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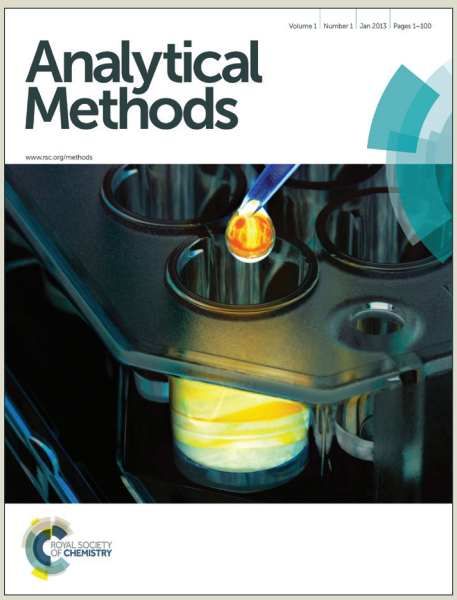

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ARTICLE TYPE

Preparation and application of carbon nanotube reinforced polyamide-based stir bar for sorptive extraction of naproxen from biological samples prior to its spectrofluorometric determination

Zahra Ayazi[a], Parvin Rafighi[b]

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A novel method for simple, fast and reproducible preparation of reinforced polyamide (PA)- based stir bar was presented. The carbon nanotube/polyamide stir bar was prepared by dispersion of appropriate amount of oxidized multiwalled carbon nanotube (MWCNTs) in a solution of PA in formic acid (FA) followed by dipping the stainless steel substrate of stir bar into the prepared solution. Finally, dipping the CNTs/PA coated substrate into the water causes the diffusion of formic acid to the water and remaining CNTs/PA forms a homogenous layer on stainless steel substrate. The surface characteristic of PA and CNTs/PA coated stir bars was investigated using scanning electron microscopy (SEM). The prepared CNTs/PA nanocomposite-based stir bar was used as an extraction device for stir bar sorptive extraction (SBSE) of naproxen (NAP) from biological samples followed by fluorimetric detection. The effect of CNTs doping level on the extraction capability of prepared coating was investigated. Important parameters influencing the extraction and desorption processes including extraction time, salt content, pH, desorption solvent, its volume and desorption time were optimized using response surface methodology (RSM). Limit of detection (LOD) was 2 ng mL^{-1} and the method precision (RSD %) with three replicates was 3.4 %. The linearity of method was 7-1000 ng mL^{-1}. The reproducibility of the presented method was evaluated by preparing eight stir bars using unit solvent and performing eight extractions from the aqueous solution applying different stir bars at the same extraction and desorption condition. The obtained RSD % was 3.0 % at 1 µg mL^{-1}. The developed method was successfully applied to the biological and tap water samples, while the relative recovery percentages (RR %) obtained to be 99%, 96 % and 95 % for urine, plasma and tap water samples respectively.

Introduction

Stir bar sorptive extraction (SBSE) was first introduced by Baltussen et al.[1] in 1999 as a novel and improved sample preparation technique based on the same principles of solid phase microextraction (SPME). SBSE has been successfully applied to monitor traces of priority organic compounds in many complex matrices[2,3], due to the very high exhibited sensitivity. These enrichment methods combine the extraction and concentration of the analytes simultaneously, having the possibility to use immersion or headspace sampling modes in only a single step. On the other hand, they reduce the manipulation and the overall time required for sample preparation. Furthermore they can be combined with the modern high sensitive instruments easily. SBSE presents advantages over liquid–liquid extraction because no solvent is used and small sample volumes can be analyzed. With respect to SPME, lower detection limits (sub-ng L^{-1} to ng L^{-1}), higher capacity, and better recoveries can be achieved. SBSE is also characterized by its high reproducibility due to minimum sample preparation and manipulation[4]. Nevertheless, once commercial polydimethysiloxane (PDMS) coated stir bars still be the most used, this option is one of the main drawbacks of SBSE, especially for the enrichment of the more polar compounds[5]. For this reason, several solutions have been proposed in the last few years, including multi-mode assays, derivatisation procedures and application of more selective sorbent phases[6-9]. Moreover, physical damage of the coating due to the direct contact with the bottom of the sample vial has also been claimed by certain authors[10], although not observed in most cases.

