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Development of cloud point extraction using pH-sensitive hydrogel for preconcentration and determination of malachite green

Morteza Bahram*, Foroogh Keshvari, Peyman Najafi-Moghaddam

Department of Chemistry, Faculty of Science, Urmia University, Urmia, Iran

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ABSTRACT

A novel and sensitive cloud point extraction procedure using pH-sensitive hydrogel was developed for preconcentration and spectrophotometric determination of trace amounts of malachite green (MG). In this extraction method, appropriate amounts of poly(styrene-*alt*-maleic acid), as a pH-sensitive hydrogel, and HCl were added respectively into the aqueous sample so a cloudy solution was formed. The cloudy phase consists of hydrogel particles distributed entirely into the aqueous phase. Organic or inorganic compounds having the potential to interact with polymer particles (chemical interaction or physical adsorption) could be extracted to cloudy phase. After centrifuging, these particles of hydrogel were sedimented in the bottom of sample tube. The sedimented hydrogel-rich phase was diluted with acetonitrile and its absorbance was measured at 617 nm (λ_{max} of malachite green in hydrogel). Central composite design and response surface method were applied to design the experiments and optimize the experimental parameters such as, concentration of hydrogel and HCl, extraction time and salting out effect. Under the optimum conditions, the linear range was 1×10^{-8}–5×10^{-7} mol L^{-1} malachite green with a correlation coefficient of 0.992. The limit of detection ($S/N = 3$) was 4.1×10^{-9} mol L^{-1}. Relative standard deviation (RSD) for 7 replicate determinations of 10^{-7} mol L^{-1} malachite green was 3.03%. In this work, the concentration factor of 20 was reached. Also the improvement factor of the proposed method was 23. The advantages of this method are simplicity of operation, rapidity and low cost.

1. Introduction

Generally in the analytical process especially in the extraction procedures, the main step is sample preparation and pretreatment (e.g. the extraction of an analyte of interest to an organic phase) that eventuates in the isolation and enrichment of interest from a sample matrix [1].

The main direction in recent researches is towards the development of efficient, economical, simple, rapid and clean sample preparation methods. Liquid–liquid extraction (LLE) is one of the oldest preconcentration methods in analytical chemistry [2]. This technique is time consuming and needs large amount of expensive and toxic organic solvent(s). Other methods like supercritical fluid extraction (SFE) and solid-phase extraction (SPE) are relatively expensive methods [3]. Solid-phase microextraction (SPME) [4–6] includes extraction and preconcentration of analyte(s) from aqueous samples or from headspace of the samples is also expensive and at most time the reproducibility of the results is low. In addition, the fiber which is used in SPME is fragile and has a limited lifetime [7]. Single drop microextraction (SDME) is inexpensive and very little solvent is used [8,9]. However, several disadvantages for this method have been reported; fast stirring would tend to break up the organic drop, air bubbles are formed [3]; extraction is time-consuming and equilibrium cannot be attained, even after a long time in most cases [9].

Cloud point extraction procedure is an easy, safe, rapid and inexpensive method which has been designed for the separation, purification and preconcentration of a variety of substances including metal ions and organic compounds [10–12]. This technique [13–15] is based on a property of most non-ionic surfactants in aqueous solutions, i.e., forming micelles and becoming turbid when being heated to the particular temperature (T_c). When the temperature is higher than T_c, the micellar solution will be separated into a surfactant-rich phase of a small volume and a diluted aqueous phase, in which the surfactant concentration is close to the critical micellar concentration (CMC). Depending on the surfactants property, the hydrophobic, amphiphilic or even ionic solutes can be extracted to the surfactant-rich phase. The advantage of CPE is the preferable use of water as the solvent, in comparison to other preconcentration procedures that still use toxic and flammable organic solvents. Also, the anionic surfactants which have been used as effective extracting agents in the cloud point extraction methods often require salt addition and adjustment of pH [16–18].

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* Corresponding author. Tel.: +98 441 2972143; fax: +98 2776707.
E-mail address: m.bahram@urmia.ac.ir (M. Bahram).

