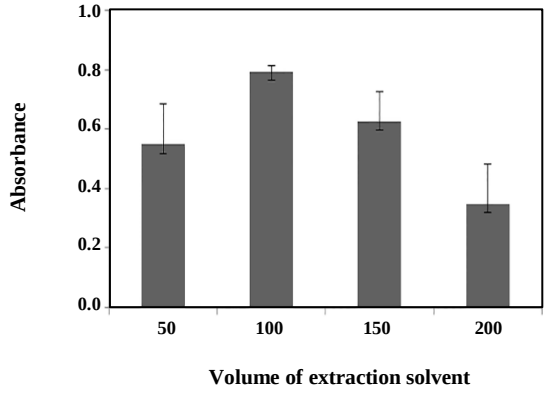

Fig. 5: Effect of volume of extraction solvent on extraction efficiency of brilliant green with modified DLLME. Conditions: sample solution, 8.0 mL; disperser solvent (methanol), 550.0 µL; extracting solvent (chloroform); amount of chitosan- zinc oxide nanoparticles, 15.0 mg; concentration of Triton X- 114, 0.2 % (v/v); time of extraction, 4.0 min; rate of centrifugation, 3000.0 rpm; time of centrifugation, 8.0 min; and pH=4.0.

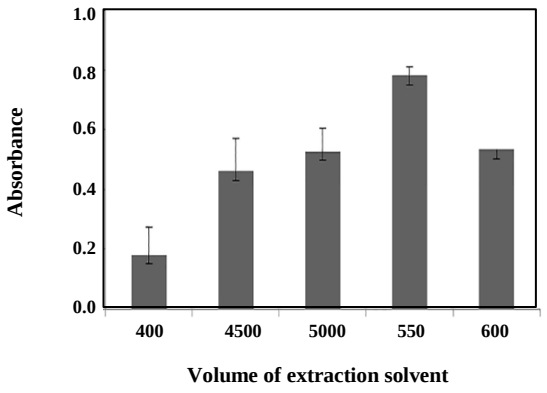

Fig. 6: Effect of volume of dispersive solvent on extraction efficiency of brilliant green with modified DLLME. Conditions: Sample solution, 8.0 mL; disperser solvent (methanol); extracting solvent (chloroform), 100.0 µL; amount of chitosan- zinc oxide nanoparticles, 15.0 mg; concentration of Triton X- 114, 0.2 % (v/v); time of extraction, 4.0 min; rate of centrifugation, 3000.0 rpm; time of centrifugation, 8.0 min; and pH=4.0.

For comparison, the effect of the mass of chitosan without zinc oxide nanoparticles on extraction efficiency, several masses of it (0, 5, 10, 15, and 20 mg) were investigated. Extractions without zinc oxide nanoparticles have a lower response is compared with experiments in the presence of chitosan- zinc oxide nanoparticles hybrid. Also, the effects of the amount of zinc oxide nanoparticles without chitosan on extraction efficiency in various masses (0, 5, 10, 15, and 20 mg) of it were studied and the result has shown that zinc oxide nanoparticles- chitosan hybrid has the best response.

Effect of concentration of Triton X- 114

To investigate the effect of the concentration of Triton X-114 on recovery, its different concentration (as is shown in Fig. 7) was examined. The concentration of Triton X-114 (as a surfactant)an effect on the extraction efficiency of dyes such as BG; so, we tried to optimize its concentration. We found that by increasing the concentration of the surfactant, the absorbance was also increased, but in the amounts of more than 0.2 % v/v of Triton X-114, a decrease in the absorbance of BG was observed (Fig. 7). This is probably due to the dilution of the analytes in larger volumes of the surfactant. Triton X-114 is a readily available surfactant with low cost; and it is also non-toxic, non-flammable, and non-ionic. Triton X-114 indicated the best effect on forming turbid solution and so increased extraction efficiency.

Effect of time of extraction

Extraction time in modified DLLME (MDLLME) was defined as the time interval between the injection of a mixture of dispersive and extraction solvents to the aquatic sample solution before starting the centrifugation (in fact, extraction time was defined as ultrasonic time). The effect of ultrasonic time was optimized in the range 0-5 min and the result showed that the best ultrasonic time was 4.0 min and after it, the signal of the analyte is approximately constant.