Recent advances in producing nanostructured materials with novel material properties have stimulated research to create multi-functional macroscopic engineering materials by designing structures at the nanometer scale. Nanocomposites can be

considered solid structures with nanometer-scale dimensional repeat distances between different phases that constitute the structure. These materials typically consist of an inorganic (host) solid containing an organic phase or vice versa. Or they can be consisting of two or more inorganic or organic phases in some combination form with the constraint that at least one of the phases or features is in the nanometer size. The nanocomposite filler can be particles (silica, metal, and other organic and inorganic particles), fibrous materials (nanofibers and nanotubes) and layered materials (graphite, layered silicate, and other layered minerals). In general, nanocomposite materials can demonstrate different mechanical, electrical, optical, electrochemical, catalytic and structural properties than those of individual components. The multifunctional behaviour of any specific property of the material is often more than the individual sum of the individual components. A morphological characteristic that is of fundamental importance in the understanding of the structure–property relationship of nanocomposites is the surface area/ volume ratio of the reinforced materials[11].

CNTs possess high flexibility, low mass density, large aspect ratio (typically>1000), extremely high tensile moduli and strengths. Indeed, this combination of mechanical and electrical properties of individual nanotubes makes them the ideal reinforcing agent in a number of applications. Because of the larger specific area and hydrophobic characteristic of the surface, CNTs have been regarded as an efficient sorbent and have been studied for preconcentration of many pollutants. MWCNTs have been characterized as superior sorbent for removing dioxins for environmental protection[12]. It is believed that reasons for its adsorption may be primarily due to their highly hydrophobic surface and unique structure with internal tube cavity and interstitial spaces between tubes. It was reported that multi-walled carbon nanotubes (MWCNTs) surface has a strong interaction with many phenolics and phthalate compounds[13]. MWCNTs had a high effective enrichment of atrazine and simazine in environmental waters[14]. Other recent studies involved CNTs for enrichment of chlorophenols[15], chlorobenzenes[16], phthalate esters[17], sulfonylurea herbicides[18], cyanazine, chlorotoluron and chlorbenzuron[19], triasulfuron and bensulfuron-methyl in water samples[20], polybrominated diphenyl ethers in milk samples[21], dicamba herbicide[22], organophorous pestiside[23], triazines, and aryloxyphenoxy propionic acid pesticides[24], chelates and organometallic compounds[25].

In this study a new simple method was developed to prepare CNTs/polyamide nanocomposite coated stir bar to overcome the discussed drawbacks of PDMS based commercial stir bars. Polyamide was applied as nanocomposite bulk polymer due to its polar characteristics and feasibility of coating methods of nanocomposite on stir bar substrate. In addition the prepared stir bars show more mechanical stability and long lifetime. Combination of advantages of PA as hosting polymer and carbon nanotubes as reinforcing agent makes the prepared nanocomposite as a high efficient extracting medium for SBSE which can create a novel aspect in this field. The applicability and capability of prepared novel stir bar was examined by sorptive extraction of naproxen from biological samples.

