



Simultaneous determination of pesticides at trace levels in water using multiwalled carbon nanotubes as solid-phase extractant and multivariate calibration

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ABSTRACT

The application of solid-phase extraction with multivariate calibration for simultaneous determination of three toxic pesticides in tap and reservoir waters was presented. The proposed analytical method was used for the determination of atrazine, methidathion, and propoxur in complex water samples without the need for chromatographic separation. Among the applied multivariate calibration methods, partial least squares (PLS-1) method was found the most effective for pesticides quantification. Multiwalled carbon nanotubes (MWCNTs) adsorbent showed a perfect extraction/preconcentration of pesticides present at trace levels. The experimental factors that affect pesticides extraction by MWCNTs adsorbent such as sample volume, eluent volume, solution pH, and extraction flow rate were studied and optimized. The figures of merit of the proposed method were: limits of detection 3, 2, and $3 \mu\text{g l}^{-1}$ and linear ranges 5–30, 3–60, and 5–40 $\mu\text{g l}^{-1}$ for atrazine, methidathion, and propoxur, respectively. A good precision was reported for the method, R.S.D. values were always less than 5.0%. Satisfactory results were reported for simultaneous determination of trace levels of pesticides in complex matrices. In tap water, the percent recoveries for pesticides were extended from 95 to 104% and R.S.D. from 1 to 3%, while lower recoveries were observed in reservoir water: 84–93% (R.S.D.: 1–3). Although the pesticides can be accurately quantified by SPE and liquid chromatography, SPE–PLS-1 method was found simpler and operated at lower running costs.

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1. Introduction

Organic pesticides including insecticides and herbicides are widely used in agriculture because of their powerful biological activity. Because they are serious environmental pollutants, plenty of analytical procedures have been developed to determine and control pesticides in surface and ground waters [1,2]. Furthermore, the presence of pesticides in fruit and vegetables was also investigated [3]. Due to their high toxicity, the minimum allowable total concentration of pesticides in water is 0.5 ppb [4].

In fact, the mobility of the studied pesticides in the terrestrial environment is high because their soil-distribution values (K_{OW} and K_{OC}) are relatively small which reflects their poor affinity toward soil and their fast migration to reach groundwater. The life times of propoxur and atrazine are high while methidathion has a short lifetime in the soil. It is important to mention that the pesticides are

all stable in solution at moderate pH conditions. Based on that, the identification and simultaneous determination of these pesticides in water is an essential task. The majority of analytical methods used for the determination of pesticides are mainly related to liquid chromatography. For example, atrazine was simultaneously quantified with other accompanying pesticides using liquid chromatography [5,6]. Prior to chromatographic analysis, the pesticides are often preconcentrated using either solid-phase extraction or liquid–liquid extraction methods to be detected by the instrument.

Recently, method development for simultaneous determination of solutes without prior separation or sample clean up has been markedly increased. This was attributed to the availability of powerful instrumentation and robust numerical analytical methods. For example, derivative spectrophotometry [7], H-point standard addition method [8], multivariate calibration methods including classical least squares (CLS) [9], principal components regression (PCR) [10], partial least squares (PLS) [9,10], and net analyte signal-based methods [11,12] have been frequently employed to resolve spectral overlap in many systems. Among the mentioned chemometric methods, partial least squares (PLS) was considered as a powerful tool that is used for the resolution of multicomponent sys-

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tems that suffer from chemical or spectral interferences. PLS-1 and PLS-2 calibration methods have been carried out using spectrophotometric [13], fluorimetric [14], infra-red [15], and polarographic data [16].

In fact, there are few studies on application of multivariate calibration for pesticides analysis [17,18] and there is no chemometric study on determination of atrazine, methidathion, and propoxur in tap and reservoir waters. Pesticides are often present in the environment at trace levels ($\mu\text{g l}^{-1}$), therefore, it was necessary to preconcentrate pesticides before their analysis. For many types of toxic pollutants, it was shown that solid-phase extraction is more effective for pollutants' preconcentration compared to classical liquid–liquid extraction method [1–3].

Due to the outstanding extraction efficiency toward many pesticides [4], carbon nanotubes (CNTs) adsorbent was selected to ensure high preconcentration factors for the studied pesticides prior to their chemometric analysis. CNTs can be divided into two types: single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) based on the principle of the hybridized carbon atom in the walls of CNTs. Solid-phase extraction, scanning probe microscopy, catalysis, hydrogen storage media, and electrochemical sensors are the most important applications of MWCNTs [4]. In fact, MWCNTs adsorbent outperformed many popular solid-phase extractants and successfully applied for preconcentration, extraction, and sorption of organic and inorganic pollutants in water as outlined in the literature [4,19–22].

In this work, CLS, PCR, and PLS-1 calibration methods were employed for simultaneous determination of three common pesticides; atrazine, methidathion, and propoxur in tap and reservoir waters. Figures of merit (limit of detection, limit of quantification, sensitivity and selectivity) for the proposed analytical method were determined. MWCNTs adsorbent was used for pesticides preconcentration prior to multivariate analysis.