Optimization of the centrifugation rate and time

The next parameter which might affect the absorbance of the proposed protocol is the collection of the sedimented phase which is related to the rate and time of centrifugation. These factors were examined in the ranges of 1000.0- 5000.0 rpm and 2.0- 10.0 min, respectively. The results explained that the absorption was increased up to 3000.0 rpm (rate of centrifugation) and 8.0 min (time of centrifugation).

Effect of volume of sample

The effect of the volume of the sample on the peak area was optimized and investigated by increasing the volume of the sample from 2.0 mL to 10.0 mL while the concentration of BG was fixed at 200.0 µg/L. Absorbance improves continuously with increasing the sample volume up to 8.0 mL

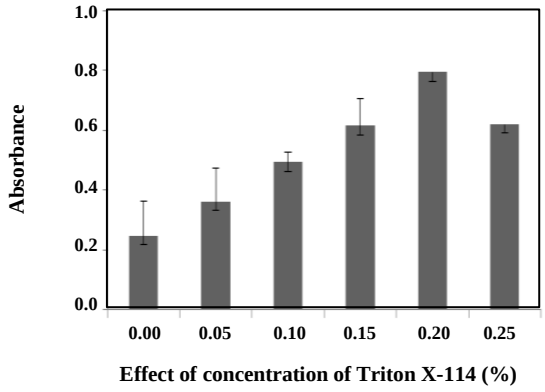

Fig. 7: Effect of concentration of Triton X- 114 on extraction efficiency of brilliant green with modified DLLME. Conditions: Sample solution, 8.0 mL; disperser solvent (methanol), 550.0 µL; extracting solvent (chloroform), 100.0 µL; amount of chitosan- zinc oxide nanoparticles, 15.0 mg; time of extraction, 4.0 min; rate of centrifugation, 3000.0 rpm; time of centrifugation, 8.0 min; and pH=4.0.

(because of increasing the number of moles of the analyte available for extraction). Further increase in sample volume causes a reduction in absorbance. It was because the BG was diluted with the increase in the volume of the sample. Hence, the volume of 8.0 mL was selected as the sample optimum volume.

Effect of solution pH

The effect of the solution pH value on the absorbance of BG was examined and optimized in the range of 2.0-8.0. The highest extraction efficiency was obtained at pH 4.0. Hence, in the next experiments, the pH was adjusted to 4.0.

Evaluation of technique

Linear range, the limit of detection and enrichment factor

With the modified DLLME (MDLLME) in this research, the calibration curves were recorded. For this purpose, a series of standard solutions of BG in the concentration range 1.0-200.0 µg/L were introduced to the microextraction procedure. The process of modifying DLLME of BG was carried out with optimal conditions according to the procedure of the research. The absorption of the analyte was registered and the calibration curve was drawn as a function of the instrument response of the analyte against its concentration. The Limit of Detection (LOD) was obtained from 3 S_d (blank) (where S_d is the standard deviation of seven consecutive measurements of the blank) [1-3]. The LOD was obtained 0.3 µg/L. Enrichment Factor (EF) and relative recovery (RR %) were achieved by applying the next equations (1, 2) [14].

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

$$RR\ \% = \left[\left(C_{found} - C_{real} \right) / \left(C_{added} \right) \right] \times 100 \quad (2)$$

That C_{sed}, C_0, C_{found}, C_{real}, and C_{added} are the concentration of the BG in the sedimented phase, the initial concentration of the analyte in the water sample, the concentration of BG after the addition of a known amount of standard in the real sample, the concentration of BG in the real sample, and the concentration of the known amount of standard that was spiked to the real sample, respectively. The enrichment factor was achieved 169.0 folds. The analytical parameters are shown in Table 1.

The intra-day precision of the evaluated technique as RSD ranged between 1.9 % to 6.3 % and-inter day reproducibility was better than 6.1 % in all cases.

Table 2 compares the characteristic data of the proposed protocol with those introduced in the literature. Comparing the obtained results for pre-concentration and analysis of brilliant green with modified DLLME combined with spectrophotometry by other protocols explains acceptable linearity and RSD, and lower LOD as indicated in Table 2. The method showed a shorter linear range in comparison with some other techniques such as Cloud Point Extraction (CPE) and Hollow Fiber Solid Phase MicroExtraction (HF- SPME) which is due to the usage of higher amounts of sorbent in BG extraction or due to exhaustive extraction for CPE and not having matrix interference for HF-SPME. The protocol showed higher LOD in comparison with SPE combined LC-MS because of the higher sensitivity of the instrument. These results show that the proposed method is simple and rapid. The method needs just a small volume of the solvent (in a microliter unit).

