

## Application of Box-Behnken Design in the Optimization of a Pipette Tip Solid Phase Extraction Using Modified Carbon Nanotubes for Spectrophotometric Determination of Malachite Green in Chabahar Bay Seawaters

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### Abstract

This paper describes trace determination of malachite green (MG) as a water pollutant dye by convenient spectrophotometry. A water-soluble hyper-branched polyamine was first prepared using the nucleophilic ring opening reaction of diepoxy and diamine monomer, which was then used for functionalization of multiwalled carbon nanotubes. This compound (named WHPA-OMCNT) was applied as a highly efficient adsorbent for the extraction of MG from seawater samples of Chabahar Bay (located in the southern east of Iran). WHPA-OMCNT was used in a pipette-tip solid phase extraction process; and for this extraction, different parameters affecting the extraction efficiency, including type and volume of eluent solvent, sample of volume, number of cycles of extraction and elution, pH of sample solution, type and amount of salt, and concentration of surfactant (triton X-114) were optimized using both one-variable-at-a-time and Box-Behnken response surface methodology techniques employing seven factors in three-levels. Under optimum conditions, the linear range of proposed method for MG was 4-250  $\mu\text{g L}^{-1}$  with a detection limit of 0.80  $\mu\text{g L}^{-1}$  and RSDs better than 6.4%.

### Keywords

Malachite Green; Box-Behnken Design; Carbon Nanotubes; Pipette Tip Solid Phase Extraction; Seawater Analysis; Chabahar Bay.

## 1. INTRODUCTION

Malachite green (MG) is a basic triphenylmethane dye with a molecular weight of 327 and chemical formula of is  $\text{C}_{23}\text{H}_{25}\text{N}_2^+$  (Fig. 1).

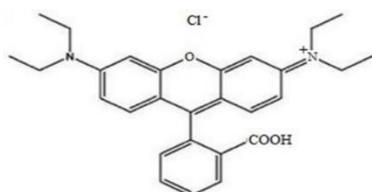


Fig. 1. Chemical structure of malachite green.

Its IUPAC name is [4-[(4-dimethylaminophenyl)-phenylmethylidene]-1 cyclohexa-2,5-dienylidene] demethylazanium. Since 1933, this compound is in use as a dye in silk, jute, wool, cotton, leather, paper and acrylic industries. MG is also applied as food coloring agent and food additive [1]. In addition, it is a biological staining agent for microscopic analysis of tissue and cell samples, as

well as direct endospores cells staining reagent. In aquaculture industries, this compound is widely in use because of its easy availability, effectiveness, inexpensiveness and less restrictive to laws [2]. Animal Health of the European Commission determined the minimum required performance limit for MG concentration as 2  $\mu\text{g Kg}^{-1}$  [3]. While MG has been used to be effective against white spot disease and ciliates and other disease in fish, fish eggs and crayfish, it may pose potential hazards to human health because it is mutagenic and carcinogenic; therefore, application of this compounds in food products has been prohibited in USA and European countries since 1983 [4-6].

As a result, determination of this compound in real samples such as seawater is of importance. The current analytical techniques for determination and detection of MG are using high performance liquid chromatography (HPLC) [7],

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gas chromatography-mass spectrometry (GC-MS) [8], liquid chromatography- mass spectrometry (LC- MS) [9], spectrophotometry [4] and capillary electrophoresis [7]. The direct determination of MG by these instruments is challenging due to the low concentration of the analyte and the severe matrix interferes in some real samples especially in seawater [10-12]. Applying a separation and preconcentration step before analysis and determination can overcome these problems [13-15]. For the extraction and preconcentration of MG, methods such as solid phase extraction (SPE) [7], magnetic solid phase extraction [9], liquid-liquid extraction (LLE), dispersive liquid-liquid microextraction (DLLME) [16-18], molecular imprinted polymer [7] and micro-cloud point extraction (CPE) [19, 20] were applied. Although each of these techniques has their own advantages, but some drawbacks still exist with them. SPE is one of the most generally applied sample pretreatment and extraction technique, but to satisfy the requirements of instrumental analysis and to handle suitable sample volumes, it requires larger quantities of sorbent and organic solvents [21]. DLLME and SPME carried out by low organic solvent consumption, but at the same time, both techniques suffer from low extraction efficiency and reproducibility; moreover, SPME fibers are expensive [13]. Pipette-tip micro-solid phase extraction (PT-SPE) has recently been used for the sample preparation and extraction of compounds of complex media [22]. The method is a miniaturized format of conventional SPE. In this technique, the analytes are micro-extracted using repeated aspiration and elution of sample solution [23]. Advantages of this method are low cost, limited consumption of organic solvents, the possibility to integrate extraction, mild extraction conditions and purification in the same step [24]. In recent years, many researches and scientists worked on the synthesis and application of carbon nanotubes (CNTs) [25, 26] for various applications. CNT has unique properties such as possessing high surface area, thermal and chemical stability and also because of its strong interaction by various molecules; particularly by those having benzene rings. These features provide CNTs excellent adsorption ability. Therefore, both functionalized and non-functionalized CNTs have been studied as sorbents for SPE alone or in conjugation with classical SPE adsorbents [27-30]. Various techniques with different agents were applied for functionalization of CNTs [31]. CNTs functionalization is simple, since it can be easily dispersed in water using either anionic, cationic or

nonionic surfactants including sodium dodecyl sulfate (SDS), sodium dodecylbenzene sulfonate (SDBS), cetyltrimethylammonium bromide (CTAB) and Triton X [32].

In analytical chemistry, multivariate experimental design methods are generally applied due to their ability to simultaneously optimize various variables; so, in comparison with traditional univariate (one-at-a-time) approaches, they are more cost effective and much faster [33]. Two levels full/fractional is one of most popular multivariate experimental design methods and every parameter of it is experimentally investigated at only two levels. Full factorial design techniques are very suitable for preliminary studies in the initial steps of an optimization; especially when the system under study contains a large number of factors [34-36]. On the other hand, the models which may be fit to these designs are relatively simple if only two levels are utilized. Among them, Box-Behnken is a second-multivariate design method based on three-level incomplete factorial designs and is usually used for investigation of critical experimental conditions, which is, maximum or minimum of response functions. The number of run (N) needed for the using of Box- Behnken design is  $N = 56 + C_0$  ( $C_0$  is replicate number of the central point) [33].

