Simultaneous Spectrophotometric Quantification of Crystal Violet and Malachite Green in Aqueous Samples: Combination of Multivariate Calibration Method and Solid Phase Extraction Based on Sodium Dodecyl Sulfate (SDS) Grafted Chitosan Nano-composite

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A simple solid phase extraction (SPE) based on sodium dodecyl sulfate grafted chitosan nanocomposite was applied for simultaneous spectrophotometric determination of crystal violet (CV) and malachite green (MG) content of water samples. For the data analysis of the overlapped spectra of the two dyes, the partial least square regression (PLSR) has been utilized. The synthesized sorbent was characterized by Fourier-transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX). The results showed the SDS was successfully cross-linked with the chitosan. Various parameters affecting the simultaneous extraction of the analytes, such as pH of sample solution, sample flow rate and the volume and flow rate of eluent were optimized by central composite design and response surface methodology (RSM). Figures of merits consisting of sensitivity, analytical sensitivity, limits of detection (LOD) and limit of quantitation (LOQ) were calculated to 0.89 ng ml^{-1}, 762.07, 0.06 ng ml^{-1} and 0.20 ng ml^{-1} for CV, and 0.15 ng ml^{-1}, 128.24, 0.17 ng ml^{-1} and 0.53 ng ml^{-1} for MG. The calibration curve showed high correlation coefficients of 0.9995 and 0.9993 for CV and MG, respectively. Moreovere, preconcentration factor and extraction recovery of the analytes were computed based on the regression coeffients of PLSR acting as a multivariate net analyte signal filter.

Keywords: Partial least square, Experimental design, Solid phase extraction, Crystal violet, Malachite green

INTRODUCTION

Synthetic dyes are widely used in different industries such as aquaculture, textile, cosmetics, and dyeing and even computer industries [1]. Despite their high stability in the environment and proved toxic effects, these dyes still are utilized due to low prices and being easily available [2]. Crystal violet (CV) and Malachite Green (MG) are triphenylmethane dyes, $C_{25}H_{30}ClN_3$ and $C_{23}H_{25}ClN_2$, respectively, that because of their anti-fungal properties have of great use in the aquaculture industries especially salmonids farming [3]. Due to several environmental

difficulties such as reducing the light transmission, and impaired photosynthesis that can endanger aquatic life, they adversly affect the human life [4]. Reportedly, these dyes may accumulate in fish tissues, enter into the food chain, and cause mutagenic and carcinogenic effects on humans [5]. It requires checking the water safety of effluents containing CV and MG before discard into the environment. Therefore, the extraction and determination of these analytes are very important [6].

One of the most common techniques for determination of the dye content is spectrophotometric technique. Despitemany advantages of this approach such as availability, simplicity, and wide application scope, some analytes may be hard to measure due to overlapping their

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signals [7]. To addrees this problem, chemometrics methods that are the combination of statistics and mathematics have been introduced [8]. One of the most widely used applications of chemometrics is partial least square regression (PLSR) method. PLSR, as one of the multivariate calibration methods, identifies the latent variables of the model considering both concentration and absorbance matrices [9]. Compared to complex and expensive sample preparation procedures, PLSR using mathematical calculations can provide simple and cost-effective strategy for simultaneous determination of analytes with severly overlapped signals [10,11].

Environmental samples often have complex matrices. Determination of the trace level of analytes in these matrices requires a sample preparation step. This step can increase the sensitivity by preconcentration of the analytes. Various methods have been used for sample preparation of dyes, such as coagulation [13], walnut adsorbent [14], cloud point extraction [15], carbon adsorbent [16] and solid phase extraction (SPE) [6,12,17]. The use of solid sorbents is a widely common technique, due to its low cost, simplicity, specificity, sensitivity and reduction of organic solvent consumption [18].

Chitosan is one of the most important natural polymers due to its excellent nontoxic, biodegradable and biocompatible properties [19]. Recently, several reports for chemical modification of chitosan have been published [20-23]. Compared to many other natural polymers, chitosan has a positive charge, which makes it eligible to interact with anionic surfactants [24]. However, there is a problem for packing the chitosan into the cartridge. Chitosan has a tendency to swell in the presence of water [25]. To overcome this problem and enhance the mechanical performance of chitosan, cross-linker reagents such as formaldehyde, glutaraldehyde and epoxy compounds have been used [26].

The present study aims toward the simultaneous spectrophotometric determination of CV and MG using PLSR. A solid phase extraction setup based on sodium dodecyl sulfate grafted chitosan was used for preconcentration of the analytes. To calculate the interaction effects of important factors on the extraction efficiency, the design matrix of the experiment was drawn based on central composite design with fractional factorial design. Due to

signal overlapping of the analytes, the combination of multivariate calibration and response surface methodology was applied to simultaneous quantification of the analytes at each experimental condition. Then, the optimal conditions of analytes preconcentrations were achived based on the analysis of multiple responses. Finally, preconcentration factor and extraction recovery of the analytes were obtained simultaneously based on the the regression coeffients of PLSR known as the multivariate net analyte signal (NAS) vector [9].

EXPERIMENTAL

Materials

All reagents were in analytical grade and used without other purification. CV and MG powders were obtained from Fluka (Switzerland). The standard stock solutions of dyes were made by dissolving an appropriate amount of MG and CV powder dyes in ultra-pure water separately. The standard stock solutions were kept in a cold and dark place to avoid any decomposition. The ultra-pure water was prepared by a Youngling ultra-pure water purification system (model Aqua Max-Ultra, South Korea). Standard solutions were prepared by dilution of the stock solution. Glutaraldehyde (50% w/w) was purchased from Merck (Germany). Sodium dodecyl sulfate, chitosan with medium molecular weight and concentrated acetic acid of ultrapure grade (TraceSELECT® Ultra) were obtained from Sigma-Aldrich (USA).