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Hydrogels are three-dimensional high-molecular weight networks composed of a polymer backbone, water and a crosslinking agent. They are gaining tremendous importance in a wide variety of applications in medical, pharmaceutical and related fields, e.g. wound dressings [19], contact lenses [20], artificial organs and drug delivery systems [21].

pH sensitive polymers are normally produced by adding pendant acidic or basic functional groups to the polymer backbone; these either accept or release protons in response to appropriate pH and/or ionic strength changes in aqueous media [22].

Hydrogels have been developed as stimuli-responsive materials, which can undergo abrupt volume change in response to small changes in environmental parameters: temperature, pH, ionic strength, etc. These unique characteristics of hydrogels are of great interest in drug delivery, cell encapsulation and tissue engineering [22].

Also the investigation of hydrogel polymer–surfactant interactions is a very active area of modern colloid science [22,23], and there is a considerable interest in the association between oppositely charged polymer–surfactant pairs. This association gives rise to soluble or insoluble complexes, the properties of which may be tuned by many variables, such as the hydrophobicity and the charge density of the polyion, the amount of the added components, and the amount of external salt.

The unique characteristic of hydrogels attracts a great interest in phase separation experiments. In order to use the pH-sensitive hydrogel for extraction and/or preconcentration propose, appropriate amounts of poly(styrene-*alt*-maleic acid), as a pH-sensitive hydrogel, and HCl should be added respectively into the aqueous sample so a cloudy solution was formed. The cloudy phase consists of the particles of hydrogel distributed entirely into the aqueous phase. The organic or inorganic compounds, which have potential to interact with polymer particles (or can be adsorbed or trapped by these particles) were extracted to cloudy phase of the hydrogel. After centrifuging, these particles of the hydrogel were sedimented in the bottom of sample tube. The sedimented hydrogel-rich phase was diluted and subjected to detection of the analyte of interest using a suitable detector. Malachite green (MG) is a triphenylmethane dye. Malachite green is still used as fungicides and antiseptic in aquaculture and fisheries [24]. It has also been used extensively as food coloring agent, dyeing cotton, wool, and leather. Malachite green is suspected to act as a tumor promoter and studies have shown that it is highly toxic to fresh water fish [25,26]. Therefore, it is very important to develop sensitive detection methods to detect the existence of MG in various water samples. Several methods for the determination of malachite green have been reported. These include spectrophotometric after solid phase extraction using magnetic affinity adsorbent [27], liquid chromatography with visible detection [28,29], liquid chromatography–mass spectrometry (LC–MS) [30,31], gas chromatography–mass spectrometry (GC–MS) [32] and capillary electrophoresis [33]. A high-sensitivity off-line coupled with on-line preconcentration method, cloud-point extraction (CPE)/cation-selective exhaustive injection (CSEI) and sweeping-MEKC has been reported for the analysis of malachite green. The variables that affect CPE were investigated [34]. Also simultaneous spectrophotometric determination of trace amount of malachite green and crystal violet in water samples after cloud point extraction using partial least squares regression has been reported by Lin An et al. [35].

This paper describes a novel, simple and sensitive cloud point extraction method for the determination of malachite green in water samples. The method is based on the extraction of malachite green mediated by pH-sensitive hydrogel (poly(styrene-*alt*-maleic acid)). The extracted-rich phase is dissolved in acetonitrile and measuring its absorbance by spectrophotometry at 617 nm. This polymeric hydrogel possesses ionic functional groups and can absorb or traps ionic dyes like malachite green.

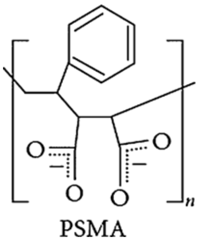

Fig. 1. Structure of poly(styrene-*alt*-maleic anhydride).

2. Experimental

2.1. Reagents and solutions

All chemicals used in the experiments were of analytical grade and were used without further purification. All solutions were prepared with distilled water. HCl, $NaNO_3$, acetonitrile and malachite green, styrene, maleic anhydride, benzoyl peroxide, tetrahydrofuran, diethyl ether, NaOH were from Merck (Darmstadt, Germany). 0.4 g hydrogel was dissolved in 100 mL distilled water to obtain 0.4% (w/v) of concentration.