In this research, for the first time, we combined water soluble hyperbranched polyamine (WHPA) with oxidized multiwalled carbon nanotubes (OMCNT) to synthesis a novel nano sorbent (WHPA-OMCNT) which was then applied for pipette-tip micro solid phase extraction of MG from seawater of Chabahar Bay and its determination by spectrophotometry. Factors affecting extraction was optimized using Box- Behnken response surface methodology (RSM) design.

## 2. EXPERIMENTAL

### 2.1. Materials and methods

All chemicals and reagents were of analytical grade and used as received. All aqueous solution was achieved using ultra-pure MilliQ® purification system. Multiwalled carbon nanotubes (outer diameter of 5-20 nm, inner diameter of 2-6 nm, length of 1-10  $\mu\text{m}$ , purity  $\geq 95\%$ ) were obtained from Plasmachem, Germany. Poly (ethylene oxide) diglycidyl ether (PEO-DGE) (Sigma Aldrich, USA) and N-ethylethylene diamine (EEDA, Alfa Aesar, UK) were used for the preparation of modified carbon nanotubes (MCNTs). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxyl succinimide (NHS)

and ethylene glycol (EG) was received from Sinopharm Chemical Reagent Beijing Co., Ltd, China. A 150  $\mu\text{L}$  filter tips (ExtraGENE, USA) were utilized as extraction column. Moreover, for increasing the CNTs surface charge, magnesium nitrate hexahydrate (Merck KGaA, Germany) was used. Malachite green, all salt, acids and organic solvents were of analytical grade and were purchased by Merck KGaA. Triton X-114 (5% v/v) solutions was prepared in doubly distilled water. Stock solution of MG ( $500 \text{ mg L}^{-1}$ ) was prepared by dissolving 0.050 g of it in doubly distilled water in a 100 mL flask. Working solutions were prepared daily by suitable dilution of this solution.

## 2.2. Apparatus

UNICO S2100 Vis spectrophotometer (China), equipped with 100  $\mu\text{L}$  quartz microcells (model Q-01701, Stara Company, UK), at wavelength of 618 nm was used for MG detection. A model 630 Metrohm (Switzerland) pH meter was applied for determination of pH of solutions.

## 2.3. Synthesis of water soluble hyper branched polyamine

Water-soluble hyper branched polyamine (WHPA) was prepared using the nucleophilic ring opening reaction of diepoxy and diamine monomer. 0.01 mol of PEO-DGE, 0.01 mol of EEDA and 40 mL of ethanol were poured in a three-neck flask equipped by a nitrogen inlet tube and reflux condenser and stirred at room temperature for 48 h and then refluxed for an additional 24 h. Then, the concentrated mixture was precipitated in n-hexane to produce a viscous liquid followed using drying at  $45^\circ\text{C}$  for 24 h in a vacuum oven [37].

## 2.4. Synthesis of oxidized multiwalled carbon nanotubes

0.5 g multiwalled carbon nanotube was treated in a mixture of concentrated  $\text{H}_2\text{SO}_4/\text{HNO}_3$  (3:1 v/v) in an ultrasonic bath at  $40^\circ\text{C}$ . After 3 h, the solution was cooled to room temperature and then multiwalled carbon nanotubes were filtered and rinsed with distilled water until the pH of the rinsing turned nearly neutral. Finally, the archived black solid was dried at  $60^\circ\text{C}$  in vacuum [37].

## 2.5. Synthesis of WHPA functionalized OMCNT

200 mg of OMCNT and 240 mg of EDC were immersed in 500 mL of phosphate buffer saline (pH 7.4). After the achieved mixture was stirred at room temperature for 2 h, 400 mg of WHPA was added and the reaction was continued for 6 h at room temperature. Finally, the obtained product was centrifuged at 9000 rpm for 20 min and

individually two times washed by water and ethanol to remove the free WHPA polymers which were not anchored to the nanotubes. The final product, named WHPA-OMCNT, was dried at  $55^\circ\text{C}$  for 24 h under vacuum.

## 2.6. Pipette-tip extraction procedure

Preconcentration of MG was performed in an Extra GENE tip mounted on a variable 150  $\mu\text{L}$  volume pipettor (Dragon Labs, USA). 50  $\mu\text{L}$  of aqueous standard solution of MG containing 150 mg of KCl and proper volume of Triton X-114 (5% v/v) with pH 3 was withdrawn into the 2 mg of WHPA-OMCNT (sorbent) and dispensed back into the same jar. The extraction and elution (with 300  $\mu\text{L}$  of acetic acid: methanol by ratio of 2:1) repeated 7 and 5 times, respectively. Finally, the desorbed liquid was directly introduced into microcell of spectrophotometer for determination.

## 3. RESULT AND DISCUSSION

### 3.1. Measurement of absorption spectra

Visible absorption spectrum of a  $100 \mu\text{g L}^{-1}$  of MG after PT-SPE for MG using proposed technique against a reagent blanks is shown in Fig. 2. The maximum absorption wavelength was determined as 618 nm.

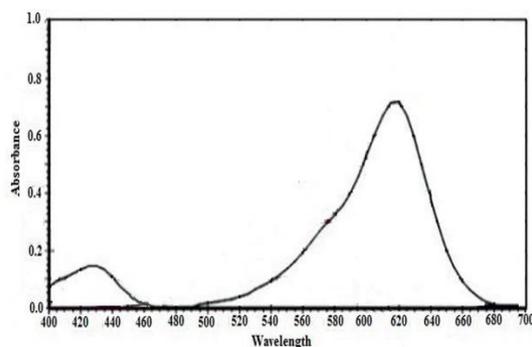


Fig. 2. Absorption spectra of  $100 \mu\text{g L}^{-1}$  of MG.

### 3.2. Optimization of PT-SPE

In order to the achieve the highest recovery of proposed method, factors affecting the extraction were investigated and optimized using both methods of one variable at a time and Box-Behnken design. A standard solution of  $100 \mu\text{g L}^{-1}$  of MG was used for this purpose. Each experiment carried out in triplicates.