Instrumentation and Software

An Agilent-8453 (Germany) UV-Vis diode-array spectrophotometer was used to record the absorption spectra with one nm spectral band pass in a 10-mm quartz cell and Agilent ChemStation software was used for data acquisition. A Metler Toledo pH meter equipped with combined Ag/AgCl electrode and its glass electrode was used for pH measurements. An ultrasonic bath, (Sonorex Super, Germany) was used during the synthesis of the sorbent. Characterization of the surface of sorbent was carried out using a scanning electron microscope (TESCAN, VEGA3 SEM, Ceska Republika) equipped with an energy dispersive spectroscopy (EDS) analyzer. An ABB Bomem (Canada) FTLA 2000 spectrometer was used to

record FT-IR spectra within the range of 400-4000 cm^{-1} using potassium bromide disks.

Statistical experimental design analysis was carried out by means of the software package, Design-Expert 11.1.0 trial version (Stat-Ease Inc., MN, USA). For the first-order multivariate calibratione, all calculations were performed in Matlab (Version 7.14), using MVC1 toolbox developed by Olivieri *et al.* that is available online (http://www.iquir-conicet.gov.ar/descargas/mvc1.zip) [27].

Preparation of the Sorbent

The sorbent was synthesized according to the previously reported methods with a little modification [26]. 2.0 g of chitosan was dissolved in 100 ml of acetic acid solution (2% v/v), and the mixture was sonicated for 2 h until an overall homogeneous solution was gained. Next, 1.5 g of SDS powder was added to the reaction flask and enough time (about 15 min) was given to it in order to well disperse. Then 15 ml of glutaraldehyde/water (50% w/w) was added to the mixture as a cross-linker and the pH was adjusted about 9.5. Then, the solution was stirred at 80 °C in the water bath for about 1 h. The precipitate was collected by centrifugation, washed with ethanol and distilled water, and dried in a vacuum oven at 50 °C.

SPE Procedure

Figure 1 represents the SPE procedure, schematically. The pH of a 150 ml solution containing certain concentrations of CV and MG was adjusted to 11 using HCl and NaOH and then the solution was introduced along the cartridge (packed with 200 mg of sorbent) using a peristaltic pump with flow rate of 6 ml min^{-1}. The analytes were entrapped onto the sorbent surface and the supernatant, limpid, was made. Then, 1.5 ml of eluent (acetic acid 5 M) was passed through the cartridge with a flow rate of 2 ml min^{-1}. After that, the cartridge was washed with ultra-pure water for 15 min with a flow rate of 1 ml min^{-1} to condition the column. A blank solution was also run under the same conditions without the addition of the analytes.

Theory

Central composit design. Design of experiment (DOE) studies the response of a process at various levels of its influencing factors to explore the real optimum conditions of the system under study. Central composite design (CCD) is a well-known methods in DOE, in which the total number of required design points (N) is as follows:

$$N = 2^f + 2f + C_p \qquad (1)$$

where f is the number of variables, $2f$ is the two level factorial design experiment, $2f$ and C_p are 'star' or 'axial' points and the number of replications in the center point, respectively.

Fractional factorial design can be used in CCD design to diminish the number of experiments.

The experimental response relationship with the factors is expressed by the following empirical second order quadratic model as in the following equatuion:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1}\sum_{j=2}^{k} \beta_{ij} X_i X_j + \varepsilon \qquad (2)$$

where Y, X_i and ε terms represent the response, factors and the residual associated with the experiments, respectively. β_0 is the intercept coefficient, β_i, β_{ii} and β_{ij} display the coefficients of the linear parameters, coefficients of the quadratic parameters and coefficients of the interaction parameters X_i and X_j, respectively. In CCD analysis, the acceptability of the model is examined by the analysis of variance (ANOVA) [28].

Multiple Response Surface Methodology

The real problems are frequently characterized by several responses of interest and the solutions that are optimal for one response model may be far from the optimal for other models. This problem can be overcome with the help of multiple response surface methodology (MRS) where all responses are simultaneously optimized. Derringer and Suich developed a procedure based on the so-called desirability function (DF) in this regard [28]. In DF method, each individually predicted response is transformed into a partial desirability function (df$_i$) in which multiple responses are reduced to a single aggregated function and then solved as a single objective optimization. Desirability function varies from zero (undesirable response) to one (optimal response) based on the Derringer and Suich equation [28]. If the response should be
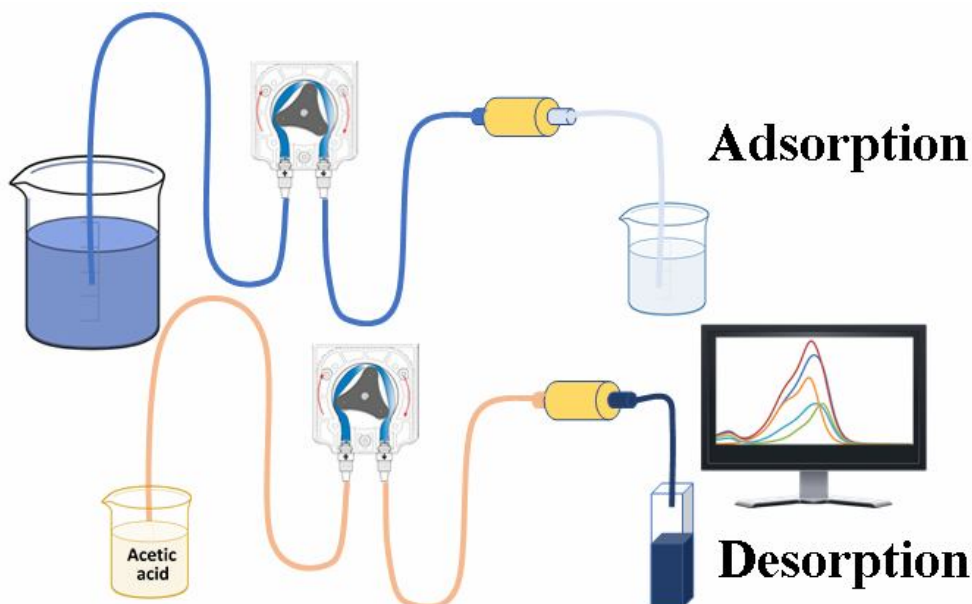
Fig. 1. Representation of the SPE procedure.