2. Experimental

2.1. Instrumentation and software

The absorbance measurements were obtained using a double beam Unicam spectrophotometer (Cary 50 UV–vis Spectrophotometer, USA). The ultraviolet spectra of pesticides were recorded over the wavelength range (200–300 nm) and digitized absorbances were transferred to a Pentium(IV) personal computer for subsequent analysis. The data treatment and chemometric calculations were carried out using MATLAB® (version 7.0). The pH measurements were made with a WTW-Inolab (Germany) pH-meter using a companion glass electrode.

2.2. Pesticides and their stock solutions

All reagents and solvents were of analytical quality unless indicated otherwise. Atrazine, methidathion, and propoxur (>99.0% purity) were supplied by Aldrich (USA). The general chemical and physical properties of pesticides were taken from *The Pesticide Manual* [23]. Atrazine: a herbicide, molecular formula $\text{C}_8\text{H}_{14}\text{N}_5\text{Cl}$, K_{oc} (organic carbon distribution value): 93.0, K_{ow} (*n*-octanol water distribution value): 320, $t_{1/2}$ (half-lifetime in soil): 146 day, and solubility in water: 33 mg l^{-1} at 25°C . Methidathion: an insecticide, molecular formula $\text{C}_6\text{H}_{11}\text{O}_5\text{N}_2\text{S}_2\text{P}$, K_{oc} : 341.0, K_{ow} : 160, $t_{1/2}$: 3 day, and solubility in water: 200 mg l^{-1} at 25°C . Propoxur: an insecticide, molecular formula $\text{C}_{10}\text{H}_{14}\text{O}_3\text{N}$, K_{oc} : 13.3, K_{ow} : 40, $t_{1/2}$: 142 day, and solubility in water: 1900 mg l^{-1} at 25°C . Stock solutions (100 mg l^{-1}) were prepared by dissolving accurately weighed 10 mg of each pesticide in 10 ml of acetonitrile. These solutions were stored at 4°C and protected from light, pesticide solutions were found stable for approximately one month. Working solutions

were prepared daily by appropriate dilution from stock solutions with acetonitrile. To prevent sorption of pesticides at the surfaces of used glassware, all glassware were initially soaked in 2.0 M HNO_3 and washed with triply distilled water and finally with acetonitrile.

2.3. Multivariate calibration

In 100.0 ml volumetric polyethylene flask, aliquots of the stock solutions were added to obtain concentrations between 0 and 3.0 mg l^{-1} for atrazine and propoxur and between 0 and 8.0 mg l^{-1} for methidathion. Before final dilution, pH was maintained at 7.0 (± 0.3) using 0.01 M HCl or 0.01 M NaOH and the mixture was diluted to the mark with acetonitrile–methanol mixture (50 vol%). The calibration matrix was obtained from recording the absorption spectra of standards over the spectral range 200–300 nm. A number of 4, 3, and 4 latent variables were found to be optimum for accurate determination of atrazine, methidathion, and propoxur by PLS-1 and 7 factors were selected for PCR calibration. The optimized PLS-1 and PCR methods were used to predict pesticides level in 18 synthetic mixtures and in complex water samples previously spiked with pesticides. In spiked real samples, the pesticides were preconcentrated using MWCNTs adsorbent prior to their determination by PLS-1.

2.4. Solid-phase extractant and solid-phase extraction procedures

Multiwalled carbon nanotubes adsorbent was purchased from Shenzhen Nanotechport Company (Shenzhen, China). MWCNTs extractant was of 1–5 μm length and 40–60 nm external diameter. Prior to extraction, the adsorbent was washed many times with triply distilled water and then dried at 70°C for 24 h. 300 mg of MWCNTs weighted exactly was loaded in a typical 6.0 ml polyethylene extraction tube. The adsorbent was then conditioned by washing with 10 ml acetonitrile followed by 10 ml triply distilled water. The conditioning solutions were forced through the adsorbent at flow rate of 0.5 ml min^{-1} to remove adsorbed impurities and activate the surface of the adsorbent. Typically, 600 ml of water sample spiked with pesticides was pumped through the extractant at flow rate of 3.0 ml min^{-1} . All solid-phase extractions were carried out with the aid of a visiprep-12-port vacuum manifold (Supelco, USA) under a controlled pressure. After completion of extraction, the extractant was washed with triply distilled water to remove any adsorbed substances other than pesticides. The trapped pesticides were eluted with 10 ml acetonitrile–methanol mixture (50 vol%), and the final solution was evaporated to dryness using a rotavapor at 40°C under a moderate vacuum (Heidolph Labo-rata 4001, Germany). The remaining residue was re-dissolved in 4.0 ml acetonitrile–methanol mixture (50 vol%) and the mixture was scanned by the spectrophotometer and the pesticides contents were quantified using PLS-1 method.