Poly(styrene-*alt*-maleic acid) as an alternating copolymer (*alt*-PSMA) is a readily synthesized copolymer of styrene and maleic anhydride followed by hydrolysis to form poly(styrene-*alt*-maleic acid) that incorporates two carboxylic groups and a phenyl group in each repeating unit (Fig. 1).

In *alt*-PSMA (120,000 average MW), the hydrophobic phenyl side group, contributed by the styrene unit is directly attached to the hydrocarbon backbone. This compound derives its anionic charge from two free carboxyl groups (pK_a 1.9 and 6.0) of maleic acid which are also directly attached to the hydrocarbon backbone instead of the aromatic ring [36] and this compound is commercially available (Sigma–Aldrich, Product no. 662631).

2.2. Preparation of poly(styrene-alt-maleic acid)

Firstly, poly(styrene-*alt*-maleic anhydride) (PSMA) was prepared through a thermally initiated free-radical polymerization of styrene and maleic anhydride according to the literature method [37]. Briefly equimolar amounts (0.005 mol) of styrene (573 mL) and maleic anhydride (0.49 g) were combined in a 100 mL round bottom flask with benzoyl peroxide (BPO) and dried tetrahydrofuran (THF). The mixture was degassed by nitrogen to remove oxygen from the reaction vessel prior to polymerization. Polymerizations were carried out overnight at 80 °C by stirring under nitrogen atmosphere. The polymerization product was diluted in THF followed by drop wise addition into a 100-fold excess (v/v) of cold diethyl ether to precipitate pure PSMA which was then filtered and dried under vacuum at room temperature. In the second stage, PSMA (1 g) was subjected to base-catalyzed hydrolysis. Briefly, 1 g of PSMA was added to 20 mL of 2 $mol\,L^{-1}$ NaOH solution and stirred for 5 h at room temperature. The copolymer was recovered from NaOH solution by acid precipitation using 1 N HCl. The precipitate was washed with distilled water several times and dried under vacuum for 24 h at 60 °C.

2.3. Instruments

The spectrophotometric measurements were done with a PG mode T80 UV-Vis double-beam spectrophotometer (Japan)

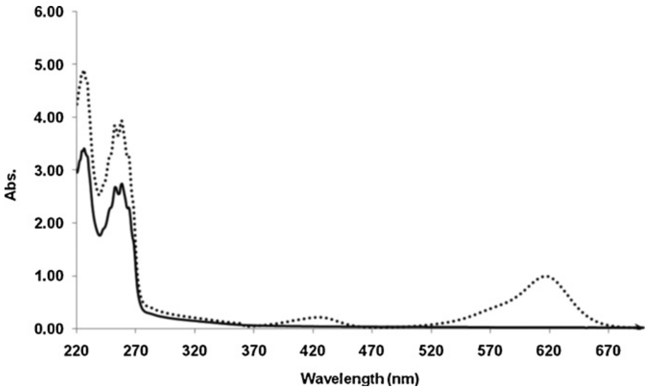

Fig. 2. The spectra of hydrogel-rich phase diluted with acetonitrile (continuum line) and hydrogel-rich phase containing malachite green (dotted line) against acetonitrile.

utilizing a 1-cm quartz cell (volume 0.5 mL). For accelerating phase separation, the Urum-Tadjihiz centrifuge (Urmia, Iran) was used.

2.4. Statistical software

Essential Regression and Experimental Design for chemists and Engineers (EREGRESS), as an MS Excel Add-in software [38,39] was used to design the experiments and to model and analyze the results.

2.5. Extraction procedure

10 mL of water sample containing an appropriate amount of MG was placed in a 15 mL falcon tube and 0.1 mL hydrogel (0.4%, w/v) was added. Then 0.5 mL HCl (0.2 mol L^{-1}) was added into the tube and was shaken gently for 5 min before centrifugation. Separation of two phases was achieved by centrifugation for 10 min at 3000 rpm. The aqueous phase was easily decanted by simply inverting the tube. The results showed that for this case, the volume of hydrogel rich phase was 0.2 mL versus 10 mL initial sample.

