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ChemTech
International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.1, No.2, pp 199-203 , April-June 2009

Trace spectrophotometric determination of brilliant green in fish farming water samples

Hossein Tavallali*, Mastaneh Ostovar

Payame Noor University PNU, Shiraz, 71365-944, Iran
**Corresponding Author: Tel: +98 917 3153520, Fax: +98 711 2292889*
Email: tavallali@pnu.ac.ir,tavallali@yahoo.com

Abstract: A micelle-mediated phase separation method for preconcentration of Brilliant Green (BG) by using spectrophotometer has been developed. The method is based on the cloud point extraction of BG in pH 3.5 mediated by micelles of nonionic surfactant Triton X-100. The extracted surfactant–rich phase was diluted with ethanol and its absorbance was measured at λ_{max} (628 nm). The effect of different variables were evaluated and optimized to enhance the sensitivity and extraction efficiency of the proposed method. The calibration graph was linear in the rang of 0.050-2.0 μg ml^{-1} of BG in the initial solution with r=0.9993. Detection limit based on three times the standard deviation of the blank was 0.015 μg ml^{-1} and the relative standard deviation for 0.10 and 1.0 μg ml^{-1} of BG were 2.7 and 1.8 % respectively. The method was applied to determine of BG in different fish farming and water samples which gave satisfactory results.

Keywords: Brilliant green determination, Cloud point extraction, Fish farming waters

1. Introduction

Brilliant green is a triphenylmethane dye that has been used in fish farming industry during many decades because of its broad anti-microbial, anti-parasitic and anti-fungal spectrum, its high efficiency in the prevention and treatment of certain fish disease and its low cost. It has been found that dyes of this family (like malachite green) can induce reproductive abnormalities in fish[1] and hepatic and renal tumors in mice[2] .Due to its carcinogenic properties, BG is not authorized by the European Union[3] and the US Food and Drug Administration (FDA). BG is often illegally used in the fish farming industry due to the low cost, easy availability, high efficiency against fungus, bacteria and parasite.

BG dye is toxic and has mutagenic and carcinogenic effects that affect aquatic biota and humans[4-8] . It causes especially eye burns, which may be responsible for permanent injury to the eyes of humans and animals. Oral consumption of the dye can cause irritation to the gastrointestinal tract and symptoms like nausea, vomiting, and diarrhea and BG may also cause irritation with redness and pain, when it comes in contact with the skin.

It is also use extensively in textile dying[9-11] and for the production of cover paper in the paper industry. About 0.8-1.0 Kg of BG is consumed per tone paper produced[12] .The use of BG has caused environmental contamination and affected human health. Therefore, developing sensitive and simple method for the determination of BG is of great importance and interest in chemical analysis.

Such this work has been done for determination of trace amounts of malachite green by spectrophotometry method[13] . But for trace amounts determination of BG hasn't worked such the present study. In the present work, a simple and sensitive cloud point extraction method introduced for the determination of BG. The method is based on the extraction of BG mediated by micelles of nonionic surfactant Triton X-100 in the presence of benzoic acid, dissolving the surfactant-rich phase in ethanol and then detected by spectrophotometer. The method was applied successfully to determine of BG in fish farming and many other water samples.

2. Experimental

2.1. Apparatus

UV-VIS absorbance spectra were recorded on a PERKIN ELMER lambda 2 UV-Vis spectrophotometer using 1cm glass cells. A JENWAY pH meter (model 3510) with a combined glass electrode was used for pH measurements. A thermostated bath (FATER WATER BATH, Model W610 B) was used for CPE experiments. Centrifuge instrument (model SHIMIFAN) with 12 ml calibrated centrifuge tubes was used to accelerate the phase separation process.