Naproxen (6-methoxy-α-methyl-2-naphthalene acetic acid) is a non-steroidal anti-inflammatory drug (NSAID) and was widely used to moderate pain relief in the treatment of many diseases[26]. Anti-inflammatory effects of naproxen are generally thought to be related to its inhibition of cyclo-oxygenase and consequent decrease in prostaglandin concentrations in various fluids and tissues[27]. Its chronic or acute administration shows toxic manifestations generally characteristic of NSAIDs, such as gastrointestinal erosion, bleeding and kidney failure[28]. Moreover, it may also increase the risk for cardiovascular events[29]. The development of a simple and sensitive method for the determination of naproxen in pharmaceuticals and biological fluids could be very useful for toxicological purposes. The analysis of naproxen has been generally based on a preconcentration step followed by chromatographic separation and detection. Several methods have been reported for the determination of this drug, including solid-phase extraction (SPE)-chromatographic/photometric determination, solid-phase microextraction coupled to liquid chromatography, fluorimetric determination, and liquid chromatography–mass spectrometry[30,31]. Also spectrophotometric methods including spectrofluorimetric and chemiluminescence methods were applied for determination of naproxen[32-35].

Experimental

Reagents and standards

MWCNTs with purity higher than 95 %, length of 1-10 µm and number of walls in the range of 3 to 15, were obtained from Plasma Chem GmbH (Germany). Naproxen, sodium chloride, acetonitrile, nitric acid, formic acid (FA), 1,4-dioxane, chloroform, dichloromethane, formamide and sodium dodecylsulfate (SDS) were supplied from Merck (Darmstadt, Germany). Methanol, ethanol and hydrochloric acid (HCl) were purchased from Sigma Aldrich (Mississauga, Canada). Potassium hydroxide (KOH) was supplied from Fluka (Buchs, Switzerland). Polyamide (nylon 6) was purchased from Kolon industries Inc. (Korea).

Instrumentation

A Varian Cary Eclipse fluorescence spectrophotometer (Springvale, Victoria, Australia) equipped with 1 cm × 1 cm quartz cell and a xenon flash lamp was used for recording the fluorescence spectra. Spectra recording were carried out with the excitation and emission slit widths of 5 nm and the excitation and emission wavelengths of 271 and 354 nm, respectively. The PMT detector was used for recording the emission lines and set on 600 V. A hot plate magnetic stirrer, Snijders 34532 (Tilburg, Holland), was used to reflux CNTs in acidic medium. An ultrasonic bath, Fungilab (Barcelona, Spain) was used to dispersion of CNTs in formic acid. Extractions were performed using Metrohm 758 stirrer (Herisau, Switzerland) and for adjusting the pH of samples a Metrohm 691 pH meter was used.

In order to characterize the morphological properties of prepared nanocomposites the SEM images were obtained by a scanning electron microscope LEO 1430 VP (Germany).

Real sample preparation

Human urine sample was collected from a healthy individual.

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Table 1. Actual and coded values of independent variables applied for response surface method.

	Effect	Symbol	-α	-1	0	+1	+α
Esxtraction step	Extraction time (min)	x_1	9.54	30	60	90	110.45
	NaCl %	x_2	5.56	10	16.5	23	27.43
	pH	x_3	0.61	3	6.5	10	12.38
Desorption step	Solvent volume (mL)	x_1	0.59	0.75	1.125	1.25	1.66
	Desorption time (min)	x_2	5.86	10	20	30	34.14

The urine sample was diluted 1:1 with water to minimize the matrix effects. Plasma sample were obtained from a healthy human subject in a local hospital and was stored at −4 ◦C until analysis. A plasma standard was prepared by adding appropriate amount of aqueous working standard to the drug-free plasma solution to yield the final desired concentrations.

The sampling procedure was carried out according to the guidelines for research ethics. All experiments on human subjects were performed in compliance with the relevant laws and institutional guidelines approved by the Medical Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran. Also written consent was obtained from volunteers prior to the experiment.

Preparation of CNTs/PA coated stir bar

First for oxidation of MWCNTs, predetermined quantities of raw MWCNTs (0.1 g) and 50 mL nitric acid were added into a round-bottomed glass flask, and the MWCNTs mixture was sonicated for 5 min in an ultrasonic bath. Next the mixture was refluxed at 100 °C for 6 h. After dilution of the mixture with distilled water, it was filtered and washed to neutral pH, dried at 40 °C for 12 h, and weighed.