#### 3.2.1. Effect of type of the desorption solvent

Various solvents were studied to elute MG from the sorbent. Ethanol, acetonitrile, methanol containing 5% acetic acid, methanol/acetic acid (1:1), methanol/acetic acid (2:1), methanol/acetic acid (1:2) acetone and HCl (0.5 M) were investigated. The results showed that methanol/acetic acid (1:2) has the best efficiency

extraction and selected for desorption of MG from of sorbent in further experiment.

### 3.2.2. Effect of amount of sorbet

The amount of sorbent for pipette tip extraction was investigated in the range of 1.0 to 2.5 mg of the modified CNT. The absorption increased to 2.0 mg and then no considerable change was achieved. So, 2.0 mg of sorbent was used for the further work.

### 3.2.3. Effect of type of salt

Normally, the solubility of organic compounds in aqueous samples decreases in traditional liquid-liquid extraction with increasing the ionic strength of solution because of salting out effect [38]. Here we tried to find the effect of addition of various

salts on the extraction efficiency; therefore, NaCl, KCl and Na<sub>2</sub>SO<sub>4</sub> were used for comparison. Among them, KCl indicated the best extraction efficiency for MG extraction and chosen as the salt of interest.

### 3.2.4. Box-Behnken design

The significant variable like pH of sample solution (X<sub>1</sub> or A), concentration of triton X- 114 (%) (X<sub>2</sub> or B), amount of KCl (mg) (X<sub>3</sub> or C), volume of solvent (X<sub>4</sub> or D), volume of sample (X<sub>5</sub> or E), the number of extraction cycles (X<sub>6</sub> or F), the number of elution (X<sub>7</sub> or G) for MG were chosen as the critical variables. In Table 1, the actual design of experiments is given.

**Table 1.** The Box-Behnken design for the optimization of the WHPA-OMCNT-PT-SPE.

A	B	C	D	E	F	G	Y <sub>observed</sub>	Y <sub>predicted</sub>	error	percent
2	0.06	150	250	10	7	5	0.354	0.344	0.010	2.756
2	0.06	150	350	10	7	5	0.332	0.333	-0.001	-0.170
2	0.08	100	300	8	7	5	0.302	0.310	-0.008	-2.556
2	0.08	100	300	12	7	5	0.284	0.298	-0.014	-5.004
2	0.08	150	300	10	5	3	0.302	0.304	-0.002	-0.602
2	0.08	150	300	10	5	7	0.312	0.314	-0.002	-0.485
2	0.08	150	300	10	9	3	0.307	0.309	-0.002	-0.607
2	0.08	150	300	10	9	7	0.329	0.327	0.002	0.599
2	0.08	200	300	8	7	5	0.356	0.349	0.007	1.924
2	0.08	200	300	12	7	5	0.332	0.325	0.007	2.242
2	0.1	150	250	10	7	5	0.342	0.339	0.003	0.975
2	0.1	150	350	10	7	5	0.323	0.323	0.000	0.080
3	0.06	100	300	10	5	5	0.501	0.501	0.000	-0.040
3	0.06	100	300	10	9	5	0.523	0.516	0.007	1.342
3	0.06	150	300	8	7	3	0.454	0.460	-0.006	-1.412
3	0.06	150	300	8	7	7	0.458	0.467	-0.009	-2.045
3	0.06	150	300	12	7	3	0.432	0.436	-0.004	-1.031
3	0.06	150	300	12	7	7	0.445	0.450	-0.005	-1.215
3	0.06	200	300	10	5	5	0.553	0.543	0.010	1.798
3	0.06	200	300	10	9	5	0.562	0.558	0.004	0.777
3	0.08	100	250	10	7	3	0.456	0.438	0.018	3.923
3	0.08	100	250	10	7	7	0.466	0.448	0.018	3.802
3	0.08	100	350	10	7	3	0.426	0.419	0.007	1.748
3	0.08	100	350	10	7	7	0.440	0.433	0.007	1.535
3	0.08	150	250	8	5	5	0.403	0.410	-0.007	-1.683
3	0.08	150	250	8	9	5	0.410	0.421	-0.011	-2.774
3	0.08	150	250	12	5	5	0.382	0.390	-0.008	-1.970
3	0.08	150	250	12	9	5	0.394	0.403	-0.009	-2.185
3	0.08	150	300	10	7	5	0.836	0.824	0.012	1.414
3	0.08	150	300	10	7	5	0.825	0.824	0.001	0.100
3	0.08	150	300	10	7	5	0.863	0.824	0.039	4.499
3	0.08	150	300	10	7	5	0.794	0.824	-0.030	-3.801
3	0.08	150	300	10	7	5	0.801	0.824	-0.023	-2.894
3	0.08	150	300	10	7	5	0.827	0.824	0.003	0.341
3	0.08	150	350	8	5	5	0.401	0.392	0.009	2.143
3	0.08	150	350	8	9	5	0.414	0.406	0.008	1.874
3	0.08	150	350	12	5	5	0.381	0.370	0.011	2.838
3	0.08	150	350	12	9	5	0.392	0.385	0.007	1.692
3	0.08	200	250	10	7	3	0.456	0.463	-0.007	-1.519
3	0.08	200	250	10	7	7	0.462	0.470	-0.008	-1.693
3	0.08	200	350	10	7	3	0.424	0.441	-0.017	-4.065

3	0.08	200	350	10	7	7	0.435	0.453	-0.018	-4.098
3	0.1	100	300	10	5	5	0.502	0.506	-0.004	-0.826
3	0.1	100	300	10	9	5	0.513	0.523	-0.010	-1.884
3	0.1	150	300	8	7	3	0.456	0.451	0.005	1.193
3	0.1	150	300	8	7	7	0.463	0.458	0.005	0.991
3	0.1	150	300	12	7	3	0.433	0.424	0.009	2.081
3	0.1	150	300	12	7	7	0.445	0.439	0.006	1.416
3	0.1	200	300	10	5	5	0.506	0.513	-0.007	-1.374
3	0.1	200	300	10	9	5	0.529	0.529	0.000	0.039
4	0.06	150	250	10	7	5	0.352	0.352	0.000	-0.077
4	0.06	150	350	10	7	5	0.333	0.336	-0.003	-0.993
4	0.08	100	300	8	7	5	0.321	0.328	-0.007	-2.306
4	0.08	100	300	12	7	5	0.309	0.315	-0.006	-2.085
4	0.08	150	300	10	5	3	0.308	0.310	-0.002	-0.621
4	0.08	150	300	10	5	7	0.312	0.310	0.002	0.600
4	0.08	150	300	10	9	3	0.322	0.320	0.002	0.477
4	0.08	150	300	10	9	7	0.331	0.329	0.002	0.573
4	0.08	200	300	8	7	5	0.355	0.339	0.016	4.373
4	0.08	200	300	12	7	5	0.322	0.314	0.008	2.445
4	0.1	150	250	10	7	5	0.344	0.343	0.001	0.167
4	0.1	150	350	10	7	5	0.314	0.323	-0.009	-2.968