2.2. Reagents

All chemical reagents used were of analytical reagent grade, and doubly distilled water was used for the experiments. All chemicals were purchased from Merck chemicals. Brilliant green stock solution (1000 μg ml^{-1}) was prepared by dissolving 0.1 gr of the reagent in water and diluting to 100 ml in a volumetric flask. A universal buffer pH 3.5 was prepared from boric acid/citric acid/phosphoric acid (0.04 M of each). The final pH was adjusted by the addition of 0.2 M sodium hydroxide. Triton X-100 stock solution 0.1 M was prepared by dissolving 6.47 gr in water and diluting to 100 ml in a volumetric flask. A solution of benzoic acid, 1 M was

prepared by dissolving 12.20 gr of benzoic acid in ethanol and diluting to 100 ml in a volumetric flask.

2.3. Procedures

For the cloud point extraction, an aliquot of 5 ml of a solution containing, 1 ml of BG (0.050-2.0 μg ml^{-1}), 0.7 ml of universal buffer solution pH 3.5, 1.25 ml of 0.1 M of Triton X-100 and 0.5 ml of 1 M benzoic acid were added to a 5 ml volumetric flask and diluted with distilled water till 5 ml. The resultant solution was kept for 10 min in the thermostatic bath at 45 °C. Separation of two phases was achieved by centrifugation for 10 min at 4000 rpm. To separate the phases completely the solution was cooled in an ice bath for 5 min, and the aqueous phase was easily decanted by simply inverting the tube. The surfactant-rich phase was diluted with 2 ml of ethanol and transferred into a 1cm glass cell to measure its absorbance at λ_{max} (628 nm).

3. Results and discussion

The spectrophotometric determination of BG by simple and sensitive cloud point extraction method was achieved. In cloud point extraction it is important to choose the correct surfactant. If the cloud point temperature (CPT) of the surfactant is too low, the phase separation is easy but the concentration efficiency is low because of the low solubility in aqueous solution[14] . At present, there are many methods of changing the CPT surfactant system, as adding an electrolyte such as chloride, sulfate, phenols, benzoic acid, and etc. It is observed that addition of benzoic acid decrease the cloud point temperature. All measurements were carried out at λ max because at recorded absorption spectra of BG after cloud point extraction with Triton X-100 maximum absorption band at this wavelength were observed. To increase performance of the method, the reagent concentration and reaction conditions must be optimized. Various experimental parameters were studied to obtain an optimized system.

3.1. Effect of pH

The pH is an important analytical parameter because, it has an effect on the BG spectral characteristics. The effect of the pH on the system absorbance was studied in the range 1.0 - 8.5. The pH of the solution was adjusted by dilute NaOH and HCl solution using pH meter. The results are shown in Fig. 1 that shows the maximum absorbance is observed at pH 3.5. At higher pH values BG becomes colorless and the absorbance decreases. Therefore pH 3.5 was selected for further work. Different buffer systems with pH 3.5 such as universal buffer and trichloroacetic acid / acetate were examined and universal buffer was selected because it dose not change the maximum wavelength of the solution after cloud point extraction. The 0.7 ml (optimized volume) of universal buffer pH 3.5 was added to the sample solutions to maintain the pH at 3.5.

3.2. Effect of nonionic surfactant Triton X-100 concentration

The effect of non ionic surfactants types such as Triton X-100 and Triton X-114 were considered, and it was found, the Triton X-100 gave the best recovery. The concentration of surfactant that is used in CPE is critical factor and the effect of Triton X-100 concentration was studied in the range of 0.01-0.05 mol L^{-1}. As Fig. 2 shows, the absorbance of the BG in surfactant-rich phase at 628 nm increase by increasing the Triton X-100 concentration up to 0.025 mol L^{-1} and remained nearly constant at higher concentration. A 0.025 mol L^{-1} surfactant concentration was selected for other experiments.

3.3. Effect of benzoic acid concentration

Studies of the effects of benzoic acid on the cloud point behavior of nonionic surfactants was showed, the CPT of the mixed system was decreased as the amount of Benzoic acid solution increased and also the extraction efficiency was increased. Based on discussion, the effect of benzoic acid on the cloud point behavior of the system was investigated and therefore benzoic acid was found to affect the recovery of extraction. Therefore, its effect in the concentration range of 0.02-0.14 mol L^{-1} was studied. As it is shown in Fig. 3 the absorption was increased up to benzoic acid concentration 0.1 mol L^{-1} and remained constant above that. Thus 0.1 mol L^{-1} of benzoic acid was selected as optimum.