2.5. Sampling and sample preparation

Natural water samples including tap and reservoir waters were collected from different local locations. Tap water samples were obtained after flowing for 15 min from various water taps in our department in different days. The collected samples were mixed together and treated as a representative sample. Representative sample for reservoir water was generated by collecting three different samples obtained from three local reservoirs which collected on different days. All reservoirs were located within Al-Zarqa area and their waters are used for irrigation purposes. The representative samples (3000 ml) were stored in polyethylene bottles at 10°C . Prior to spiking with pesticides, the samples were filtered using cellulose membrane filter (Millipore) of $0.45 \mu\text{m}$ pore size to remove any suspended matters. Furthermore, the initial pH of samples was adjusted to 7.0. Finally, the content of the pesticides in natural sam-

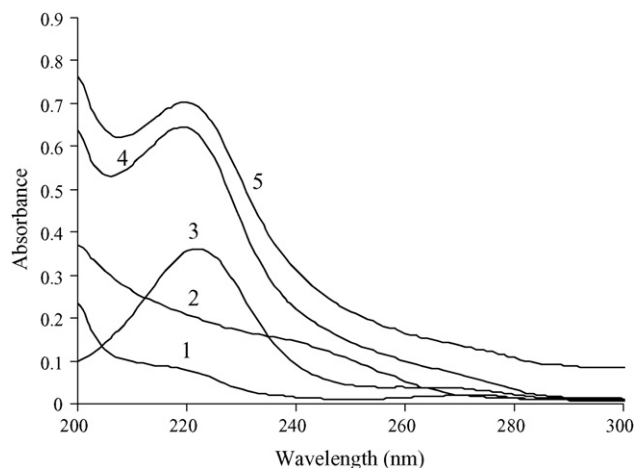


Fig. 1. Absorption spectra of pesticides. Absorption spectra of 2.0 mg l⁻¹ of propoxur (1), 8.0 mg l⁻¹ of methidathion (2), 2.0 mg l⁻¹ of atrazine (3), pesticides mixture (4), and the numerically added signals from pure substances (5). Concentrations of pesticides in the mixture: 2.0 mg l⁻¹ for atrazine and propoxur and 8.0 mg l⁻¹ for methidathion at pH 7.0.

ples were determined according to the general procedure outlined in Section 2.4.

3. Results and discussion

3.1. Spectral overlap and importance of multivariate calibration

The absorption spectra of pesticide and their mixture were given in Fig. 1. As indicated in Fig. 1, the pesticides were active within the spectral region 210–250 nm. After 250 nm, the three pesticides gave insignificant absorbances. The difficulties that arise when mixtures of these pesticides are to be determined are realized by studying the individual spectrum given in Fig. 1.

The spectra of pesticides were strongly overlapped within the spectral region (200–260 nm) and no wavelength can be found where any of the solutes is the only absorber. This indicates that conventional calibration procedures would have a limited application for quantitative determination. Determinations are further complicated by interaction within the solutions. This is indicated in Fig. 1, when the absorption spectrum of the pesticides (spectrum 4) is compared with the spectrum expected from the mixture, i.e., a summation of the individual spectra (spectrum 5). As shown, spectra 4 and 5 significantly differed in intensity, which reflected the nonlinear relationship between signal intensity and concentration. Therefore, the simultaneous determination of these pesticides requires: (a) the application of a powerful separation techniques like liquid chromatography or capillary electrophoresis before their determination, or (b) the application of multivariate calibration techniques for resolution of spectral overlap. The second option was chosen in this study owing to its simplicity, rapidity and low cost. The concentrations of atrazine, methidathion, and propoxur in their mixtures were estimated using different chemometric approaches including classical least squares (CLS), principal component regression (PCR), and partial least squares (PLS-1).

3.2. Effect of chemical variables

There are many chemical variables that affect the absorption characteristics, stability, and hydrolysis of pesticides in water. Among these variables, pH is the most influential and, therefore, should be investigated. The influence of pH on the absorption

characteristics of pesticides was studied over the pH range 2–8 and initial concentration of 3.0 mg l⁻¹. The initial pH was adjusted using 0.01 M HCl or 0.01 M NaOH solutions. The effect of solution pH on absorption of atrazine was insignificant, however, at solution pH 2 there was a large decrease in the compound absorption. The absorption value at 220 nm was decreased by 60% when pH reduced from 6 to 2. A similar behavior was observed for propoxur but there was a high increase in its absorption at pH 2. There was no variation in the methidathion absorption over the studied pH range. Accordingly, pH 7.0 was selected to be the optimum value for measuring the absorption of pesticides in solution.

3.3. Determination of pesticides using multivariate calibration methods

A comparison had been made between the performances of chemometric methods for quantitative spectral analysis, the comparison revealed that it is very difficult to generalize about the superiority of one method against the others [9]. The effectiveness of any chemometric method is highly dependant on the particular data set under analysis. Three popular chemometric methods (CLS, PCR, and PLS-1) were evaluated for spectral resolution of pesticides. Moreover, a comparison study of the prediction powers of these methods was also carried out.

3.4. Experimental design of the calibration–validation sets, selection of the optimum wavelength range, and outliers determination

A training set of 29 mixtures was taken. The composition of the calibration set was selected according to the five-level fractional factor designed to ensure that no correlation existed between the concentrations of pesticides. Collinear components in the calibration set tend to cause over-fitting in chemometric analysis. Atrazine and propoxur concentration ranges were varied between 0 and 3.0 mg l⁻¹ and methidathion level was varied within 0 and 8 mg l⁻¹. The composition of validation set was within the ranges used in building calibration set but randomly designed. The calibration and validation sets were presented as 3D plot in Fig. 2 so as to view the homogeneity between them.