The sedimented phase was diluted with 0.3 mL acetonitrile and transferred into a 0.5 mL quartz cell to measure the absorbance of the solution at 617 nm against a reagent blank. The spectra of hydrogel-rich phase and hydrogel-rich phase containing extracted malachite green (both diluted with 0.3 mL acetonitrile) against acetonitrile are shown in Fig. 2.

2.6. Central composite design

There are different factors that affect the extraction process. In order to obtain the optimum conditions, the effect of different parameters such as concentration of HCl, concentration of hydrogel (HG), salting out effect and extraction time was investigated and

Table 1
The variables and values used for central composite design (CCD).

Coded factor levels						
Variable name		−1.607(low)	−1	0	1	+1.607(high)
F_1	HCl (mol L^{-1})	0.0016	0.004	0.008	0.012	0.0144
F_2	HG (%w/v)	0.0048	0.012	0.024	0.036	0.044
F_3	NaNO$_3$ (mol L^{-1})	0.002	0.020	0.050	0.080	0.098
F_4	Time (min)	1.8	3	5	7	8.2

Table 2
List of experiments in the CCD (coded values) and the response of each run.

Design points	Factors				
	F_1	F_2	F_3	F_4	Response
1	−1	1	−1	1	0.183
2	0	0	1.607	0	0.894
3	1	1	−1	1	0.814
4	0	−1.607	0	0	1.844
5	1	1	1	−1	0.95
6	−1	1	1	−1	0.352
7	0	0	0	−1.607	0.648
8$^{(cp)}$	0	0	0	0	0.938
9	1.607	0	0	0	1.034
10	1	1	1	1	0.91
11	0	1.607	0	0	0.558
12	−1	1	1	1	0.6
13$^{(cp)}$	0	0	0	0	0.926
14	1	−1	−1	−1	1.26
15	−1	−1	−1	1	0.984
16	−1	−1	−1	−1	0.77
17	1	−1	1	1	1.294
18	1	−1	−1	1	1.75
19	−1	1	−1	−1	0.213
20$^{(cp)}$	0	0	0	0	0.478
21	0	0	0	1.607	0.658
22	−1	−1	1	−1	0.816
23	0	0	−1.607	0	0.922
24$^{(cp)}$	0	0	0	0	1.148
25	−1	−1	1	1	1.508
26	1	−1	1	−1	1.16
27	−1.607	0	0	0	0
28	1	1	−1	−1	0.636

(cp) indicates 4 repeat of the center point.

Table 3
Some characteristics of the constructed models.

Regression equation		Coefficient	Value
Response = $b_0 + b_1 \times$ HG + $b_2 \times$ HCl + $b_3 \times$ HG × HG + $b_4 \times$ time + $b_5 \times$ HCl × HCl + $b_6 \times$ HG × time + $b_7 \times$ HG × $NaNO_3$ + $b_8 \times$ HCl × $NaNO_3$		b_0	0.608
Absorbance = $b_0 + b_1 \times$ HCl + $b_2 \times$ HG + $b_3 \times$ time + $b_4 \times$ HCl × HCl + $b_5 \times$ HCl × $NaNO_3$ + $b_6 \times$ HG × HG + $b_7 \times$ HG × $NaNO_3$ + $b_8 \times$ HG × time			
|R|	0.928	b_1	193.65
R^2	0.862	b_2	−74.85
R^2 adjusted	0.803	b_3	0.118
Standard error	0.09637	b_4	−6834.9
Points no.	28	b_5	−502.27
PRESS	0.34	b_6	1079.9
R^2 for prediction	0.732	b_7	218.69
		b_8	−3.057

Table 4
Optimum conditions obtained by response surface modelling.

Variable name		Optimum values	Selected values
F_1	HCl (mol L^{-1})	0.007–0.012	0.01
F_2	HG (%w/v)	0.004–0.008	0.004
F_3	($NaNO_3$/mol L^{-1})	Low effect	0
F_4	Time (min)	5–7 (low effect, depends on HCl)	5

the optimum conditions were selected. Four independent variables, namely the concentration of HCl (F_1), HG (F_2), $NaNO_3$ (F_3) and time of shaking (F_4) were studied at five levels with four repeats at the central point. Preliminary studies showed that the poly(styrene-*alt*-maleic acid) solution becomes cloudy at acidic pH, therefore instead of pH optimization; the effect of concentration of HCl on the preconcentration procedure was studied. Circumscribed CCD was used to design the experiments. For each of the four studied variables, high coded values and low-coded values were selected as +1.607 and −1.607, respectively to construct an orthogonal design (Table 1). Also, the coded values of designed experiments based on CCD methodology which have been achieved using EREGRESS software are represented in Table 2.