maximized, the one-sided desirability function will be applied, written as:

$$df_i = 0, \quad \hat{y}_i < \alpha$$

$$df_i = (y_i - \alpha/\beta - \alpha)^{wi}, \quad \alpha \le \hat{y}_i \le \beta$$

$$df_i = 1, \quad \hat{y}_i > \beta \tag{3}$$

where $\hat{y}_i$ and w_i are the fitted value of ith response and the weight of that response. α and β are the lowest and highest values of $\hat{y}_i$, respectively. DF as the overall optimization function is calculated by the geometric mean of different df_i values as follows:

$$DF = \left[df_1^{w1} \times df_2^{w2} \times \cdots \times df_n^{wi} \right]^{\frac{1}{n}}, \quad 0 \le wi \le 1 \, (i = 1, 2, \cdots, n) \tag{4}$$

$$\sum_{i=1}^{n} wi = 1$$

where df_i designates the individual desirability of the response y_i (i = 1, 2, 3,.., n) and wi displays the importance of ith response. Actually, DF is the aggregated measure of all responses yi's which is optimized over the independent variable area by any existing univariate method.

Partial Leas Squares Regression

PLSR method is a first-order calibration method correlating variations in a signal d with variation of property of interest. In the present work, d is the UV-Vis spectrum of sample and the property to be modeled is the concentration of the desired analyte. This method involves a two-step procedure: (1) calibration, where the relationship between spectra and analyte concentrations is established from a set of calibration samples, and (2) prediction, in which the calibration results are applied to estimate the component concentrations in unknown or prediction samples.

In PLSR, the spectral information about the analyte concentration is ''extracted'' by

$$c = r^T b \tag{5}$$

The vector of regression coefficients b (J × 1) is obtained from the spectra of I calibration samples measured at J wavelengths, R (I × J), and the reference concentrations to these calibration samples. A more detailed discussion of the PLSR algorithm can be found elsewhere [9]. The most

important task in conducting PLSR is the accurate selection of the number of components. For this purpose, leave one-out-cross validation (LOOCV) method can be employed in the calibration step to find the number of components [9]. In this method, the number of latent variables becomes available with left out each sample from the calibration set and predicting the concentration using a model build with the data for the remaining samples. The predicted error sum of squares (PRESS) parameters can be calculated based on the sum of squared error for the prediction of the left out a sample as followed in Eq. (6) [29],

$$PRESS = \sum_{i=1}^{I}(c_i - c_{i,pred})^2 \tag{6}$$

where I is the total number of calibration samples, c_i and $c_{i,pred}$ are the real and predicted concentrations of the ith sample, respectively. The data set is analyzed with a high number of latent calibration variables, larger than the expected to be optimum. Finally, the number of latent variables with minimum PRESS is selected for furture analysis.

The concentration of each analyte in an unknown sample is predicted using the calibration model and response vector of the sample.

The statistical parameters such as R^2, the root of the mean squared error of calibration samples (RMSEC) and prediction samples (RMSEP) values can be used to evaluate the PLSR model and its prediction ability for the analysis of unknown samples. These parameters are calculated as follows:

$$R^2 = 1 - \left(\frac{\sum_{i=1}^{n}(c_i - \hat{c}_i)^2}{\sum_{i=1}^{n}(c_i - \overline{c}_i)^2} \right) \tag{7}$$

$$RMSEC = \sqrt{\frac{\sum_{i=1}^{n}(c_i - \hat{c}_i)^2}{I - 1}} \tag{8}$$

where I, $\hat{c}_i$ and $\overline{c}_i$ are the total number of samples in the calibration set, the real and predicted concentrations of the given analyte in the ith calibration sample.

$$RMSEP = \sqrt{\frac{\sum_{j=1}^{F}(c_j - \hat{c}_j)^2}{F - 1}} \tag{9}$$

Where F, c_j and $\hat{c}_j$ are the total number of samples in the prediction set, the real and predicted concentrations of the given analyte in the jth prediction sample.

The other figures of merits including sensitivity (SEN), analytical sensitivity , limit of detection (LOD) and limit of quantitation (LOQ) for calibration are computed based on uncertainty propagation as described in Eq. (10),

$$SEN = \frac{1}{\|b\|} \tag{10}$$

where b is the same vector as in Eq. (5) and $\| \|$ designates the norm of the vector.

It should be noted that the unit of SEN value is (signal × concentration^{-1}) and therefore this parameter is not applicable for comparing the sensitivities derived from spectral and electrochemical determinations on an equal basis; due to the dependence of the signal type on the calibration method. Consequently, analytical sensitivity γ is introduced as the most useful indicator, which defines sensitivity divided to instrumental noise with unit of concentration^{-1}:

$$\gamma = \frac{SEN}{\sigma_r} \tag{11}$$

where σ_r is the constant uncertainty in instrumental noise. Generally, any prediction process should include an assessment of the uncertainty in the predicted value(s) which can be computed from the data used to fit the model. Prediction uncertainty analysis focuses on the effect of a three-term expression according to Eq. (12), which accounts for the instrumental signal in the test sample data, instrumental signals in the calibration data and calibration concentrations:

$$\sigma_{c,un}^2 = SEN^{-2}\sigma_r^2 + h_0 SEN^{-2}\sigma_r^2 + h_0\sigma_{ccal}^2 \tag{12}$$