In multicomponent spectrophotometric analysis, the optimum wavelength range should be carefully chosen for getting acceptable accuracy and precision in the results. The selection of the optimum wavelength range can be made using several criteria such as

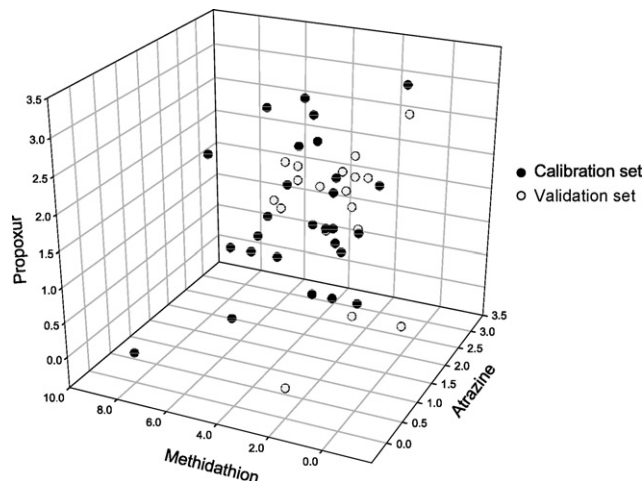


Fig. 2. A 3D plot of calibration and validation mixtures.

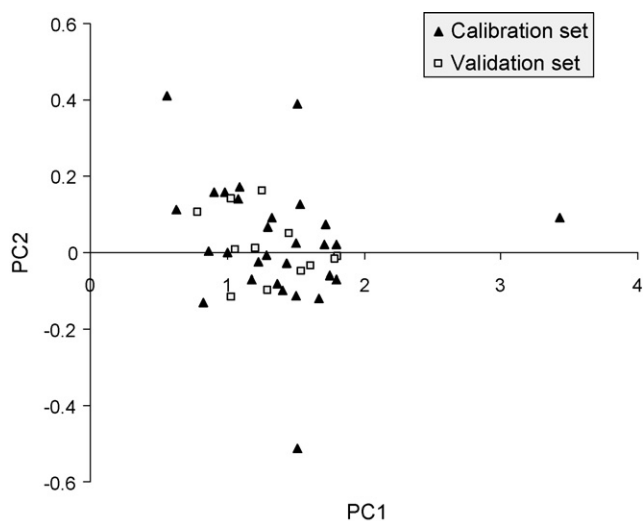


Fig. 3. PC1–PC2 plot. PC1–PC2 plot for detecting outliers in standard mixtures.

maximum signal/noise ratio method [18], changeable size moving window [24], and the minimum condition number of the calibration matrix ($\text{cond}(\mathbf{K})$) [25] and the last method was adopted in this work due to its ease of implementation. In fact, error propagation is important in establishing the optimal wavelength range for quantitation. This error can be represented by the condition number of the calibration matrix, $\text{cond}(\mathbf{K})$ [18]; a large condition number represents a large error in estimating the analyte concentration as well as a high degree of non-orthogonality in the spectra. Instead of selecting a wavelength range with the best sensitivity, it must be selected with the smallest error amplification, i.e., smallest $\text{cond}(\mathbf{K})$ [26]. The $\text{cond}(\mathbf{K})$ value was calculated for a set of different wavelength ranges between 200 and 300 nm, which is the interval where the pesticides present absorbance (see Fig. 1). The calibration matrix used in the numerical analysis for estimation of $\text{cond}(\mathbf{K})$ was the one obtained from CLS analysis [18,24,25], similar results were obtained for $\text{cond}(\mathbf{K})$ values while using the \mathbf{K} matrix of absorptivities of pure solutes. For the spectral ranges: 200–300, 200–290, 200–280, 210–300, 210–280, 210–250, and 220–280 nm the obtained $\text{cond}(\mathbf{K})$ values were 11, 8, 7, 10, 7, 2, and 6, respectively. Obviously, the lowest $\text{cond}(\mathbf{K})$ value was 2 which obtained for 210–250 nm range, therefore, this range was selected for pesticides analysis.

Detecting outliers and careful selection of the informative spectral ranges are necessary to improve the calibration power of PLS-1 [27–29]. PCA was used to detect outliers in calibration and validation sets. Using principal component analysis [29], five (84.7, 0.73, 0.12, 0.007, 0.003) non-zero eigenvalues were obtained after decomposing both calibration and validation matrices. As can be noted from the magnitudes of the eigenvalues, the entire data can be represented using the first two eigenvalues. Statistical analysis revealed that 92.6–98.3% of the total variances in the multivariate data can be presented by the first two eigenvectors (first two principal components). The scatter plot of PC1 (first principal component or score vector 1) against PC2 (second principal component or score vector 2) reveals evident pattern in the pesticides samples and facilitate detection any potential outlier(s). PC1–PC2 plots were depicted in Fig. 2 for the current system. As shown in Fig. 3, four samples (all back to calibration set) were located away from the cluster of the samples. Accordingly, these four samples were removed prior to chemometric calibration.