HF- SPME, hollow fiber solid-phase microextraction; SPE, Solid-phase extraction; CPE, Cloud point extraction; IL-DLLME- ZCDSP, Ionic liquid-based dispersive liquid-liquid microextraction followed by Zero-crossing first derivative spectrophotometric method

Determination of BG in real samples

Determination of BG in seawater

To examine the utilization of the suggested procedure, determination of BG in seawater samples of two station

Table 1: Analytical characteristic of suggested technique for determination of BG after modified DLLME.

Analyte	Linearity range (µg/L)	Equation of calibration curve[a]	Determination coefficient (R^2)	LOD (µg/L)	Enrichment factor (EF)
brilliant green (BG)	1.0-200.0	A = 0.0041 C – 0.0115	0.9962	0.3	169.0

a) Where C and A are concentration of BG and response spectrophotometry, respectively

Table 2: Comparison of suggested method by other technique for determination of brilliant green.

Extraction method	Detection method	LOD (µg/L)	Linear range (µg/L)	RSD (%)	Reference
IL-DLLME- ZCDSP	UV- Vis	2.7	10.0- 500.0	4.7	[6]
SPE	LC- MS	0.07- 0.24 (µg/g)	0.5- 10.0 (µg/g)	14.8	[9]
HF- SPME	UV- Vis detection	0.5	1.0- 10000.0	8.3	[11]
CPE	UV- Vis	15.0	50.0- 2000.0	2.7	[24]
Modified DLLME	UV- Vis	0.3	1.0- 200.0	6.1	This work

Table 3: Recovery results for modified DLLME method for determination of brilliant green from of seawater and fish sample.

Sample	Analyte added (µg/L)	Analyte found (µg/L)	Recovery (%)	RSD (%, n=3)
Seawater (station 1, Beris)	0.0	6.3	Not available	1.1
Seawater (station 1, Beris)	20.0	25.9	98.0	2.0
Seawater (station 1, Beris)	50.0	56.2	99.8	2.7
Seawater (station 2, Pasabandar)	0.0	5.4	Not available	2.3
Seawater (station 2, Pasabandar)	20.0	23.7	91.5	6.1
Seawater (station 2, Pasabandar)	50.0	53.1	95.4	2.4
Fish	0.0	< LOD	Not available	Not available
Fish	20.0	19.2	96.0	5.9
Fish	50.0	46.7	93.4	4.4

of Chabahar Bay was carried out. For examination of modified MDLLME coupled Vis spectrophotometry for seawater samples, these samples spiked by 20 and 50 µg/L of BG, and the observations are shown in Table 3. A relative standard deviation (n= 3) better than 6.1 % and recoveries between 91.5 and 99.8 % were achieved for the determination of BG in the real sample.

Determination of BG in fish

To evaluate the use of the modified DLLME method; it was applied for the determination of BG in *Sphyraena jello* (fish) sample.

The extraction of BG of fish muscle was performed using the standard of EPA 3550C with a few changes [25]. In this method, 1.0 g of the dried fish muscle sample was spiked to 5.0 mL of methanol. By sonication for 25.0 min in an ultrasonic bath, the mixture was shaken. The mixture was centrifuged for 15.0 min at 3500.0 rpm and after that, the supernatant was decanted in to 15.0 mL of the conical glass sample tube (not detected BG) and preserved for the following step.

In order to evaluate the applicability of the proposed MDLLME for various concentrations, these samples were spiked with different levels of BG (Table 3). Good recoveries 93.4 and 96.0 % were obtained. A relative standard deviation (n=3) better than 5.9 % was observed for the determination of BG in fish samples.

The obtained observations of the determination of BG in different real samples including seawater and fish samples proved that the modified DLLME can be successfully applied for preconcentration of the analyte in the complicated matrix.