where σ_r is the same as in Eq. (11), h_0 is the sample leverage as described in [30], and σ_{ccal} is the uncertainty in
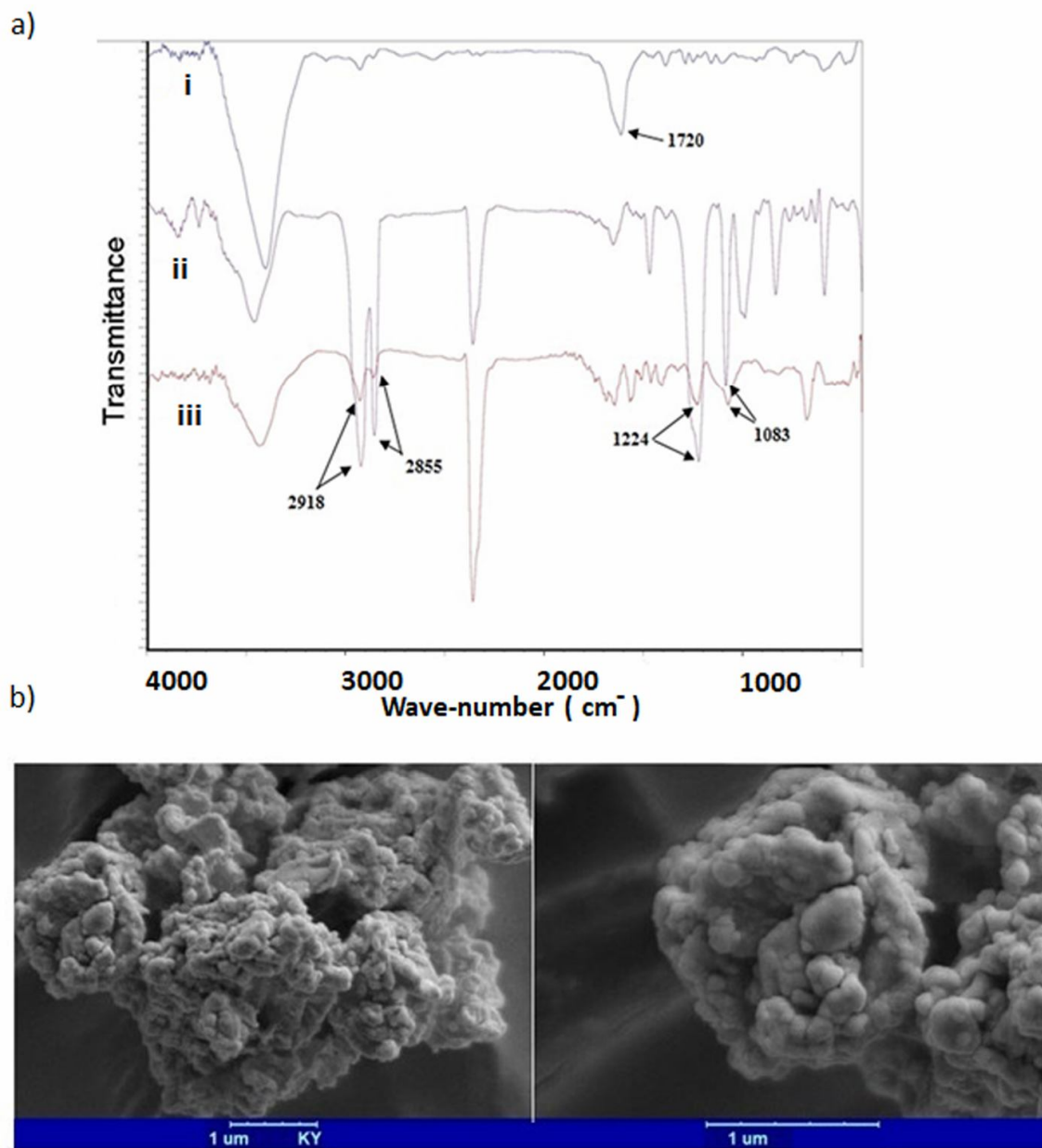
Fig. 2. (a) FT-IR spectra of chitosan (i), sodium dodecyl sulfate (ii), SDS grafted chitosan (iii); and (b) SEM image of SDS grafted chitosan.

calibration concentrations.

LOD and LOQ are estimated as the concentration level, which are 3.3, and 10 times root square of prediction error in Eq. (12), respectively. The minimum and maximum in LOD and LOQ are related to minimum and maximum leverage for a blank sample (h_{0min} and h_{0max}), respectively.

RESULT AND DISCUSSION

Characterization of Sorbent

Figure 2a shows the FT-IR spectra of chitosan, SDS and SDS grafted chitosan. The broad peak around 3200-3500 cm^{-1} in Fig. 2a (i) verifies the stretching vibration of N-H
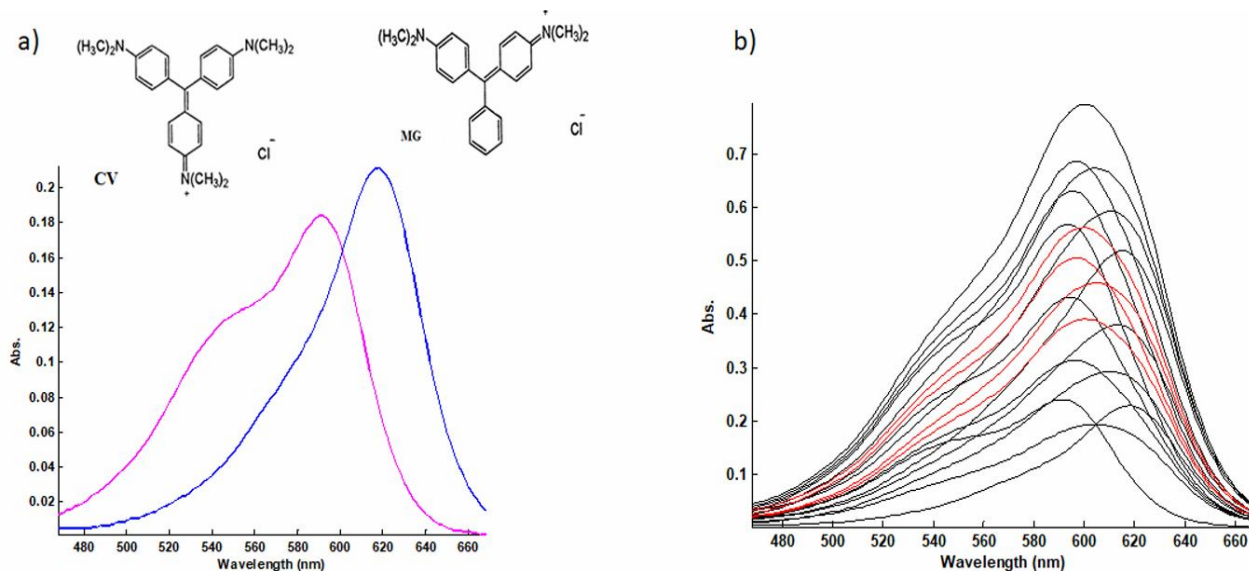
Fig. 3. (a) UV-Vis normalized pure spectra of CV and MG and their structures; the spectral data of calibration (black color) and prediction set (red color).

and O-H groups of chitosan [22]. In addition, the peak at 1720 cm^{-1} is attributed to chitosan C=O groups [23]. In Fig. 2a (ii), peaks at 1224 and 1083 cm^{-1} are attributed to asymmetric and symmetric stretching of S=O groups of SDS [31]. Figure 2c (iii) shows a combination of Fig. 2a and 2b implying a good distribution of SDS molecules in the polymeric network of cross-linked chitosan.