3.5. Selection of the optimum number of factors (h) for PCR and PLS-1 methods

The optimum number of factors and latent variables should be selected in order to avoid overfitting when using PCR and PLS-1 methods. This can be carried out by applying leave-one-out cross validation procedure as developed by Haaland and Thomas [9]. For each factor, the concentration predicted (C_{pred}) for each sample is compared with the actual concentration (C_{act}) of this reference sample. The sum of squared prediction errors for all calibration samples, or PRESS = $\sum (C_{i,\text{pred}} - C_{i,\text{act}})^2$ was calculated each time a new factor (h) is added to the PCR or PLS-1 model. The optimum number of factors was obtained as described in literature [29]. In our case, 4, 3, and 4 latent variables were found optimum for simultaneous determination of atrazine, methidathion, and propoxur using PLS-1 calibration method and 7 factors was chosen for spectral resolution of pesticides using PCR. For PCR, the PRESS values at $h = 1$ were 412, 293, and 89 for propoxur, methidathion, and atrazine respectively, while the corresponding PRESS values at $h = 7$ were 0.021, 0.013, and 0.015 which indicated the importance of finding the optimum number of factors before multivariate calibration.

3.6. Statistical assessment of multivariate calibration methods

Using the cross-validation method the following statistical parameters were obtained: (1) the values of root mean square difference (RMSD), which indicate the average error in the analysis for each solute, (2) the square of the correlation coefficients (r^2), which measures the degree of linearity between actual and predicted concentrations, and (3) the relative error of prediction (REP) with regard to the average true value (\bar{C}_t), which measures the predictive ability of the analytical method for determination of pesticides in the mixture. Table 1 summarized the statistical parameters for the employed chemometric calibration methods.

As shown in Table 1, the applied chemometric methods exhibited different prediction powers for the determination of the pesticides in their mixtures. It seems that CLS method has a limited application for the determination of current biocide system, the obtained REP% values were relatively high (8–15%). On the other hand, PLS-1 was the most effective method for simultaneous determination of the pesticides. The lowest values of REP% were reported for this method and were in the range: 2.0–3.5%. As can be noted from Table 1, the correlation coefficient (r^2) values obtained for PLS-1 were fairly close to unity which reflects the similarity

Table 1
Statistical assessment of CLS, PCR, and PLS-1 methods for pesticides analysis.^a

Method/pesticide	Factor (h)	PRESS	RMSD	r^2	REP%
CLS					
Atrazine		1.3779	0.2180	0.6623	12.1788
Methidathion		1.8774	0.2544	0.8952	8.0264
Propoxur		2.0254	0.2643	0.6124	14.7654
PCR					
Atrazine	7	0.1752	0.0777	0.94623	4.3423
Methidathion		0.1475	0.0713	0.9595	2.2498
Propoxur		0.1625	0.0749	0.9493	4.1819
PLS-1					
Atrazine	4	0.1103	0.0617	0.9889	3.4469
Methidathion	3	0.1325	0.0676	0.9925	2.1323
Propoxur	4	0.1132	0.0625	0.9775	3.4916

$$^a \text{RMSD} = (\text{PRESS}/m)^{1/2}; r^2 = 1 - \left(\frac{\sum_{i=1}^m (C_{i,\text{pred}} - C_{i,\text{act}})^2}{\sum_{i=1}^m (C_{i,\text{act}} - \bar{C})^2} \right)$$

\bar{C} is the average solute concentration in the m calibration samples (29 samples), REP% = RMSD / \bar{C} [31]. Calibration by CLS, PCR, and PLS-1 was carried out according to the algorithms described in refs. [9,10]

between predicted and actual values. Relatively speaking, the PCR and PLS-1 chemometric methods can be employed for simultaneous determination of the three pesticides with an acceptable degree of accuracy without the need for prior separation of solutes. Ni et al. [30] have applied a number of chemometric methods including PCR, PLS, and radial basis function–artificial neural networks (RBF–ANN) methods for simultaneous determination of propoxur, isoprocarb, carbaryl and carbofuran in water and wastewater samples without prior separation and with using electrochemical detection. The RBF–ANN outperformed the rest of the methods and gave 100% recovery and 5.6 REP% [30]. In a similar chemometric study, Ferré et al. [17] have effectively applied CLS method for simultaneous determination of carbaryl, carbofurane, propoxur and isoprocarb using flow injection analysis and spectrophotometric detection.

3.7. Figures of merit of multivariate calibration and simultaneous determination of pesticides in synthetic mixtures

Figures of merit like sensitivity, selectivity, limit of detection LOD, limit of quantification LOQ and linearity range for multivariate calibration were obtained as outlined elsewhere [11,17,31–33]. Usually, the multivariate selectivity values range between zero (complete overlap between analyte and other analytes or interferences) and unity (no or small overlap between analyte and other analytes or interferences) [32]. Low selectivity values are associated with unstable estimations of the concentrations and large prediction errors for new samples. Higher values of sensitivity are an indication of higher accuracy in determination of the solute in the mixture. The obtained sensitivities, selectivities, LOD (mg l^{-1}), LOQ (mg l^{-1}), linearity ranges (mg l^{-1}) and accuracies (as REP%) were 0.0413, 0.321, 0.31, 0.54, 0.54–4.0, and 3.45 for atrazine and 0.0623, 0.545, 0.22, 0.31, 0.31–7.5, and 2.13 for methidathion and 0.0392, 0.298, 0.41, 0.61, 0.61–5.0, and 3.49 for propoxur. As indicated from the results, methidathion was the compound that has the highest selectivity (0.545) and sensitivity (0.0623) while the rest of pesticides have a lower and closer values and this may be attributed to their high spectral overlap. In order to take into account all the causes of variability of the method, accuracy in pesticides determination was assessed by comparing the REP% values of a set of n validation samples not included in the calibration set, which were analyzed in reproducible conditions using the proposed PLS-