3.4. Effect of temperature

The effects of incubation time and equilibration temperature in cloud point extraction were investigated, because they are necessary to complete extraction and to achieve easy phase separation. Therefore the effect of equilibration temperature and incubation time was studied in the range of 30-60 °C and 10-20 min, respectively. As can be seen in Fig. 4, the maximum quantitative extraction was obtained at 45 °C and equilibration time of 10 min.

3.5. Effect of others experimental parameters

The effect of centrifugation rate and time also was investigated on extraction efficiency with in the range of 3000-6000 rpm and 5-30 min respectively. A centrifuge time of 10 min at 4000 rpm was selected for the entire procedure. The CPE optimized parameters were shown in Table 1.

Table 1: CPE peractical parameters

Equilibration temperature	**45 °C**
Equilibration time (before and after centrifugation)	10 min
Centrifugation time	10 min
Centrifugation (rpm)	4000
Experiment pH	3.5
Surfactant	0.025 M
Benzoic Acid	0.100 M

3.6. Analytical performance

From measurements made under the optimum conditions described above, the calibration graph was linear in the range 0.050-2.0 μg ml^{-1}. The equation for the line is A = 0.4428 C_{BG} + 0.0302 with regression coefficient (r = 0.9993, n = 5) where A is the absorbance at 628 nm and CBG is the concentration of BG in μg ml^{-1}. The limit of detection based on three time of standard deviation of the blank (3Sb/m) [15] was 0.015 μg ml^{-1} and the relative standard deviation (R.S.D.) for 0.10 and 1.0 μg ml^{-1} of BG was 2.7 and 1.8 % (n = 5) respectively.

3.7. Interference studies

Performing the procedure in the presence of foreign ions mostly those present in water samples validated the selectivity of the cloud point extraction for BG. Ions were considered as interfere, when it caused a variation in the absorbance of the sample greater than ±5% in the determination of BG by the proposed method. Solution containing 0.10 μg ml^{-1} of BG and various amounts of foreign ions were prepared and the general procedure was followed. As the results show in Table 2 large excess amounts, of common cations and anions do not interfere on the determination of trace quantities of BG. Thus can be seen a very good selectivity is achieved.

Table 2: The effect of foreign ions on the determination of 0.10 μg ml^{-1} of BG

Foreign ions	Tolerance ratio[a]
SO_4^{2-}, Pb^{2+}, Cd^{2+}, SCN^-,Co^{2+}, Al^{3+}, Na^+, K^+, Li+, Fe2+	1000
Cu^{2+}, Zn^{2+}, Mg^{2+}, Ag^+, Mn^{2+}	700
Cr^{3+}, $CH_3CO_2^-$, Cl^-, Br^-, Ni^{2+}	500
Hg^{2+}	400

[a] Amount of foreign ion / amount of BG

Table 3. Determination of BG in water samples

Sample	BG concentration (μg ml^{-1})		%Recovery (n = 5)
	Taken	Found	
River water	-	ND	-
	0.050	0.049	98.0
	0.100	0.102	102.0
	0.150	0.149	99.3
Tap water	-	ND	-
	0.060	0.059	98.3
	0.070	0.072	102.8
	0.100	0.105	105.0
Persian Golf water	-	ND	-
	0.050	0.051	101.6
	0.100	0.098	98.2
	0.150	0.015	100.1

ND: Not Detected

Table 4. Determination of BG in fish farming samples

Sample	BG concentration (μg ml^{-1})		Recovery (n = 5)%
	Taken	Found	
Fish farming water 1	-	0.195	-
	0.100	0.298	99.3
	0.200	0.396	99.0
	0.300	0.505	101.1
Fish farming water 2	-	0.196	-
	0.100	0.291	97.0
	0.200	0.404	101.0
	0.300	0.502	100.5
Fish farming water 3	-	0.099	-
	0.100	0.201	100.7
	0.150	0.241	96.6
	0.200	0.303	101.1