CONCLUSIONS

In the paper, modified DLLME combined with Vis spectrophotometry was optimized and employed for the extraction and determination of Brilliant Green (BG) from different real samples including fish and seawater samples. The result showed that the method was efficient. The technique is faster, inexpensive, and easier to operate. Good recoveries for the spiked concentration of BG in the different real samples were achieved. Also, good linearity with a suitable enrichment factor was obtained.

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REFERENCES

[1] Hashemi S.H., Kaykhaii M., Keikha A.J., Sajjadi Z., Application of Box-Behnken Design In Response Surface Methodology for the Molecularly Imprinted Polymer Pipette-Tip Solid Phase Extraction of Methyl Red from Seawater Samples and Its Determination by Spectrophotometry, *Marine Poll. Bull*., **137:** 306-314 (2018).

[2] Hashemi S.H., Kaykhaii M., Keikha A.J., Parkaz A., Application of Response Surface Methodology to Optimize Pipette Tip Micro-Solid Phase Extraction of Dyes from Seawater by Molecularly Imprinted Polymer and Their Determination by HPLC, *J. Iran Chem. Soc*., **16:** 2613-2627 (2019).

[3] Hashemi S.H., Kaykhaii M., Keikha A.J., Mirmoradzehi E., Box–Behnken Design Optimization of Pipette Tip Solid Phase Extraction For Methyl Orange and Acid Red Determination By Spectrophotometry in Seawater Samples Using Graphite Based Magnetic $NiFe_2O_4$ Decorated Exfoliated as Sorbent, *Spectrochim. Acta A Mol. Biomol. Spectrosc*., **213:** 218-227 (2019).

[4] Mohammad Zadeh Kakhki R., Heydari S., A Simple Conductometric Method for Trace Level Determination of Brilliant Green in Water Based on β-Cyclodextrin and Silver Nitrate and Determination of Their Thermodynamic Parameters, *Arab. J. Chem*., **7:** 1086-1090 (2014).

[5] Mariah G.K., Pak K.S., Removal of Brilliant Green Dye from Aqueous Solution by Electrocoagulation Using Response Surface Methodology, *Materials Today: Proceedings*, **20:** 488-492 (2020).

[6] Sadeghi S., Nasehi Z., Simultaneous Determination of Brilliant Green and Crystal Violet Dyes in Fish and Water Samples with Dispersive Liquid-Liquid Micro-Extraction Using Ionic Liquid Followed by Zero Crossing First Derivative Spectrophotometric Analysis Method, *Spectrochim. Acta A Mol. Biomol. Spectrosc*., **201:** 134-142 (2018).

[7] Yi H., Qu W., Huang W., Electrochemical Determination of Malachite Green Using a Multiwall Carbon Nanotube Modified Glassy Carbon Electrode, *Microchim. Acta*, **160:** 291–296 (2008).

[8] Huang B., Zhou X., Chen J., Wu G., Lu X., Determination of Malachite Green in Fish Based on Magnetic Molecularly Imprinted Polymer Extraction Followed by Electrochemiluminescence, *Talanta*, **142:** 228–234 (2015).

[9] Andersen W.C., Turnipseed S.B., Karbiwnyk C.M., Lee R.H., Clark S.B., Rowe W.D., Madson M.R., Miller K.E., Multiresidue Method for the Triphenylmethane Dyes in Fish: Malachite Green, Crystal (Gentian) Violet, and Brilliant Green, *Anal. Chim. Acta*, **637:** 279-289 (2009).

[10] Shen Y.D., Deng X.F., Xu Z.L., Wang Y., Lei H.T., Wang H., Yang J.Y., Xiao Z.L., Sun Y.M., Simultaneous Determination of Malachite Green, Brilliant Green and Crystal Violet in Grass Carp Tissues by a Broad-Specificity Indirect Competitive Enzyme-Linked Immunosorbent Assay, *Anal. Chim. Acta*, **707:** 148-154 (2011).

[11] Es'haghi Z., Ahmadi-Kalateh Khooni M., Heidari T., Determination of Brilliant Green from Fish Pond Water Using Carbon Nanotube Assisted Pseudo-Stir Bar Solid/Liquid Microextraction Combined with UV–Vis Spectroscopy–Diode Array Detection, *Spectrochim. Acta A Mol. Biomol. Spectrosc*., **79:** 603-607 (2011).