1 calibration method. The values of REP% that estimated from the validation set indicated that the most accurate estimation was for methidathion where the obtained REP% value was 2.13. In general, the studied pesticides can be simultaneously analyzed with an acceptable degree of accuracy with satisfactory linearity ranges as given earlier. The multivariate limits of detection (LOD) for pesticides were in the range: 0.22 and 0.41 mg l^{-1} . The lower LOD of methidathion (0.22 mg l^{-1}) was expected as this pesticide has the highest values of sensitivity and selectivity. One set of 18 synthetic mixtures (with one, two, and three solutes in different amounts, containing from 0 to 2.5 mg l^{-1} of atrazine, from 0 to 4.4 mg l^{-1} of methidathion, and from 0 to 3.0 mg l^{-1} of propoxur) were analyzed using both PCR and PLS-1 proposed calibration methods. The composition of the validation set and the recoveries obtained using PCR and PLS-1 calibration were summarized in Table 2.

As can be noted, the analytical results obtained from both chemometric methods are not significantly different from each other which extended the findings of other researchers that PCR and PLS (types 1 and 2) all have identical calibration powers [28,29]. PLS-1 has slightly outperformed PCR method, the obtained recoveries of pesticides were 99.3–100.8 with R.S.D. values less than 5.0% in all cases. Based on that, PLS-1 will be adopted for analysis of pesticides in real samples where many unknown interferences are present.

3.8. Optimization of the solid-phase extraction process of pesticides

As mentioned in Section 3.7, the pesticides were quantified in acetonitrile/methanol mixture using PLS-1 with limits of quantification ranging between 0.33 and 0.63 mg l^{-1} . As mentioned earlier, the studied pesticides are usually present in natural environment at trace levels ($\mu\text{g l}^{-1}$). Accordingly, pesticides should be preconcentrated (as large as possible) so they could be detected and analyzed by the proposed PLS-1 method. To ensure large enrichment factors (high preconcentration levels) of pesticides, the process of pesticides extraction by MWCNTs was optimized beforehand. Sample flow rate, eluent type and volume, sample volume, and solution pH are the main factors that influence the extraction and the enrichment efficiency of any SPE process. A number of organic solvents including methanol, acetonitrile, dichloromethane and acetone

Table 2
Recoveries found in synthetic mixtures by PCR and PLS-1 methods.

Content (mg l^{-1})			Recovery (%)					
			PCR			PLS-1		
Atrazine	Methidathion	Propoxur	Atrazine	Methidathion	Propoxur	Atrazine	Methidathion	Propoxur
1.8	1.6	1.4	96.7	96.1	98.2	100.2	100.7	98.1
1.4	2.6	2.1	98.2	102.3	94.3	99.5	104.2	94.5
2.5	2.6	1.8	103.3	103.5	96.5	100.1	97.8	96.8
1.6	4.4	1.6	95.0	98.2	103.3	101.2	101.3	96.6
2.1	4.4	2.0	96.2	102.2	96.1	99.8	99.2	99.8
2.1	4.4	1.8	96.3	103.6	95.8	100.2	100.8	99.6
2.1	1.6	2.0	106.5	99.8	98.3	99.1	99.5	100.2
1.4	1.6	2.1	99.8	95.5	106.2	115.2	103.2	103.3
1.4	4.4	1.8	117.3	103.9	98.3	94.3	99.1	99.2
2.5	2.6	2.1	101.3	105.2	98.5	100.6	102.3	102.3
1.6	2.6	1.4	98.6	97.3	95.1	101.0	99.3	101.3
1.6	1.6	1.8	99.5	105.5	99.2	99.6	98.6	97.8
2.0	0.0	0.0	105.8	-0.12 ^a	0.03 ^a	101.3	-0.12 ^a	-0.36 ^a
0.0	2.0	0.0	0.45 ^a	96.3	-0.82 ^a	0.45 ^a	99.6	-0.52 ^a
0.0	0.0	3.0	0.13 ^a	-0.04 ^a	95.2	0.16 ^a	0.05 ^a	99.6
2.0	2.0	0.0	105.6	103.6	-0.22 ^a	101.6	100.2	-0.05 ^a
2.0	0.0	3.0	102.3	0.03 ^a	96.2	98.5	0.32 ^a	99.6
0.0	2.0	3.0	0.05 ^a	107.2	95.3	0.03 ^a	99.6	100.3
Mean recovery (R.S.D.)			101.5 (5.7)	101.3 (3.8)	97.8 (3.2)	100.8 (4.3)	100.4 (1.8)	99.3 (2.3)

^a The predicted value from the calibration method when no pesticide is present.