In a system involving seven significant independent variable including  $X_1, X_2, X_3, X_4, X_5, X_6$  and  $X_7$  the mathematical relationship of the response on these variables can be approximated employing the quadratic (second degree) polynomial equation:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

Where  $Y$  is predicted response,  $\beta_0$  is the constant,  $X_1, X_2, X_3, X_4, X_5, X_6$  and  $X_7$  are the coded independent variables,  $\beta_i$  is the linear effect,  $\beta_{ii}$  is the quadratic effect,  $\beta_{ij}$  demonstrates the coefficient of the interaction factor,  $\varepsilon$  is the random error or allows for description or uncertainties between predicated and determined data [39].

A multiple regression analysis is done to achieve the coefficients and the equation can be applied to predict the response. The degree of experiments selection of the study was Box-Behnken for seven independent variables. It is applicable once the critical variables have been determined. A total of 62 experiments were needed to estimate of the full model.

Table 1 shows the matrix, the observed and predicated responses as analytical signals and error for MG. The following equation indicates the relationship between seven variables, i.e. pH of the sample solution (A), concentration of triton X-114 (B), concentration of KCl (C), eluent volume (D), volume of sample (E), the number of extraction cycles (F), and the number of elution cycles (G) with analytical response (Y).

$$Y = \text{Absorbance} = -9.72887 + (1.11833 \times A) + (20.90181 \times B) + (8.16572 \times 10^{-3} \times C) + (0.023270 \times D) + (0.51219 \times E) + (0.27013 \times F) + (0.21331 \times G) - (0.034065 \times A \times B) - (1.23918 \times 10^{-4} \times A \times C) - (1.78278 \times 10^{-5} \times A \times D) - (1.22972 \times 10^{-4} \times A \times E) + (6.05200 \times 10^{-4} \times A \times$$

$$F) - (1.06663 \times 10^{-3} \times A \times G) - (6.05056 \times 10^{-3} \times B \times C) - (9.51992 \times 10^{-4} \times B \times D) - (0.013770 \times C \times E) + (7.20920 \times 10^{-3} \times B \times F) + (4.50399 \times 10^{-3} \times B \times G) - (1.18874 \times 10^{-7} \times C \times D) - (2.68895 \times 10^{-5} \times C \times E) - (1.35058 \times 10^{-6} \times C \times F) - (6.47730 \times 10^{-6} \times C \times G) - (4.92920 \times 10^{-6} \times D \times E) + (4.88431 \times 10^{-6} \times D \times F) + (9.04797 \times 10^{-6} \times D \times G) + (8.77326 \times 10^{-5} \times E \times F) + (3.35841 \times 10^{-4} \times E \times G) + (4.61516 \times 10^{-4} \times F \times G) - (0.18125 \times A^2) - (123.39200 \times B^2) - (2.26585 \times 10^{-5} \times C^2) - (3.88160 \times 10^{-5} \times D^2) - (0.025579 \times E^2) - (0.019593 \times F^2) - (0.021676 \times G^2) \quad (2)$$

The critical point in the surface response are found with solving these equation systems for the condition of  $\partial(Y)/\partial(A) = 0, \partial(Y)/\partial(B) = 0, \partial(Y)/\partial(C) = 0, \partial(Y)/\partial(D) = 0, \partial(Y)/\partial(E) = 0, \partial(Y)/\partial(F) = 0, \partial(Y)/\partial(G) = 0$ . The way of calculating these critical points has been showed by Santelli et al. [40]. The summary of the analysis of variance (ANOVA) is indicated in Table 2. The calculated values for the critical point are as follows: pH (A) = 3, concentration of triton X- 114 (%) (B) = 0.08, concentration of KCl (mg) = 150, eluent volume (D) = 300  $\mu$ L, volume of sample (E) = 10, the number of extraction cycles (G) = 7, the number of elution cycles (G) = 5 for MG.

The Model F-value of 139.53 implied the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicated model terms are significant.

In this case C, D, E, F,  $A^2, B^2, C^2, D^2, E^2, F^2, G^2$  are significant model terms. Values greater than 0.1000 indicated the model terms are not significant.

The "Lack of Fit F-value" of 0.59 implies the Lack of Fit is not significant relative to the pure error. There is a 82.16% chance that a "Lack of Fit F-value" this large could occur due to noise. The "Predicated R-Squared" of 0.9710 is in reasonable agreement with the "Adj R-Squared" of 0.9876.

Applying the sum of squares (SS) values corresponding term as equation 3, the percent contribution (PC %) of each of the individual term in final model were achieved [41].