The surface morphological images of the SDS grafted chitosan was obtained by SEM and are shown in Fig. 2b. As seen, the surface morphology of the sorbent confirms the good distribution of SDS into the polymeric network of chitosan, indicating the increase of analyte adsorption from sample solutions. Moreover, based on EDS analysis, the atomic percentages of 52.3 wt.% (C), 38.1 wt.% (O), 4.2 wt.% (S) and 4.4 wt.% (N) confirm the successful grafting of SDS by the polymeric network of chitosan.

QAUNTIFICATION OF BINARY MIXTURES OF CV AND MG USING PLSR METHOD

The pure normalized spectra of CV, MG and their structures are shown in Fig. 3a. As seen, there is a notable overlap of the absorption spectra of these analytes, so their concentrations cannot be determined in mixtures without previous chemical separation. Therefore, PLSR strategy can be employed for data analysis to overcome this problem.

In PLSR method, two data sets are required: calibration and prediction. Here, eighteen samples containing different concentrations of dyes were synthesized (Table 1): sixteen of which were designed based on the full factorial design at four levels of two dyes and two of which were the standard solution of each dye.

On the basis of pKa of CV and MG, the pH of all sample solutions was adjusted to five to make the PLS model applicable for the determination of analytes in the desorbed solutions. Four out of eighteen synthesized samples were selected for prediction set, and the others were used as a calibration set. The spectrum of each sample was recorded in the wavelength range 400-800 with 2 nm intervals and the data at wavelength range 470-670 was selected for further studies (Fig. 3b). The calibration data set is a matrix with dimension 101×14.

The LOOCV method was applied in the calibration step to find the number of latent variables (LV) in the analysis. Figures 4a and b represent the plots of PRESS, and LOG PRESS against the number of LVs. As seen, the PRESS is minimal when the number of LVs is two. In Fig. 4, the

Table 1. Calibration Set for PLSR Analysis

RUN	Concentration of CV ($\mu g\ ml^{-1}$)	Concentration of MG ($\mu g\ ml^{-1}$)
1	0.5	3
2	1	3
3	1.5	3
4	2	3
5	0.5	5
6	1	5
7	1.5	5
8	2	5
9	0.5	7
10	1	7
11	1.5	7
12	2	7
13	0.5	10
14	1	10
15	1.5	10
16	2	10
17	1	0
18	0	5

calibration curve of CV (c) and MG (d) were ploted at the optimal number of LVs.

In the prediction step, the prediction data were pretreated the same as calibration data and the concentration of each analyte was obtained based on the calibration model.

The figures of merits including R^2, RMSEC, RMSEP, sensitivity, analytical sensitivity, LOD and LOQ for each analyte calibration were calculated based on the presented equations in theory section which are reported in Table 2.

Optimizating the Preconcentration Processes of CV and MG

In this study, five factors affect the adsorption processes, as follows: sample volume, pH, adsorption flow rate, desorption flow rate and dyes concentrations and three important factors in the desorption step, as follows: sample volume, eluent type and mobile phase concentration. Based on the preliminary experiments, 150 ml of sample volume and 5 M of acetic acid as eluent type were selected and the other factors were optimized using the CCD method. To reduce the number of investigated factors in CCD design, which in turn, reduces the number of experiments, the concentration of dyes with ratio 1:1 was considerd as a factor instead of applying CV and MG concentration as two independent factors.

According to Eq. (1), the overall data points in CCD involves 45 experiments. However, in this work, the
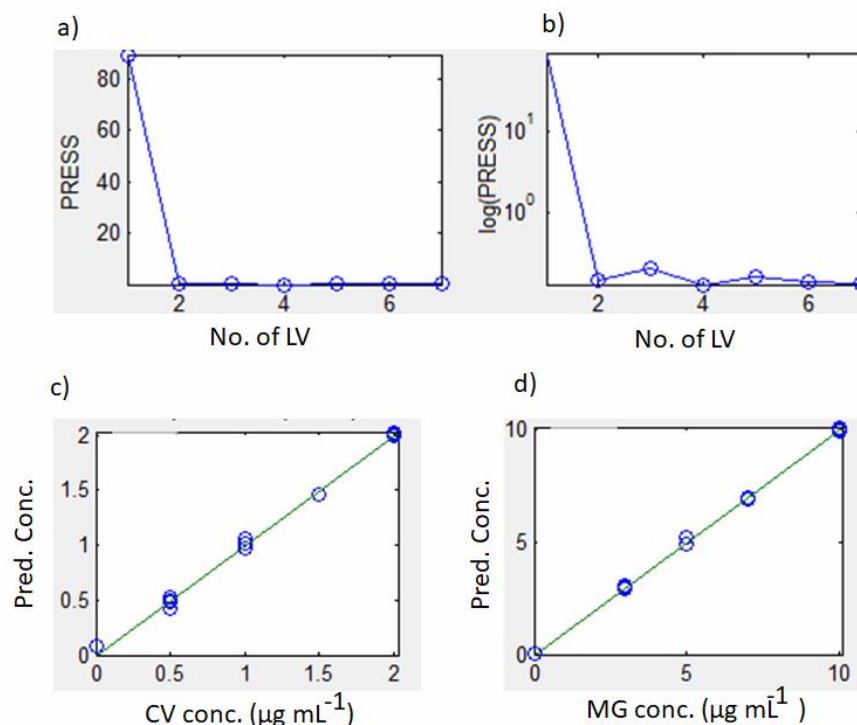
Fig. 4. Plots of PRESS (a) and LOG PRESS (b) *vs.* No. of LV, calibration curve of CV (c) and MG (d).