Table 3
Influence of various parameters on the preconcentration recovery of pesticides using MWCNTs.

Parameter	Preconcentration recovery(%)		
	Atrazine	Methidathion	Propoxur
Eluent volume (ml)			
4.0	85.6	86.4	65.3
6.0	90.4	92.8	75.4
8.0	96.3	97.0	85.6
10.0	>99	>99	>99
12.0	>99	>99	>99
Flow rate (ml min ⁻¹)			
1.0	>99	>99	>99
3.0	>99	>99	>99
4.0	>99	>99	>99
5.0	95.3	>99	93.2
6.0	82.7	94.6	87.3
9.0	79.4	89.3	81.4
Sample volume (ml)			
200	n.d.	n.d.	n.d.
300	n.d.	94.3	n.d.
500	>99	>99	>99
600	>99	>99	>99
700	>99	>99	>99

Mass of extractant: 0.3 g, flow rate: 8.0 ml min⁻¹, elution flow rate: 1.0 ml min⁻¹, pH: 7.0, sample volume 500 ml, and pesticides concentration 5.0 µg l⁻¹. n.d.: not detected by the proposed method.

were tested for elution of pesticides from the surface of MWCNTs adsorbent. The results indicated that acetonitrile–methanol mixture (50:50, vol%) was the best reagent and, therefore, it was used as eluent in all extraction studies. Furthermore, the elution flow rate was kept constant at 1.0 ml min⁻¹ for all experiments. The amount of eluent used for pesticides elution has a high effect on recoveries of pesticides as indicated in Table 3.

As indicated in Table 3, for the three pesticides the preconcentration recoveries increased by increasing eluent volume until reaching the maximum recovery. Almost a complete elution of the pesticides was achieved using 10 ml volume of the eluent, therefore, this volume was used in subsequent extractions. The influence of sample flow rate on pesticides preconcentration was studied over the range 1.0–8.0 ml min⁻¹ and the results were summarized in Table 3. As shown in Table 3, almost a complete recovery was obtained within the flow rate 1.0–4.0 ml min⁻¹. At flow rates higher than 5.0 ml min⁻¹, the enrichment efficiencies were decreased for all pesticides. Based on that, a flow rate of 3.0 ml min⁻¹ was selected as the optimum one to achieve maximum pesticides enrichment and save analysis time. Sample volume is an important factor to be investigated when optimizing the extraction process. The influence of sample volume on pesticides enrichment was studied over the range 100–1000 ml while keeping the other factors constant; flow rate at 3.0 ml min⁻¹, eluent volume 10 ml, pH at 7.0, and elution rate at 1.0 ml min⁻¹. The results were given in Table 3. A complete recovery of pesticides was obtained for sample volumes between 500 and 700 ml. However, the method was ineffective for detection of pesticides when sample volume was between 200 and 300 ml. For complete recovery of pesticides with reasonable time, a 600 ml sample was chosen. Effect of pH on pesticides extraction and preconcentration was investigated over a wide range 3–8. pH range 5–7 was found to be optimum for complete recovery of pesticides from solution (the data are not provided).

3.9. Analytical characteristics of the proposed SPE-multivariate calibration and analysis of real water samples

Some important figures of merit were determined for the proposed SPE-multivariate calibration method. LOD (µg l⁻¹), corre-

lation coefficients r^2 , precision (as R.S.D., $n = 5$), and linear dynamic range (µg l⁻¹) were 3.0, 0.9975, 2.6, and 5.0–30 for atrazine and 2.0, 0.9988, 2.1, and 3.0–60 for methidathion, and 3.0, 0.9992, 2.5, and 5.0–40 for propoxur. Linear ranges for pesticides analysis were obtained by determining a set of purified water samples spiked with different trace levels of the pesticides covering the range 1.0–50 µg l⁻¹. A comparable linearity range was obtained for atrazine and propoxur, while the analytical range for methidathion was extended over higher concentrations. The detection limits of the pesticides as obtained from the multivariate calibration analysis were in the range 3–5 µg l⁻¹. Five replicate determinations of 2.5 µg of the pesticides present in 500 ml by the proposed method gave a satisfactory R.S.D. values (< 3%) which indicated the high precision of the proposed method. Obviously, the next step was to determine how well the proposed analytical method will work when applied to the analysis of pesticides in different water samples, such as tap and reservoir waters. Natural waters were carefully analyzed by the proposed analytical method and the results revealed the absence of the studied pesticides in the samples. Water samples (600 ml) were spiked with different levels of the three pesticides and then subjected to the SPE-PLS-1 proposed analytical method. The obtained recoveries of the pesticides from natural water samples were given in Table 4.

As indicated in Table 4, the recovery of the pesticides present in tap water was ranged from 94 to 103 (R.S.D.: 1–3), while a lower recovery range for pesticides was obtained in reservoir water 84–93 (R.S.D.: 1–3). Taking into account that no prior separation step was introduced in the analysis, the obtained recoveries were satisfactory which reflects the applicability of the proposed method for simultaneous pesticides determination in complex water samples. Relatively speaking, the obtained recoveries of the pesticides in reservoir water was lower compared to tap water system which was attributed to the high complexity of reservoir water sample and the presence of many interfering substances which affected the preconcentration of the pesticides by nanotube activated carbon and also reduced the resolution power of PLS-1 as these interferences were not accounted in the calibration step.