For stir bar preparation, the stainless steel wires with length of 2 cm and thickness of 2 mm were prepared and were washed with acetone in ultrasonic bath for 30 min. Appropriate amount of oxidized and pristine MWCNTs were dispersed in a 2 mL SDS solution (1 %) of formic acid for 2 h under sonication to prepare the MWCNTs suspension. Afterward 0.2 g PA was added to the CNTs suspension and was sonicated for further 1 h. The pretreated stir bar substrate was immersed into the MWCNTs/PA suspension for a few seconds, and then it was immersed in double distilled water. This procedure led to production of a layer of CNTs/PA nanocomposite which coated on the stainless steel substrate.

The SBSE procedure

In a typical assay, 10 mL of distilled water spiked at the 1 µg mL^{-1} level was introduced into a glass vial, a prepared stir bar was immersed and the vial closed. The extraction was performed on stirrer for 30 min. After the extraction, for liquid desorption (LD) purposes, the stir bar was removed with clean tweezers, dried with a lint-free tissue, placed into a desorption vial containing 1 mL of the desorption solvent, ensuring its total immersion prior to stirring for 30 min at room temperature. To guarantee a total desorption of the analytes it was necessary that the polymer be completely deep in the solvent. After back-extraction, the stir bar was removed using a magnetic rod and washed with methanol and water. Finally desorption solvent was introduced to the fluorescence cell and determination was performed at excitation wavelength of 271 nm and emission wavelength of 354 nm.

Experimental design

In SBSE method there are two separated steps, extraction and back extraction. These two steps are affected by some factors, which can be considered separately, therefore in this study first the extraction condition was studied and afterward the parameters affecting the solvent desorption process was optimized using central composite design (CCD). Three parameters affecting the extraction condition (the number of factors, k =3) have been introduced as RSM input variables including salt content, pH and extraction time, which their experimental ranges in coded and actual values are presented in Table 1. The extraction recovery was calculated as response of model. Also, for studying the desorption step, two influencing factors (solvent volume and desorption time) are selected as RSM input variables and their coded and actual values are reported in Table 1. The desorption recovery was calculated as response of model.

Central composite design, used extensively in RSM experimental design, was employed to evaluate the individual and interactive effects of controllable variables on the output response. CCD for studying the extraction process, with three input variables consists of 20 experiments with 8 (2k) orthogonal two levels full factorial design points (coded as ±1), 6 (2k) axial points (or star point coded as ±α = 1.68179) and 6 replications of the central points to provide an estimation of the experimental error variance. CCD for studying the desorption process, with two factor consists of 14 experiments with 4 full factorial design points (coded as ±1), 4 axial points (coded as ±α = 1.41421) and 6 replications of the central points. The design of experiments and experimental data analysis were performed using Minintab 15 software.

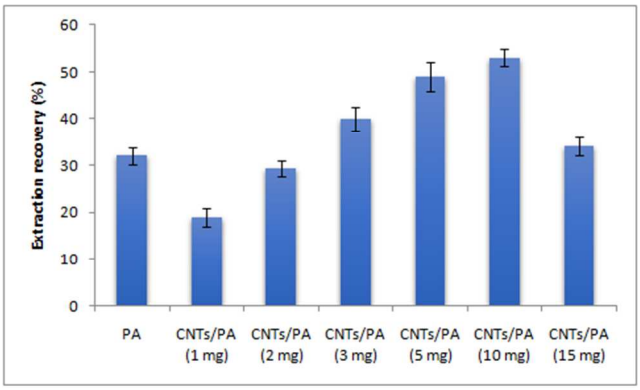

Fig. 1 Effect of CNT doping level on prepared stir bar extraction capability. Extractions were performed using 10 mL sample solution at concentration of 1 μL mL^{-1}, for 30 min. Desorption was performed using 1 mL of methanol for 30 min.