Polynomial equations and response surfaces were produced using EREGRESS. For an experimental design with four factors, the model including linear, quadratic, and cross terms can be expressed as Eq. (1).

$$\begin{aligned}\text{Response} &= b_0 + b_1 \times F_1 + b_2 \times F_2 + b_3 \times F_3 + b_4 \times F_4 + b_5 \times F_1 \\ &\times F_1 + b_6 \times F_2 \times F_2 + b_7 \times F_3 \times F_3 + b_8 \times F_4 \times F_4 + b_9 \times F_1 \times F_2 \\ &+ b_{10} \times F_1 \times F_3 + b_{11} \times F_1 \times F_4 + b_{12} \times F_2 \times F_3 + b_{13} \times F_2 \times F_4 \\ &+ b_{14} \times F_3 \times F_4 \qquad (1)\end{aligned}$$

F_1–F_4 are the variable parameters, and b_0–b_{14} are the coefficient values obtained through multiple linear regressions. The response surface plots were obtained through statistical process that describes the design and the modelled CCD data. Response surface method graphically illustrates the relationships between couple of parameters and the response(s) and is the way to obtain an exact optimum [38,39].

In order to show the fitness of the model, the squared regression coefficient (R^2) is used. However, the adjusted regression coefficient (R^2_{adj}) and the prediction regression coefficient (R^2_{pred}) are better criteria than absolute regression coefficient (R^2). Since regression coefficient (R^2) always decreases when a regression variable is eliminated from the model in statistical modelling, the (R^2_{adj}), which takes the number of regression variables into account, is usually selected [36,37]. In addition, R^2_{pred}, which indicates the predictive power of the model, is chosen for the same reason. This parameter was approximated using prediction error sum of squares (PRESS) that is calculated from residuals. Therefore, the regression coefficient (R^2), adjusted regression coefficient (R^2_{adj}), and R^2 for prediction (R^2_{pred}) altogether are very convenient to get a quick impression of the overall fit and the prediction power of a constructed model.

3. Result and discussion

3.1. Experimental design

The aims of this CCD strategy were: (i) study of the effect of concentration of hydrogel, concentration of HCl, extraction time and salting out effect on the preconcentration and determination of

Table 5
Analytical characteristics of the proposed method.

Regression equation[a]	Abs = 2×10^6C–0.008
R^2[b]	0.992
Linear range (mol L^{-1})	1×10^{-8}–5×10^{-7}
LOD (mol L^{-1}) [d]	4.1×10^{-9}
Repeatability (RSD)[c]	3.03
Regression equation before extraction[a]	Abs = 86883X + 0.0146
Concentration factor	20
Improvement factor[e]	23.02

[a] Concentration of malachite green in mol L^{-1}.
[b] Squared regression coefficient.
[c] Relative standard deviation for 7 replicate determination of 10^{-7} mol L^{-1} malachite green.
[d] Limit of detection for $S/N = 3$.
[e] The ratio of the slope of the calibration graph for the pH-sensitized hydrogel extraction method to that of the slope of the calibration graph without preconcentration.

Table 6
Preconcentration and spectrophotometric determination of malachite green in the spiked water samples by the proposed method.

Sample	Added (mol L^{-1})	Found (mol L^{-1})	Recovery (%)
Tap water 1 (Urmia, Iran)	2×10^{-7}	1.88×10^{-7}	94
	6×10^{-7}	5.80×10^{-7}	96
Tap water 2 (Nazlou, Urmia-Iran)	2×10^{-7}	1.09×10^{-7}	95
	6×10^{-7}	5.90×10^{-7}	98

malachite green using pH-sensitive extraction; (ii) identification of the variables that have a higher impact on the extraction; (iii) giving an insight on the robustness of the method close to the optimum conditions; and (iv) eventually showing the possible interactions between the variables.