3.10. Chemometry against HPLC for pesticides analysis

The validity of the proposed chemometric method was further confirmed by analyzing the pesticides in extracted solutions using popular HPLC methodology. In fact, the three pesticides (atrazine, methidathion, and propoxur) were simultaneously determined using HPLC (UV-detection at 210 nm) after their solid-phase extraction using acid-treated-MWCNTs in our laboratory [34]. The analytical parameters obtained from the previous study were 0.9990 (r^2), 1.5 (R.S.D., $n = 5$), 0.057 µg l⁻¹ (detection limit), 10–100 µg l⁻¹ (linear range), and 93.1–108 (recovery from reservoir water) for atrazine. For methidathion: 0.9993, 1.4, 0.037, 10–50, and 95.7–104.0. For propoxur: 0.9740, 1.7, 0.22, 10–50, and 85.0–107.0. The comparison between HPLC results and the results given in Section 3.9 revealed that both methods gave similar linearity ranges for methidathion and propoxur, while a wider analytical range was observed for atrazine (10–100 µg l⁻¹) using SPE-HPLC method. On the other hand, a much lower detection limit for pesticides was obtained by SPE-HPLC method, for example, the obtained detection limit of methidathion using SPE-HPLC method was 54-fold lower than that obtained from SPE-PLS-1 method. The low detection limits reported in SPE-HPLC was expected because solutes were completely separated before being analyzed, while no separation was attempted in analysis by SPE-PLS-1 method which lowers the sensitivity of the analyses. The results obtained for pesticides determination by SPE-PLS-1 were compared to those obtained from SPE-HPLC method. In tap water, the recovery results were almost similar for atrazine and propoxur, while a better recovery for

Table 4
Determination of pesticides in different mixtures after preconcentration with MWCNTs using PLS-1 calibration method.^a

Mixture	Added ($\mu\text{g l}^{-1}$)	Tap water		Reservoir water	
		Found ($\mu\text{g l}^{-1}$)	Recovery (R.S.D) ^b	Found ($\mu\text{g l}^{-1}$)	Recovery (R.S.D) ^b
Atrazine	5.0	5.2	104.2 (1.2)	4.2	84.3 (1.7)
Methidathion	5.0	4.8	97.0 (2.2)	4.5	89.6 (2.2)
Propoxur	5.0	4.7	95.2 (1.1)	4.6	90.9 (1.4)
Atrazine	30	28.3	93.8 (1.2)	26.4	88.3 (3.2)
Methidathion	10	9.5	95.3 (2.8)	9.1	91.4 (0.9)
Propoxur	10	10.3	103.0 (2.1)	8.9	89.3 (2.1)
Atrazine	20	19.3	96.7 (1.4)	18.5	93.0 (1.2)
Methidathion	50	47.4	95.2 (0.9)	43.0	86.4 (2.3)
Propoxur	20	20.2	101.3 (3.1)	17.4	86.6 (2.8)
Atrazine	30	29.2	96.8 (1.7)	26.4	88.0 (1.8)
Methidathion	60	57.6	96.4 (2.3)	55.6	93.3 (2.3)
Propoxur	40	38.4	96.1 (3.1)	34.0	84.7 (3.4)

^a Sample volume: 600 ml, eluent volume: 10.0 ml, elution rate: 1.0 ml min⁻¹, flow rate: 3.0 ml min⁻¹, and pH 7.0.

^b Average of three trials.

methidathion was observed using chemometric method (95–97%). In reservoir water case, SPE–HPLC method and for the three pesticides recoveries were more satisfactory. Compared to SPE–HPLC method, the proposed SPE–PLS-1 method was rapid, easy and of low cost for the quantification of toxic pesticides in water using simple UV-spectrophotometry. The current proposed method could be used for the screening of atrazine, methidathion, and propoxur in natural waters (e.g., in situ analyses) or as a quantification method in cases where the chromatographic ones cannot be implemented owing to cost limitations or even lack of analytical instrumentation.

4. Conclusions

A simple analytical method was developed for simultaneous determination of atrazine, methidathion, and propoxur in real water samples without the need for chromatographic separation. PLS-1 calibration method was found effective for simultaneous determination of pesticides in reservoir water, the mean recoveries and R.S.D. values of 84–94 and 1.0–3.5%. Multiwalled carbon nanotubes adsorbent was found a perfect extractant for preconcentration of trace levels of pesticides. With the aid of MWCNTs adsorbent and multivariate calibration, the pesticides were reproducibly detected with detection limits of 3, 2, and 3 $\mu\text{g l}^{-1}$ and linear ranges of 5–30, 3–60, and 5–40 $\mu\text{g l}^{-1}$ for atrazine, methidathion, and propoxur; respectively. PLS-1 calibration offers a simple and a reasonable substitute for expensive liquid chromatography for pesticides quantification in reservoir water.

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