**Table 2.** ANOVA for preconcentration of MG.

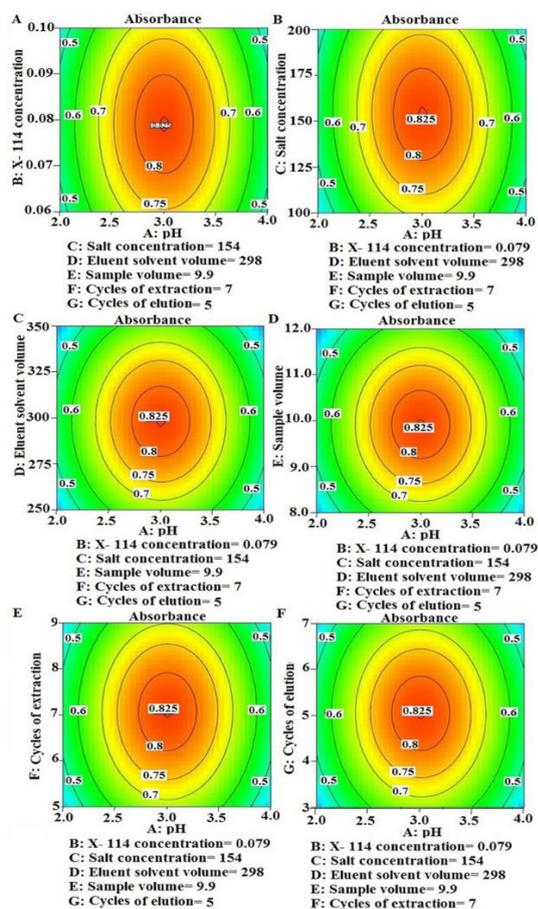
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	% PC
Model	0.61	35	0.017	139.53	< 0.0001	
A-pH	$8.02 \times 10^{-5}$	1	$8.02 \times 10^{-5}$	0.64	0.43	$8.02 \times 10^{-5}$
B-x-114 concentration	0.00039	1	0.00039	3.10	0.090	0.040
C-Salt concentration	0.0016	1	0.0016	13.20	0.0012	0.16
D-Eluent solvent volume	0.0011	1	0.00113	9.03	0.0058	0.11
E-Sample volume	0.00159	1	0.0016	12.74	0.0014	0.16
F-Cycles of extraction	0.00068	1	0.00068	5.46	0.027	0.070
G-Cycles of elution	0.00040	1	0.00040	3.18	0.086	0.041
AB	$3.71 \times 10^{-6}$	1	$3.71 \times 10^{-6}$	0.030	0.86	$3.71 \times 10^{-6}$
AC	0.00031	1	0.00031	2.45	0.13	0.032
AD	$6.36 \times 10^{-6}$	1	$6.36 \times 10^{-6}$	0.051	0.82	$6.36 \times 10^{-6}$
AE	$4.84 \times 10^{-7}$	1	$4.84 \times 10^{-7}$	0.0039	0.95	$4.84 \times 10^{-7}$
AF	$1.17 \times 10^{-5}$	1	$1.17 \times 10^{-5}$	0.094	0.76	$1.17 \times 10^{-5}$
AG	$3.64 \times 10^{-5}$	1	$3.64 \times 10^{-5}$	0.29	0.59	$3.64 \times 10^{-5}$
BC	0.00029	1	0.00029	2.34	0.14	0.030
BD	$7.25 \times 10^{-6}$	1	$7.25 \times 10^{-6}$	0.058	0.81	$7.25 \times 10^{-6}$
BE	$2.43 \times 10^{-6}$	1	$2.43 \times 10^{-6}$	0.019	0.89	$2.43 \times 10^{-6}$
BF	$6.65 \times 10^{-7}$	1	$6.65 \times 10^{-7}$	0.0053	0.94	$6.65 \times 10^{-7}$
BG	$2.6 \times 10^{-7}$	1	$2.60 \times 10^{-7}$	0.0021	0.96	$2.60 \times 10^{-7}$
CD	$7.07 \times 10^{-7}$	1	$7.07 \times 10^{-7}$	0.0056	0.94	$7.07 \times 10^{-7}$
CE	$5.78 \times 10^{-5}$	1	$5.78 \times 10^{-5}$	0.46	0.50	$5.78 \times 10^{-5}$
CF	$1.46 \times 10^{-7}$	1	$1.46 \times 10^{-7}$	0.0012	0.97	$1.46 \times 10^{-7}$
CG	$3.36 \times 10^{-6}$	1	$3.36 \times 10^{-6}$	0.0268	0.87	$3.36 \times 10^{-6}$
DE	$1.94 \times 10^{-6}$	1	$1.94 \times 10^{-6}$	0.015	0.90	$1.94 \times 10^{-6}$
DF	$1.91 \times 10^{-6}$	1	$1.91 \times 10^{-6}$	0.015	0.90	$1.91 \times 10^{-6}$
DG	$6.55 \times 10^{-6}$	1	$6.55 \times 10^{-6}$	0.052	0.82	$6.55 \times 10^{-6}$
EF	$9.85 \times 10^{-7}$	1	$9.85 \times 10^{-7}$	0.0079	0.93	$9.85 \times 10^{-7}$
EG	$1.44 \times 10^{-5}$	1	$1.44 \times 10^{-5}$	0.11	0.74	$1.44 \times 10^{-5}$
FG	$2.73 \times 10^{-5}$	1	$2.73 \times 10^{-5}$	0.22	0.64	$2.73 \times 10^{-5}$
A^2	0.44	1	0.44	3542.52	< 0.0001	45.11
B^2	0.033	1	0.033	262.69	< 0.0001	3.38
C^2	0.043	1	0.043	346.01	< 0.0001	4.41
D^2	0.13	1	0.13	1015.42	< 0.0001	13.33
E^2	0.14	1	0.14	1128.81	< 0.0001	14.35
F^2	0.083	1	0.083	662.33	< 0.0001	8.51
G^2	0.10	1	0.10	810.63	< 0.0001	10.25
Residual	0.0032	26	0.00012			
Lack of Fit	0.0023	21	0.00011	0.59	0.82	
Pure Error	0.00094	5	0.00019			

$$\% \text{ PC} = (\text{SS} / \sum \text{SS}) \times 100 \quad (3)$$

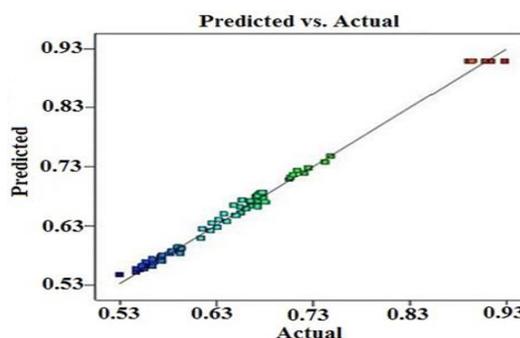
The value of adjusted  $R^2$  (0.9876) showed that 1.24 % of the total variations were not described

using the model. The value of determination ( $R^2=0.9947$ ) indicated good relation between the experimental and predicted data. The lack-of-fit

determined the failure of the model to represent values in the regression. The non-significant value of lack-of-fit ( $>0.05$ ) revealed which this model is statistically significant for the response. The "Lack of Fit F-value" determination ( $R^2=0.9079$ ) indicated good relation between the experimental and predicted data. The lack-of-fit determined the failure of the model to represent values in the regression. A high degree of precision and a good deal of the reliability of the conducted experiments were showed using a low value of the coefficient of variation ( $CV=1.71$ ) [41]. The ANOVA of the regression model showed which the quadratic model was significant, as was evident from the Fisher's  $F$  test ( $F_{\text{model}}=139.53$ ) by a very low probability value ( $P$ ) ( $P_{\text{model}} \leq 0.0001$ ) [39]. In Fig. 3 are showed two dimensional response surface as the functions of two variables at the center level of other variables. Fig. 4 indicates the correlation between the observed and predicted value extraction efficiency of MG. As can be seen, the points cluster around the diagonal line showed a good fit of the model, since the deviation between the observed and predicted values was less.