Results and discussion

Effect of CNT doping level

The effectiveness of carbon nanotube as a reinforcing filler in polymeric matrix depends not only on their content within the hosting system according to traditional micro-mechanics of composites but also by the level of dispersion within the final nanocomposite. In a CNT/polymer composite, aggregation and agglomeration of CNTs, due to high surface free energy and existence of inter-tube van der Waals forces, is presently a major obstacle for the realization of their technological potential[36]. To obtain processable CNT, two important approaches have been reported (i) covalent attachment of CNT with alkyl chains[37] and (ii) CNT/polymer composite preparation[38]. Among these, covalent chemical approach is known to disrupt the extended p-network at CNT surfaces resulting in their poor mechanical and electrical properties[39]. On the other hand, CNT/polymer composite preparation via non-covalent supra-molecular approach helps to use their unique properties and makes them attractive building blocks for development of novel nanomaterials for desired applications. This approach involves the wrapping of CNT with high molecular weight polymers which could disrupt the van der Waals interactions without affecting the p-network of CNT that would otherwise cause the aggregation of CNT into bundles. This leads to the uniform dispersion of CNT in a desired polymer matrix.

The effect of oxidation of CNTs on extraction efficiency was evaluated by preparation of a stir bar which coated by 10 mg pristine CNTs doped nanocomposite. The result shows that oxidized CNTs has led to better extraction efficiency due to the better dispersion of CNTs in polymer solution and also due to the introduction of polar moiety to the CNTs structure in oxidation process.

To investigate the effect of CNTs doping level on the CNTs/PA nanocomposite extraction capability, the composites were prepared with CNTs doping level of 0, 1, 2, 3, 5, 10 and 15 mg (0, 0.25, 0.5, 0.75, 1.25, 2.5 and 3.75 % w/w). Afterward each prepared stir bar was applied for extraction of NAP from spiked double distilled water. According to the results depicted in Fig. 1, it can be observed that the CNTs doping level has an important role in the sorbent extraction ability. Adding CNTs at the doping level of 1 mg to the polyamide, causes a decrement in extraction capability, after that increasing the CNTs doping level leads to an improvement in extraction efficiency, while the increase of doped CNTs amount, above 10 mg has a negative influence on the extraction capability. These results are a bit contradictive considering our last experiences[11,24]. The surface characteristic of PA and CNTs/PA nanocomposites is investigated using SEM (Fig. 2). According to the SEM images of prepared nanocomposites, it can be observed that CNTs are not in the nanocomposte surface. Therefore they can not participate in the extraction of naproxen directly. But they can increase the specific surface area and then increase the extraction efficiency. Considering Fig. 2b and 2c, it is clear that adding CNTs to the polyamide bulk leads to a morphological change of polymer. There are a lot of cavities in the structure of bulk polyamide (Fig. 2b) which are eliminated by adding CNTs (Fig. 2c). Therefore presence of CNTs in low doping level in nanocomposite leads to decrease of specific surface area and decrease of extraction capability. Increasing the CNTs doping level up to 10 mg causes to an improvement in morphological characteristics and leads to increase of extraction efficiency (Fig. 2e). In the case of CNTs doping level of 15 mg, no more improvement in surface characteristics was observed, which might be due to agglomeration of CNTs bundles in higher CNTs doping level[40].