In order to find the important factors and build a model to optimize the procedure, a full quadratic model including all terms of Eq. (1) was constructed at first step. Then, in order to obtain a simple and yet a realistic model, the insignificant terms were eliminated from the model through '*backward elimination*' process. By the elimination of insignificant terms of Eq. (1) from the constructed model, calibration R^2 decreased to 0.862 but adjusted R^2 (R^2_{adj}), and R^2 of prediction (R^2_{pred}) increased to 0.803 and 0.732, respectively. The characteristics of the abstracted model including R^2 values, PRESS, standard error and significant linear, quadratic and interaction coefficients are shown in Table 3.

3.2. *Response surface method and selection of the optimum conditions*

In order to gain insight about the effect of each variable, the three dimensional (3D) plots for the predicted responses were formed based on the model function. Some of the response surface plots are represented in Fig. 3, which show the 3D plots of absorbance of samples (617 nm) versus pairs of variables while 2 other variables were kept in center levels. As shown in Fig. 3, there was a non-linear relation between the response and the variables F_1–F_4, because the surface plots of the response are curvature. From the coefficients represented in Table 3, it is clear that several linear, squared and interaction parameters are statistically significant. Using the response surfaces the optimum conditions are reached and represented in Table 4.

From the constructed models (the results of Table 3 and the response surfaces of Fig. 3), the following results were concluded: HCl (F_1), HG (F_2) affect significantly the proposed preconcentration method both by linear and quadratic terms. Also the response surface showed that there are interactions between hydrogel concentration and $NaNO_3$ concentration, hydrogel concentration and time of extraction and also HCl and $NaNO_3$ concentrations. In addition, Fig. 3 shows that the effect of $NaNO_3$ is negligible. However, it is clear from the results that in low concentration of HCl, the extraction efficiency is affected by salting out term.

3.3. *Analytical characteristics*

Table 5 summarizes the analytical characteristics of the optimized method, including regression equation, linear range, limit of detection, reproducibility, preconcentration, and improvement factors. The limit of detection, defined as $C_L = 3S_B/m$ (where S_B and m are standard deviation of the blank and slope of the calibration graph, respectively) was 4.1×10^{-9}. Because the amount of malachite green in 10 mL of initial sample solution is measured after preconcentration by pH-sensitive hydrogel extraction in a final volume of 0.5 mL (0.2 mL hydrogel rich-phase + 0.3 mL acetonitrile), the solution is concentrated by a factor of 20. The improvement factor, defined as the ratio of the slope of the calibration graph for the pH-sensitive hydrogel extraction method to the slope of the calibration graph in the presence of hydrogel without preconcentration was 23.02 (for this case, in order to avoid the samples to be clouded, the pH of the solutions was adjusted above 4.).

3.4. *Application of the proposed method*

The proposed method was applied to the determination of malachite green in several spiked water samples. The results are