**Fig. 3.** Response surface-2D contours indicating the effect of independent variable on absorbance of MG.



**Fig. 4.** Parity plot show the correlation between the observed and predicted data.

### 3.3. Analytical performance

Percent extraction ( $E\%$ ) of MG was calculated from the following equation [13]:

$$E\% = 100 (C_A / C_B)$$

Where  $C_A$  and  $C_B$  are the concentration of MG in the solution after and before extraction, respectively.

To obtain a high enrichment factor (EF), the effect of the sample volume on the recovery was investigated in the range of 2 to 12 mL of MG. The results showed that the extraction efficiency of MG was very efficient ( $>97\%$ ) in a sample volume of 10 mL and more than that, recovery decreases. By considering the final elution of 300  $\mu\text{L}$  and the sample volume of 10 mL, EF should be 33.3. The real enrichment factor that was experimentally obtained was 32.3. Under the optimum conditions described, the calibration curve was linear ( $R^2=0.992$ ) over a concentration range of 4 to 250  $\mu\text{g L}^{-1}$  for MG, and the least square equation at this dynamic linear range was  $A=0.0067C+0.0451$ , where  $C$  and  $A$  are the concentration of MG and spectrophotometry response, respectively.

The limit of detection (LOD) of the proposed technique for the determination of MG was studied under the optimal experimental conditions. The LOD achieved from  $3S_b m^{-1}$  equation was  $0.80 \mu\text{g L}^{-1}$ , where  $S_b$  is the standard deviation of 7 consecutive measurements of the blank and  $m$  is the slope of the calibration curve. A comparison of the proposed method with other extraction techniques reported in the literatures for MG determination can be found in Table 3.

In order to assess the applicability of the technique to real samples with complicated matrices, it was applied to the extraction and separation of MG from seawater, sampled from four locations in Chabahar Bay in southern east part of Iran. To study the effect of sample media on recovery, these real samples were spiked with  $10 \mu\text{g L}^{-1}$  of MG. The recoveries are presents in Table 4. Reproducibility of method as relative standard deviation (RSD %) was in the range of 1.9-6.4%. These results show that the developed

**Table 3.** Comparison of the published techniques for MG determination with the proposed method in this research.

Extraction method	Detection method	LOD ( $\mu\text{g L}^{-1}$ )	Linear range ( $\mu\text{g L}^{-1}$ )	Ref.
Molecularly imprinted polymer	HPLC	0.17	0- 200	(Lian <i>et al.</i> , 2012)
DLLME	Spectrophotometry	3.60	15-182	(Bidari <i>et al.</i> , 2011)
Micro-cloud point	Spectrophotometry	4.10	60- 600	(Ghasemi and Kaykhaei 2016b)
Molecularly imprinted polymer	HPLC	0.11	0- 10	(Long <i>et al.</i> , 2009)
WHPA-OMCNT-PT-SPE	Spectrophotometry	0.80	5- 250	This work

**Table 4.** Recovery results for real sample obtained from different of seawater of Chabahar Bay (Iran).

Sampling location	Recovery % at spiked level of 10 ( $\mu\text{g L}^{-1}$ )	MG found	RSD (%) <sup>b</sup>
Station 1, Konarak <sup>a</sup>	-	1.3	1.1
Station 1, Konarak	97.0	11.0	2.8
Station 2, Tis <sup>a</sup>	-	1.5	2.3
Station 2, Tis	86.0	10.1	3.0
Station 3, Chabahar Maritime University <sup>a</sup>	-	1.6	3.1
Station 3, Chabahar Maritime University	9.6	11.2	6.4
Station 4, Kalantary <sup>a</sup>	-	2.7	3.4
Station 4, Kalantary	95.0	12.2	2.5

<sup>a</sup>No spiking; <sup>b</sup>RSD, relative standard deviation for seven replicate measurements.

method can be successfully used for the determination of MG in very complicated samples including seawaters.

#### 4. CONCLUSION

In the presented study, a novel nanometer-sized modified carbon nanotube was synthesized and used for PT-SPE preconcentration of MG from four stations of seawater of Chabahar Bay (located in southern east part of Iran) prior to the determination by Vis spectrophotometry. Parameters affecting extraction were optimized by Box-Behnken design. The LOD of the proposed technique was  $0.80 \mu\text{g L}^{-1}$ , which is comparable by LODs previously reported [8, 20], while wider linear dynamic and sensitivity were observed in this research in comparison to most cases. Moreover, the technique has advantages including as simplicity, repeatability, rapid separation, low cost, low consumption of organic solvents and ease of operation for the preconcentration and determination of organic dyes such as MG.

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#### REFERENCES

[1] L. Liu, F. Zhao, F. Xiao and B. Zeng, Improved voltametric response of malachite green at a multi-walled carbon nanotubes coated glassy carbon electrode in the present

of surfactant, *Int. J. Electrochem. Sci.* 4 (2009) 525-534.