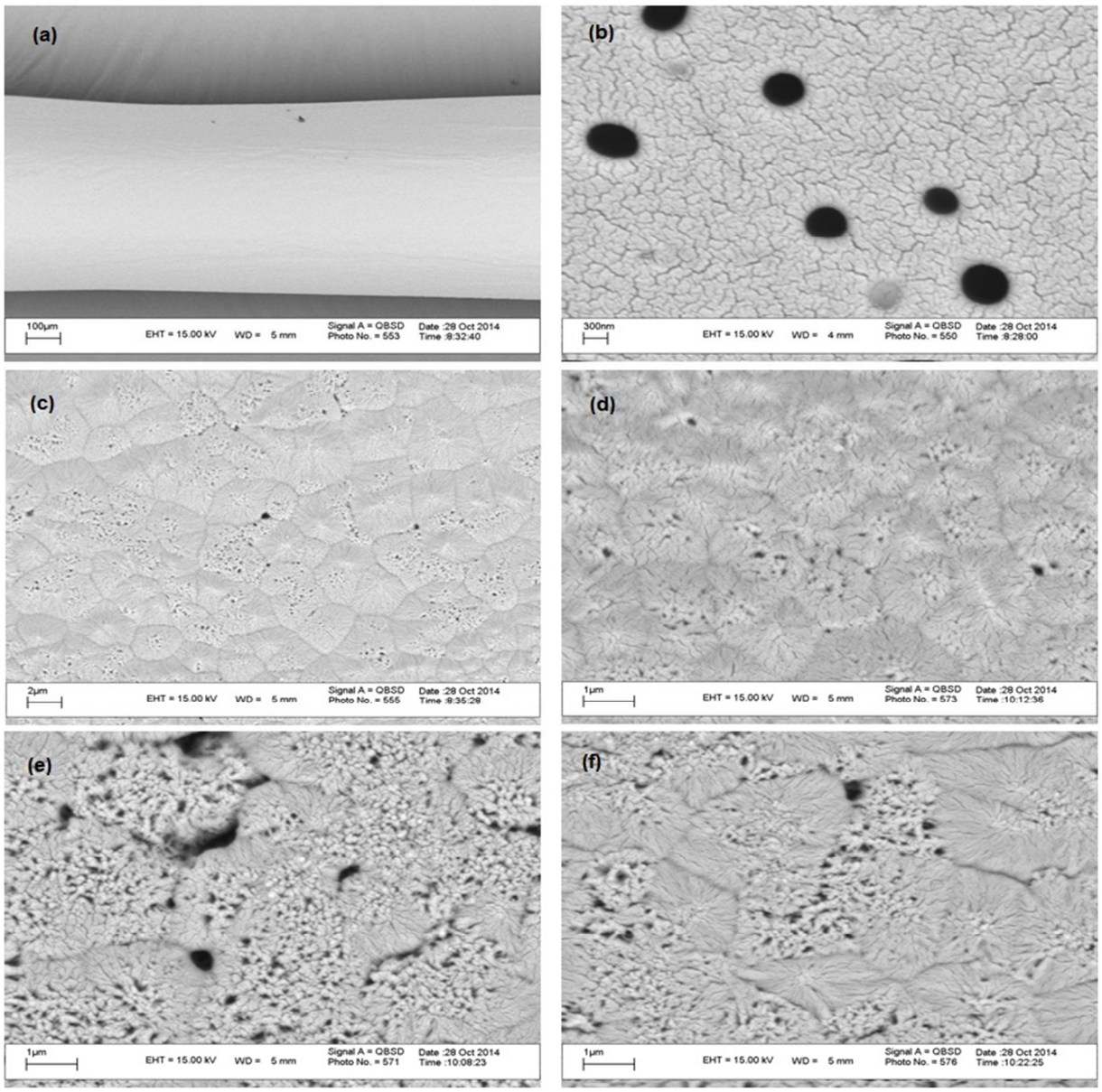

Fig. 2. SEM image of (a) prepared stir bar with the coating of CNTs/PA with CNT doping level of 1 mg (b) bulk PA, (c, d) CNTs/PA with CNT doping level of 1 mg, (e) CNTs/PA with CNT doping level of 10 mg and (f) CNTs/PA with CNT doping level of 15 mg.

Table 2. Analysis of variance (ANOVA) for the developed models.