Table 2. Figures of Merites for CV and MG Obtained from PLSR Method

Analyte	R^2	RMSEC	RMSEP	SEN	Anal SEN	LOD	LOQ
CV	0.9995	0.0334	0.0173	0.89	762.07	0.06 (±0.01)	0.20 (±0.04)
MG	0.9993	0.0900	0.0808	0.15	128.24	0.17 (±0.02)	0.53 (±0.11)

number of extraction conditions based on half-fractional factorial design was decreased to 27 (Table 3). Moreover, three pH levels were used based on preliminary experiments.

In each extraction experiment, UV-Vis spectrum was obtained in the same wavelength range of calibration data in the PLSR model. Then, the concentrations of MG and CV were predicted from the PLSR model and considered as responses in CCD design, reported in Table 3.

The obtained data from the experimental design were evaluated by ANOVA and the results of the analysis were reported in Table 4. Based on the ANOVA analysis, at 95% confidence level, the responses for CV (y_{CV}) and MG (y_{MG})

were correlated with five parameters according to the following coded Eqs.:

$$y_{CV} = 0.82 - 0.11X_1 - 0.024X_2 + 0.11X_3 - 0.20X_4 + 0.15X_5 - 0.05X_1X_2 + 0.07X_1X_4 + 0.036X_2X_5 - 0.08X_3X_4 - 0.035X_4X_5 - 0.95X_2^2 - 0.074X_3^2 - 0.048X_4^2$$

(13)

$$y_{MG} = 1.35 - 0.33X_1 + 0.28X_3 - 0.27X_4 + 0.38X_5 - 0.17X_1X_2 - 0.14X_1X_3 + 0.2X_1X_4 - 0.11X_1X_5 + 0.18X_2X_3 + 0.18X_2X_5 - 0.16X_3X_4 + 0.18X_3X_5 - 0.14X_4X_5 - 0.15X_2^2 - 0.15X_3^2 - 0.11X_4^2$$

(14)

where X_1, X_2, X_4 and X_5 were the pH of the samples,

Table 3. Experimental Conditions Based on 2^{5-1} Central Composite Design in SPE Processes and Responses of Analytes

Factors			Levels			
		$-\alpha$	-1	0	1	$+\alpha$
X_1: pH			7	9	11	
X_2: Adsorption flow rate (ml min^{-1})		2	3	5	6	8
X_3: Concentration (ng ml^{-1})		20	35	50	65	80
X_4: Desorption flow rate (ml min^{-1})		1	2	3	4	5
X_5: Desorption volume (ml)		1	1.5	2	2.5	3

			Factor			Response	
Run	X_1	X_2	X_3	X_4	X_5	CV (μg ml^{-1})	MG (μg ml^{-1})
1	11.00	3.00	35.00	2.00	1.50	0.79	1.21
2	11.00	6.00	65.00	2.00	1.50	1.48	3.60
3	9.00	2.00	50.00	3.00	2.00	0.53	0.68
4	9.00	5.00	50.00	3.00	2.00	0.86	1.30
5	9.00	5.00	50.00	5.00	2.00	0.45	0.40
6	9.00	8.00	50.00	3.00	2.00	0.38	0.95
7	9.00	5.00	20.00	3.00	2.00	0.35	0.38
8	9.00	5.00	50.00	3.00	2.00	0.90	1.70
9	7.00	3.00	65.00	4.00	2.50	0.28	0.36
10	11.00	3.00	65.00	2.00	2.50	0.36	0.89
11	7.00	6.00	35.00	4.00	2.50	0.21	0.27
12	11.00	6.00	65.00	4.00	2.50	0.60	0.98
13	11.00	6.00	35.00	2.00	2.50	0.52	0.62
14	7.00	6.00	65.00	4.00	1.50	0.65	0.59
15	11.00	3.00	35.00	4.00	2.50	0.45	0.74
16	9.00	5.00	50.00	3.00	3.00	0.31	0.50
17	7.00	3.00	35.00	4.00	1.50	0.41	0.56
18	7.00	3.00	65.00	2.00	1.50	0.92	0.99
19	7.00	3.00	35.00	2.00	2.50	0.19	0.39
20	9.00	5.00	80.00	3.00	2.00	0.71	1.20
21	9.00	5.00	50.00	3.00	1.00	0.96	1.46
22	9.00	5.00	50.00	3.00	2.00	0.78	1.39
23	7.00	6.00	65.00	2.00	2.50	0.43	0.64
24	9.00	5.00	50.00	1.00	2.00	1.09	2.08
25	11.00	6.00	35.00	4.00	1.50	0.66	0.62
26	7.00	6.00	35.00	2.00	1.50	0.59	0.66
27	11.00	3.00	65.00	4.00	1.50	1.01	1.25

Table 4. The Results of ANOVA Analysis for CV and MG

	Source	SS[a]	D.F.[b]	MSS[c]	F-value	p-value
CV	Model	2.34	13	0.18	25.74	<0.0001
	Residual	0.091	13	6.991E-003		
	Lack of fit	0.084	11	7.599E-003	2.08	0.3687
	Pure error	7.292E-003	2	3.646E-003		
	Cor total	2.43	26			
	R-Squared	0.9626				
	Adj. R-Squared	0.9252				
MG	Model	11.86	16	0.74	14.14	<0.0001
	Residual	0.52	10	0.052		
	Lack of fit	0.44	8	0.055	1.28	0.5114
	Pure error	0.086	2	0.043		
	Cor total	12.39	26			
	R-Squared	0.9577				
	Adj. R-Square	0.8899				

[a]Sum of square. [b]Degree of freedom. [c]Mean sum of square.

adsorption and desorption flow rates and eluent volume, respectively. X_3 belongs to dyes mixture concentration.

The p-value of the regression smaller than 0.05 indicates the model signification at a 95% confidence level. Furthermore, the p-value of the lack of fit was far bigger than 0.05 and it approves the model significance. The coefficient of determination R^2 and adjusted correlation coefficient R^2_{adj} are used to express the fitting quality of the polynomial model in Eqs. (13) and (14).