- [2] O. Brandt and J.D. Hoheisel, Peptide nucleic acids on microarrays and other biosensors, *Trends Biotechnol.* 22 (2004) 617 – 622.
- [3] Y.C. Wong and T.C. Cheung, Performance assessment for determining malachite green and leuco-malachite green in swamp eel (*Monopterus albus*) muscle using assigned reference values in a proficiency test, *Food Addit. Contam.* 26 (2009) 1472-1481.
- [4] E. Sudova, J. Machova, Z. Svobodova and T. Vesely, Negative effects of malachite green and possibilities of its replacement in the treatment of fish eggs and fish: a review, *Veterinary Medicinal* 52 (2007) 527-539.
- [5] A.A. Bergwerff, R.V. Kniper and P. Scherpenisse, Persistence of residues of malachite green in juvenile eels (*Anguilla anguilla*), *Aquaculture* 233 (2004) 55-63.
- [6] Y. Jiang, P. Xie and G. Liang, Distribution and depuration of the potentially carcinogenic malachite green in tissues of three fresh water farmed Chinese fish with different food habits, *Aquaculture* 288 (2009) 1-6.
- [7] Z. Lian and J. Wang, Molecularly imprinted polymer for selective extraction of malachite green from seawater and seafood coupled with high-performance liquid chromatographic determination, *Marine Poll. Bull.* 64 (2012) 2656–2662.

- [8] A. Bidari, M.R. Ganjali and P. Norouzi, Surfactant Enhance DLLME/FO-LADS: Assay of Malachite Green level in aquatic environment of trout fish, *Clean: Soil, Air, Wat.* 39 (2011) 83–87.
- [9] K. Mitrowska, A. Posyniak and J. Zmudzki, Determination of malachite green and leucomalachite green residues in water using liquid chromatography with visible and fluorescence detection and confirmation by tandem mass spectrometry, *J. Chromatogr. A* 1207 (2008) 94–100
- [10] S.H. Hashemi and Z. Monfaredzadeh, Molecularly imprinted stir bar sorptive extraction coupled with high-performance liquid chromatography for trace analysis of diclofenac in different real samples, *Iran. J. Chem. Chem. Eng.* Articles in Press.
- [11] M. Khajeh, M. Bohlooli and H. Hashemi, Imprinted polymer particles for aluminum uptake: synthesis and analytical applications, *J. Macromol. Sci.* 46 (2009) 1-7.
- [12] M. Kaykhaii, M. Khajeh and S.H. Hashemi, Magnetic molecularly imprinted polymer nanoparticles for selective extraction of copper from aqueous solutions prior to its flame atomic absorption determination, *J. Anal. Chem.* 70 (2015) 1318-1329.
- [13] S.H. Hashemi, M. Kaykhaii and F. Tabehzar, Molecularly imprinted stir bar sorptive extraction coupled with high performance liquid chromatography for trace analysis of naphthalene sulfonates in seawater, *J. Iran Chem. Soc.* 13 (2016) 733-741.
- [14] H. Hashemi, M. Khajeh and M. Kaykhaii, Molecularly imprinted stir bar sorptive extraction coupled with atomic absorption spectrometry for trace analysis of copper in drinking water samples, *Anal. Methods* 5 (2013) 2778-2783.
- [15] S.H. Hashemi and M. Kaykhaii, Developments in methods of analysis for naphthalene sulfonates, *Crit. Rev. Anal. Chem.* 47 (2016) 127-137.
- [16] B. Xu, D. Song, Y. Wang, Y. Gao, B. Cao, H. Zhang and Y. Sun, In situ ionic-liquid-dispersive liquid-liquid microextraction of Sudan dyes from liquid samples, *J. Sep. Sci.* 37 (2014) 1967–1973.
- [17] Z. Gao, T. Liu, X. Yan, C. Sun, H. He and S. Yang, Application of ionic liquid-based microwave-assisted extraction of malachite green and crystal violet from water samples, *J. Sep. Sci.* 36 (2013) 1112–1118.
- [18] P. Biparva, E. Ranjbari and M.R. Hadjmohammadi, Application of dispersive liquid-liquid microextraction and spectrophotometric detection to the rapid determination of rhodamine 6G in industrial effluents, *Anal. Chim. Acta* 674 (2010) 206–210.
- [19] E. Ghasemi and M. Kaykhaii, Application of a novel micro-cloud point extraction for preconcentration and spectrophotometric determination of azo dyes, *J. Braz. Chem. Soc.* 27 (2016) 1521-1526.
- [20] E. Ghasemi and M. Kaykhaii, Application of micro-cloud point extraction for spectrophotometric determination of Malachite green, Crystal violet and Rhodamine B in aqueous samples, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 164 (2016) 93–97.
- [21] Y. Wen, M. Zhang, Q. Zhao and Y. Feng, Monitoring of five sulfonamide antibacterial residues in milk by in-tube solid-phase microextraction coupled to high-performance liquid chromatography, *J. Agr. Food Chem.* 53 (2005) 8468–8473.
- [22] M. Kaykhaii, H. Yahyavi, M. Hashemi and M.R. Khoshroo, A simple graphene-based pipette tip solid-phase extraction of malondialdehyde from human plasma and its determination by spectrofluorometry, *Anal. Bioanal. Chem.* 408 (2016) 4907–4915.
- [23] M.R. Rezaei-Kakhkha, M.R. Oveisi, M. Kaykhaii and B. Rezaei-Kakhkha, Determination of carbamazepine in urine and water samples using amino-functionalized metal-organic framework as sorbent, *Chem. Cent. J* 12:77 (2017) 1-12.
- [24] M.R. Rezaei-Kakhkha, S. Daliran, M.R. Oveisi, M. Kaykhaii and Z. Sepehri, The mesoporous porphyrinic zirconium metal-organic framework for pipette-tip solid-phase extraction of mercury from fish samples followed by cold vapor atomic absorption spectrometric determination, *Food Anal. Methods* 10 (2017) 2175–2184.
- [25] S. Deng, Q. Zhang, Y. Nie, H. Wei, Bin Wang, J. Huang, G. Yu and B. Xing, Sorption mechanisms of perfluorinated compounds on carbon nanotubes, *Environ. Pollut.* 168 (2014) 138–144.
- [26] J.E.N. Dolatabadi, Y. Omid and D. Losic, Carbon nanotubes as an advanced drug and gene delivery nanosystem, *Cur. Nanosci.* 7 (2011) 297–314.