[12] Rezaee M., Assadi Y., Hosseini M.M., Aghaee E., Ahmadi F., Berijani S., Determination of Organic Compounds in Water Using Dispersive Liquid-Liquid Microextraction, *J. Chromatogr. A*, **1116:** 1-9 (2006).

[13] Mirmoghaddam M., Kaykhaii M., Hashemi M., Keikha A.J., Hashemi S.H., Yahyavi H., Application of Response Surface Modeling for Optimization and Determination of Malondiaaldehyde by Vortex-Assisted Dispersive Liquid-Liquid Microextraction and GC-FID, *J. Chil. Chem. Soc*., **64:** 4531-4537 (2019).

[14] Nassiri M., Kaykhaii M., Hashemi S.H., Sepahi M., Spectrophotometric Determination of Formaldehyde in Seawater Samples After in-situ Derivatization and Dispersive Liquid-Liquid Microextraction, *Iran J. Chem. Chem. Eng. (IJCCE)*, **37:** 89-97 (2018).

[15] Ziyaadini M., Hashemi S.H., Zahedi M.M., Bashande S., Application of Response Surface Methodology to Optimise Vortex-Assisted Dispersive Liquid-Liquid Microextraction Combined with HPLC for Determination of Naproxen in Chabahar Bay, *Int. J. Environ. Anal. Chem*., (2019) in press.

[16] Berijani S., Ahmadi G., Ultrasound Assisted Surfactant Enhanced Emulsification Microextraction and Spectrofluorimetry for Determination of Oxadiazon in Agricultural Water Samples, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **33**: 41-49 (2014).

[17] Chamsaz M., Hossein-Poor-Zaryab M., Arab-Zavar M.H., Darroudi A., Dispersive Liquid-Liquid Microextraction Based on Solidification of Floating Organic Drop Combined with Flame Atomic Absorption Spectrometry for Preconcentration and Determination of Thallium (III) in Water Samples, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **33**: 59-66 (2014).

[18] Soleiman B., Zakerian R., Ionic Liquid Based Dispersive Liquid Liquid Microextraction and Enhanced Determination of the Palladium in Water, Soil and Vegetable Samples by FAAS, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **33:** 51-58 (2014).

[19] Gharanjik R., Nassiri M., Hashemi S.H., Spectrophotometric Determination of Copper and Nickel in Marine Brown Algae after Preconcentration with Surfactant Assisted Dispersive Liquid-Liquid Microextraction, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **39:** 117-125 (2020).

[20] Khajeh M., Golzary A.R., Synthesis of Zinc Oxide Nanoparticles–Chitosan for Extraction of Methyl Orange from Water Samples: Cuckoo Optimization Algorithm–Artificial Neural Network, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **131:** 189-194 (2014).

[21] Hashemi S.H., Kaykhaii M., Keikha A.J., Parkaz A., Application of Box–Behnken Design in the Optimization of a Simple Graphene Oxide/Zinc Oxide Nanocomposite-Based Pipette Tip Micro-Solid Phase Extraction for the Determination of Rhodamine B and Malachite Green in Seawater Samples by Spectrophotometry, *Anal. Methods*, **10:** 5707-5714 (2018).

[22] Hashemi S.H., Keykha F., Application of the Response Surface Methodology in the Optimization of Modified Molecularly Imprinted Polymer Based Pipette-Tip Micro-Solid Phase Extraction for Spectrophotometric Determination of Nicotine in Seawater and Human Plasma, *Anal. Methods*, **11:** 5405-5412 (2019).

[23] Hashemi S.H., Keykha F., Spectrophotometric Determination of Nalidixic Acid in Some Real Samples After Preconcentration with GO/ZnO Nanocomposite-Based Pipette Tip Micro-Solid Phase Microextraction, *Iran J. Anal. Chem*., **7:** 21-29 (2020).

[24] Tavallali H., Ostovar M., Trace Spectrophotometric Determination of Brilliant Green in Fish Farming Water Samples, *Int. J. Chem. Tech. Res*., **1:** 199-203 (2009).

[25] Ziyaadini, M., Zahedi, M.M., Khosravinia, S., Ultrasound-Assisted Extraction Combined with Reverse Phase-Dispersive Liquid-Liquid Micro Extraction as a New Approach for Preconcentration and Spectrophotometric Determination of Total Phenol in Marine Sediments of Chabahar Bay, *Marine Poll. Bull*., **109:** 104–109 (2016).