The magnitudes of the coefficients in Eqs. (13) and (14) can illustrate the statistical importance of the experimental variables [30]. Here, the coefficient of X_5 is the highest one, which means this factor has the most influence on the extraction response.

The relationship between the levels of the two factors

and a response can be represented by a surface in three dimensions as known the response surface, with the target optimum being the top of the 'mountain' [32]. For a better interpretation of the relation between the response and parameters, some three-dimensional plots of the response surface methodology (RSM) are represented in Fig. 5; when the amount of two factors is fixed at the central point and the others are allowed to be varied. In these figures, the curvatures of the plots and the mutual interaction among parameters are displayed [33]. For example, it can be seen in Fig. 5b that there is a non-linear relationship between the response and eluent volume. The response is improved with the increase of the eluent volume to 1.5 ml. Increasing the eluent volume is initially necessary because desorption of the dyes from the sorbent increased, but more increasing the
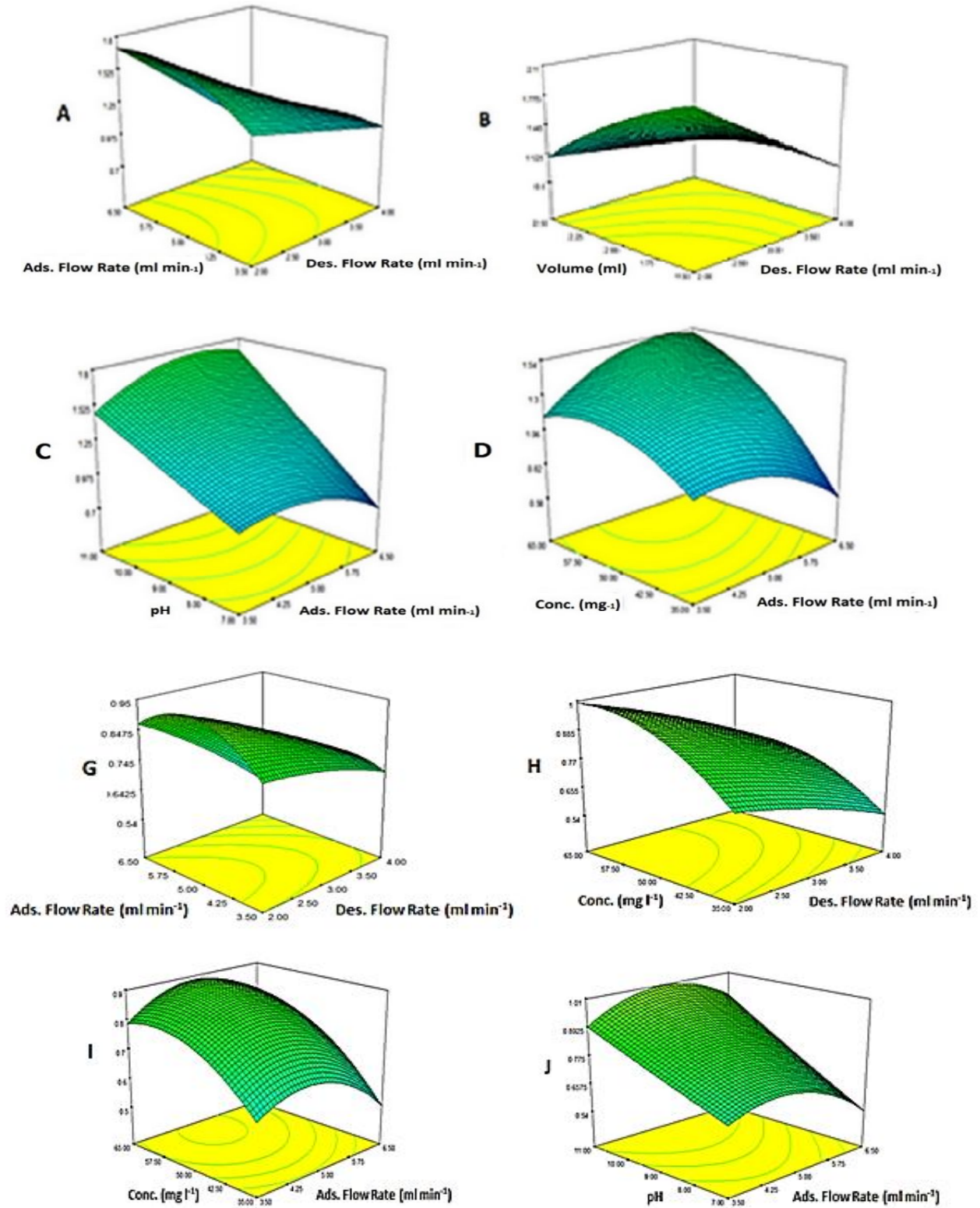
Fig. 5. Three-dimensional RSM plot for preconcentration of MG (A-F) and CV (G-J).

eluent volume causes a significant dilution in the concentration of the final solution and consequently reduce the responses (Fig. 5a and g). For the range of 0.035-0.065 μg ml^{-1}, the higher dyes concentrations are, the greater amount of dyes are extracted. However, concentration more than 0.065 μg ml^{-1}, the amount of extraction is reduced due to the overloading of sorbent (Figs. 5D-I). In Figure 6g, the response is maximum at the highest adsorption flow rate with the lowest desorption flow rate, since there is more interaction time of analyte with the active sites of the sorbent.

Generally, the color of organic dyes can be influenced by pH changes in acidic or basic limit and is one of the crucial factors playing an important role in the adsorption of dyes on synthesized sorbent *via* affecting the surface charge of the sorbent. In acidic pH, the positive charge repulsion of cationic dyes with each other may prevent them to be adsorbed, and therefore, the maximal adsorption occurs at basic pHs, which is clearly shown in Figs. 5D and J.

It is worth noting that the effect of the factors and their interactions for each dye may be different as a case in our study. The interaction effect is greater or less than that of the straight factor effects. Therefore, it is not supposed that the individual optimization to be equivalent to simultaneous optimization and collective effect is greater or less than what is expected from the straight factor effects. The aim of this study is finding the experimental condition in which both CV and MG dyes can be extracted from aqueous solutions with high efficiency, a fact which demands applying DF method as described in theory section.