- [27] V.F. Samanidou, Carbon nanotubes: application in pharmaceuticals, *Pharmaceut. Anal. Acta* 3 (2012) 1-2.
- [28] H. He, L.A. Pham-Huy, P. Dramou, D. Xiao, P. Zuo and C. Pham-Huy, Carbon nanotubes: applications in pharmacy and medicine, *Biomed. Res. Int.* 2013 (2013) 1-12.
- [29] A.H. El-Sheikh and J.A. Sweileh, Recent applications of carbon nanotubes in solid phase extraction and preconcentration: a review, *Jordan J. Chem.* 6 (2011) 1-16.
- [30] D. Xiao, P. Dramou and N. Xiong, Development of novel molecularly imprinted magnetic solid-phase extraction materials based on magnetic carbon nanotubes and their application for the determination of gatifloxacin in serum samples coupled with high performance liquid chromatography, *J. Chromatogr. A* 1274 (2013) 44-53.
- [31] G.D. Sheng, D.D. Shao, X.M. Ren, X.Q. Wang, J.X. Li, Y.X. Chen and X.K. Wang, Kinetics and thermodynamics of adsorption of ionizable aromatic compounds from aqueous solutions by as-prepared and oxidized multiwalled carbon nanotubes, *J. Hazard. Mater.* 178 (2010) 505-516.
- [32] X. Liu, M. Wang, S. Zhang and B. Pan, Application potential of carbon nanotubes in water treatment: A review, *J. Environ. Sci.* 25 (2013) 1263-1280.
- [33] D.C. Montgomery, Design and Analysis of experiments, fourth ed. Wiley, New York, (1997).
- [34] G.E.P. Box, W.G. Hunter and J.S. Hunter, Statistics for experimenters, Wiley, New York, (1997).
- [35] N. Jalbani, T.G. Kazi, M.K. Jamali, M.B. Arain, H.I. Afridi, S.T. Sheerazi and R. Ansari, Application of fractional factorial design and Doehlert matrix in the optimization of experimental variables associated with the ultrasonic-assisted acid digestion of chocolate samples for aluminum determination by atomic absorption spectrometry, *J. AOAC Int.* 90 (2007) 1682-1688.
- [36] M.B. Arain, T.G. Kazi, M.K. Jamali, N. Jalbani, H.I. Afridi, Kandhro, G.A.R. Ansari and R.A. Sarfraz, Hazardous impact of toxic metals on tobacco leaves grown in contaminated soil by ultrasonic assisted pseudo-digestion: multivariate study, *J. Hazard. Mater.* 155 (2008) 216-224.
- [37] L. Hu, Z. Yang, Y. Wang, Y. Li, D. Fan, D. Wu, Q. Wei and B. Du, Facile preparation of water soluble hyperbranched polyamine functionalized multiwalled carbon nanotubes for high-efficiency organic dye removal from aqueous solution, *Sci. Reports* 7 (2017) 3611-3618.
- [38] S.H. Hashemi, M. Kaykhaii and F. Tabezhar, Spectrophotometric determination of four naphthalene sulfonates in seawater after their molecularly imprinted stir bar sorptive extraction, *J. Chil. Chem. Soc.* 63 (2018) 4032-4038.
- [39] V. Hosseinpour, M. Kazemeini and A. Mohammadrezaee, Optimization of Ru-promoted Ir-catalyzed methanol carbonylation utilizing response surface methodology, *Appl. Catal. A* 394 (2011) 166-175.
- [40] R.E. Santelli, M.A. Bezerra, O.D. SantAna, R.J. Cassella and S.L.C. Ferreira, Multivariate technique for optimization of digestion procedure by focussed microwave system for determination of Mn, Zn and Fe in food samples using FAAS, *Talanta* 68 (2006) 1083-1088.
- [41] M. Khajeh and F. Musavi Zadeh, Response surface modeling of ultrasound-assisted dispersive liquid-liquid microextraction for determination of benzene, toluene and xylenes in water samples: Box-Behnken design, *Bull. Environ. Contam. Toxicol.* 89 (2012) 38-43.
- [42] C. Long, Z. Mai, Y. Yang, B. Zhu, X. Xu, L. Lu and X. Zou, Determination of multi-residue for malachite green, gentian violet and their metabolites in aquatic products by high-performance liquid chromatography coupled with molecularly imprinted solid-phase extraction, *J. Chromatogr. A* 1216 (2009) 2275-2281.

## استفاده از طراحی باکس-بنکن در بهینه سازی استخراج فاز جامد در سرسمپلر با جاذب نانولوله کربن اصلاح شده برای تعیین اسپکتروفتومتری مالاشیت سبز در آبهای خلیج چابهار

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### چکیده

در این کار تحقیقاتی تعیین مالاشیت سبز، که یک رنگ آلوده کننده آب میباشد، توسط اسپکتروفتومتری شرح داده شده است. ابتدا یک پلی آمین انشعاب دار محلول در آب با استفاده از واکنش باز کردن حلقه ی نوکلئوفیلی دیپوکسی و مونومر دیامین تهیه شده و برای عامل دار کردن نانوتیوب های کربن چندجداره استفاده گردید. این ترکیب (با علامت اختصاری WHPA-OMCNT) به عنوان یک جاذب کارآمد برای استخراج مالاشیت سبز از نمونه های آب دریای خلیج چابهار به کار رفت. از WHPA-OMCNT در جاذب استخراج فاز جامد سرسمپلر استفاده شد و پارامترهای مختلف موثر بر کارائی استخراج، از جمله نوع و حجم حلال شوینده، حجم نمونه، تعداد سیکل های استخراج و شویش، pH محلول نمونه، نوع و مقدار نمک و غلظت ماده فعال سطحی (تریتون X-۱۱۴) با استفاده از روش های یک متغیر در یک زمان و سطح پاسخ (باکس-بنکن) با کاربرد ۷ فاکتور در سه سطح بهینه شدند. تحت شرایط بهینه، محدوده خطی منحنی درجه بندی روش پیشنهادی برای مالاشیت سبز ۲۵۰-۴ میکروگرم بر لیتر بدست آمد و حد تشخیص روش ۰/۸۰ میکروگرم بر لیتر با تکرارپذیری (RSD) بهتر از ۶/۴٪ محاسبه گردید.

### واژه های کلیدی

مالاشیت سبز؛ طراحی باکس بنکن؛ نانولوله کربنی؛ استخراج فاز جامد در سرسمپلر؛ تجزیه آب دریا؛ خلیج چابهار.