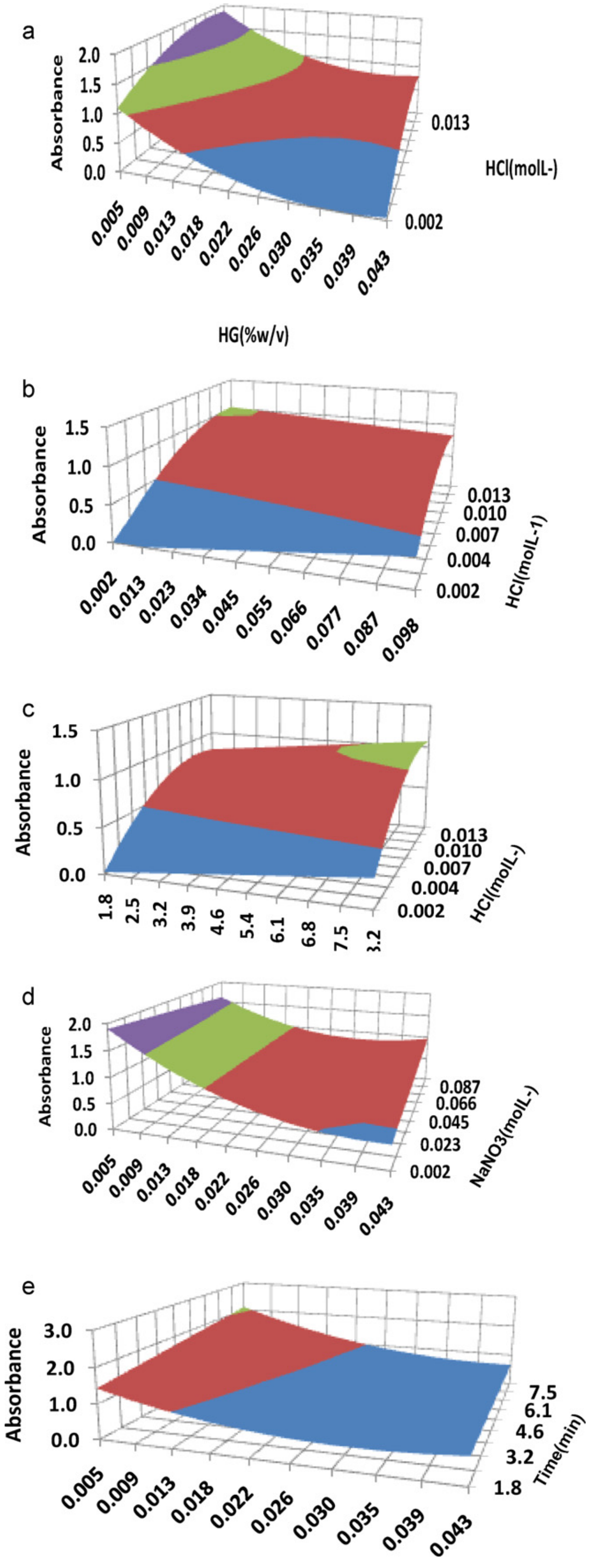

Fig. 3. Response surface plots.

Table 7
Comparison of some analytical characteristics of the proposed methods with those of previously reported.

Method	Analyzed sample	Linear range	LOD	Ref.
Simultaneous spectrophotometric determination of malachite green and crystal violet in water after cloud point extraction by applying PLS	Water samples	9.9–800 ng mL^{-1}	2.9 ng mL^{-1}	[35]
Surface-enhanced Raman microfluidic sensor	Malachite green in a polydimethylsiloxane microfluidic sensor	1–100 ng mL^{-1}	1–2 ng mL^{-1}	[40]
HPLC determination and MS confirmation	Residues of malachite green, gentian violet and their leuco metabolites in channel catfish muscle	0.5–16.0 ng g^{-1}	0.38 ng/g	[41]
Spectrophtometric after cloud point extraction using nonionic surfactant Triton X-100	Malachite green in fish farming water	4–500 ng mL^{-1}	1.2 ng mL^{-1}	[42]
Electrochemical determination using a multi-wall carbon nanotube modified glassy carbon electrode	Water samples	5.0×10^{-8}–8.0×10^{-6} mol L^{-1}	6.0×10^{-9} mol L^{-1}	[43]
Cloud point extraction using pH-sensitive hydrogel	Water samples	1×10^{-8}–5×10^{-7} mol L^{-1}	3×10^{-9} mol L^{-1}	This work

presented in Table 6. The recoveries are close to 100% and indicate that the proposed method was helpful for the preconcentration and determination purpose.

Also in Table 7, the analytical performance of the proposed method for extraction and determination of malachite green was compared with those of previously reported. As seen, the proposed method based on applying pH-sensitive hydrogel for preconcentration may provide an attractive alternative for determination of malachite green. The proposed extraction method in this work shows a lower or comparable detection limit with a relatively wider dynamic range than previously reported electrodes.

4. Conclusion

The newly proposed procedure gives a very simple and low-cost spectrophotometric procedure for preconcentration and determination of organic compounds that can be applied to real samples analysis. By using pH sensitive hydrogel extraction, toxic solvent extraction has been avoided. The performance of the proposed method was illustrated with the preconcentration and determination of malachite green (which is a water pollutant compound) as a case organic compound. The proposed method was successfully applied to the determination of malachite green in several spiked water samples.

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