Based on desirability function, the predicted values of the optimal experimental conditions for extraction of CV and MG were achieved at: pH = 11, desorption volume = 1.5 ml, concentration dyes = 65 ng ml^{1-} and adsorption and desorption flow rate of 6 and 2 ml min^{-1}, respectively (Fig. 6).

Adsorption Capacity

The total adsorption capacities of CV and MG were measured separately through a batch procedure. For each dye, a 100 ml solution containing 20 μg ml^{-1} of analyte and 100 mg of sorbent was stirred with a magnetic stirrer for 1 h. After that, the sorbent was collected by centrifuge at 4000 rpm and the supernatant was transferred to the quartz cell for measuring the remaining concentration of analytes with spectrophotometric technique. Then, the adsorption capacities were calculated to be 33.2 for CV and 31.5 for MG using the following Eq.:

$$Adsorption\ Capacity\ = \frac{(C_i - C_f) \times V}{m} \qquad (15)$$

where C_i and C_f are the concentration of analyte (μg ml^{-1}) before and after the adsorption procedure, respectively, V is the volume of the sample solution (L) and *m* is the sorbent amount (g).

The Figures of Merits for SPE Method

Preconcentration factor (PF) and extraction recovery percentage (ER%) were calculated based on Eqs. (17) and (18):

$$PF = \frac{slop\ of\ extraction\ calibration}{slop\ of\ direct\ calibration} \qquad (16)$$

$$ER\% = PF \times \frac{Final\ sample\ Volume}{Initial\ sample\ Volume} \qquad (17)$$

The above Eqs. have been presented for univariate analysis in which the parameters in Eq. (16) can be obtained simply using the univariate calibration curve. In this study, the norm of correlation coefficient vector b in Eq. (5) was used as the slope of direct calibration, because this vector acts as a net analyte signal filter [9]. The obtained PF and ER% of analytes are reported in Table 5. Furthermore, the results of the present work are compared with the previous studies in this table.

CONCLUSIONS

In this study, a simple SPE method based on SDS grafted chitosan nanocomposite sorbent was applied for the simultaneous preconcentration of crystal violet and malachite green in mixtures followed by the spectrophotometric techniques coupled with PLSR method for data analysis. The synthesized sorbent was characterized by SEM, EDS and FT-IR. The important and main influencing factors on the extraction efficiencies were
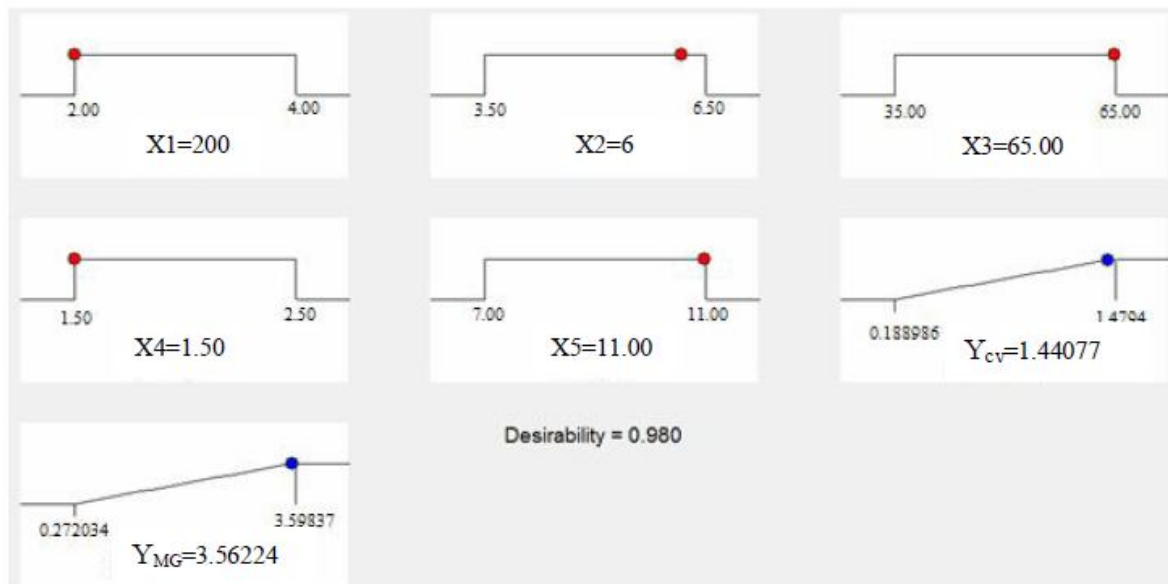
Fig. 6. Desirability plot for preconcentration of CV and MG.

Table 5. Comparisons between the Results Obtained in this Study and others in Literature

Method	Analyte	Linear range (ng ml^{-1})	LOD (ng ml^{-1})	PF	Ref.
CPE-PLSR	CV	9.9-800	2.9	20	[10]
	MG	16-1000	4.8	20	
SPE-HPLC	CV	1-100	0.12-0.28	NR	[35]
	MG	1-100	0.12-0.28	NR	
CPE-Spectrophotometer	MG	4-500	1.2	25	[15]
SPE- Spectrophotometer	CV	5-225	1.8	NR	[24]
SPE-PLSR	CV	20-80	0.06	47	This work
	MG	20-80	0.17	26	

Abbreviation NR: not reported, CPE: cloud point extraction.

optimized by central composite design with fractional factorial design. Under the optimum conditions, high adsorption capacities of 33.2 mg g^{-1} and 31.5 mg g^{-1} were obtained for CV and MG, respectively. In addition, the proposed method was used to calculate the analytical figure of merit in multivariate calibration. By using of NAS calculations to the multivariate regression data, the PF and ER% were calculated simply. The method presented good

extraction recoveries, low LOD, good accuracies, precisions, and good dynamic linear ranges for the analytes. These results show the combination of SPE and PLSR methods is a suitable alternative for the selective and efficient simultaneous extraction of CV and MG in aqueous mixtures without tedious pre-separation procedures and expensive instrumentation. On the other hand, the LOD of the present work is lower than that obtained by extraction and chromatographic methods.

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