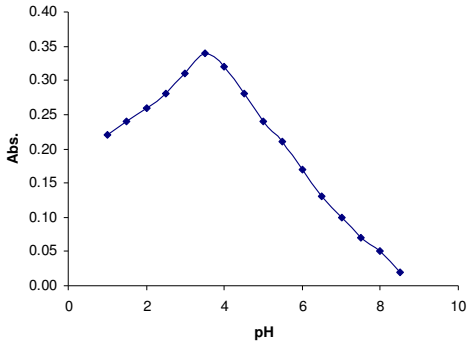

Fig.1.The effect of pH on the absorption of 0.5 μg $ml^{-1}$of BG after cloud point extraction with 0.02 mol L^{-1} of Triton X-100.

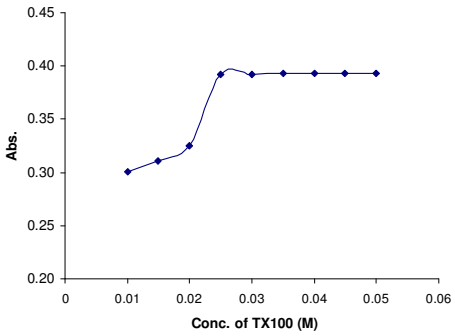

Fig.2. The influence of Triton X-100 concentration on the absorbance of 0.5 μg ml^{-1} of BG after cloud point extraction at pH 3.5.

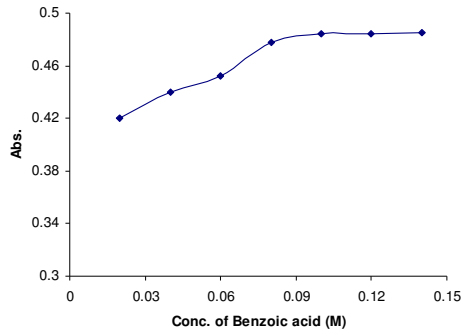

Fig.3. The effect of benzoic acid concentration on the absorption of 0.5 µg ml^{-1} of BG after cloud point extraction at pH 3.5 and 0.025 mol L^{-1} Triton X-100.

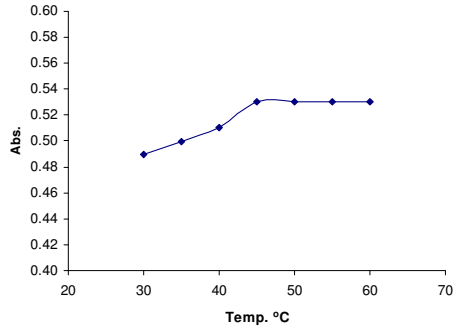

Fig.4. The effect of temperature on the absorption of 0.5 µg ml^{-1} of BG after cloud point extraction at pH 3.5, 0.025 mol L^{-1} Triton X-100 and 0.1 mol L^{-1} benzoic acid .

4. Application

In order to evaluate the analytical applicability of the proposed method, it was applied to the determination of concentration of BG in natural water and fish farming water samples. 1 ml of each of the samples was treated under proposed procedure. For calibration purposes, the working standard solutions were subjected to the same preconcentration procedure as used for the sample solutions. Spiking BG to the samples performed the validity of the procedure. The results are shown in Table 3, 4. The good agreement between the results and known values indicated the successfully applicability of the proposed method for determination of BG in environmental samples.

5. Conclusion

The cloud point extraction offers a simple, sensitive, inexpensive, and non-polluting and environmentally begins methodology which is alternative to other separation/ preconcentration technique in various water samples. Triton X-100 was chosen for the formation are the surfactant-rich phase due to its low CP temperature, high density of the surfactant-rich phase, which facilitates phase separation. The proposed CPE method gives very low LOD, good SD'S, more eco-friendly and uses nontoxic surfactant which agrees with the green chemistry principles The proposed method is successfully applied for the determination of BG in water samples.

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