	Extraction step					Desorption step				
Source	DF	Seq SS	Adj MS	F-value	P-value	DF	Seq SS	Adj MS	F-value	P-value
Regression	9	12185.3	1353.92	18.88	0.000	5	2713.4	542.67	9.98	0.004
Residual error	7	502.0	71.72	-	-	7	380.6	54.37	-	-
Lack-of-fit	2	197.0	98.50	1.61	0.288	3	140.4	46.80	0.78	0.564
Pure error	5	305.0	61.00	-	-	4	240.2	60.05	-	-

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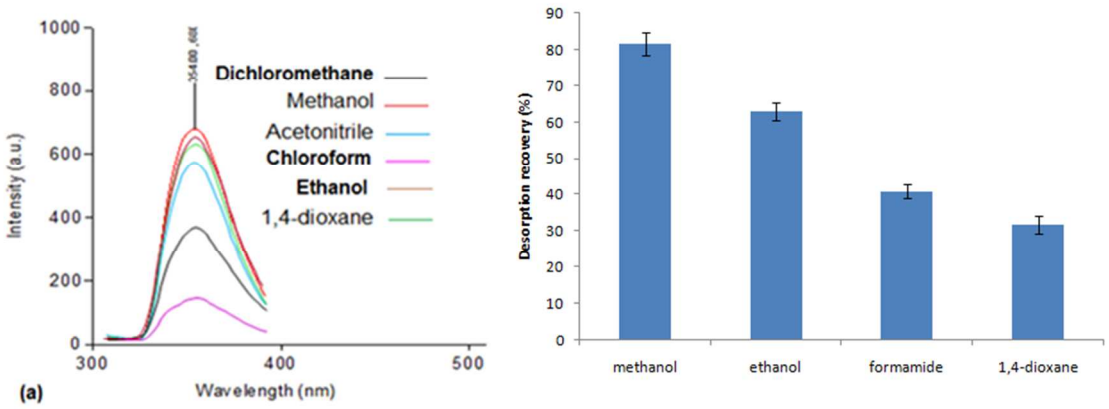

Fig. 3. (a) Quenching effect of various solvents on NAP fluorimetric spectra at concentration of 1 µg mL^{-1}. Influence of various solvents on the desorption efficiency. Extractions were performed using 10 mL sample containing analytes at level of 1 µL mL^{-1}, for 30 min. Desorption was performed using 1 mL of various solvents.

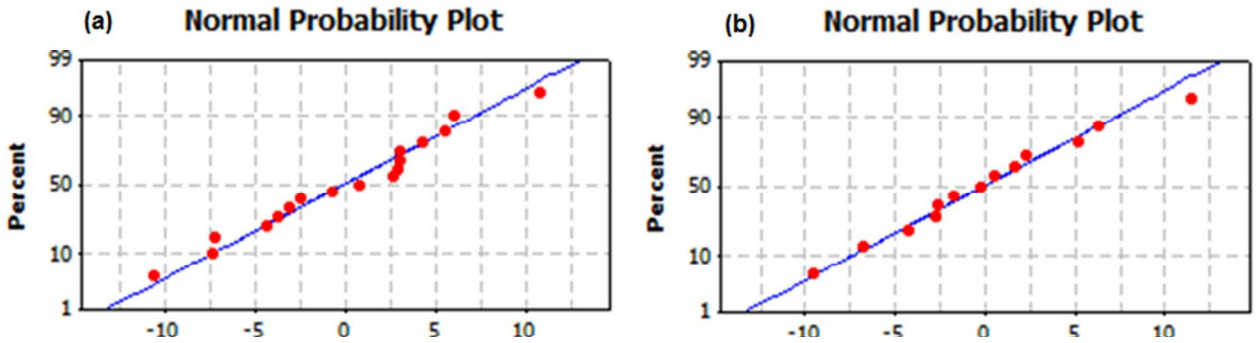

Fig. 4. Normal probability plots of residuals for (a) extraction recovery of NAP by CNTs/PA stir bar and (b) desorption recovery of NAP from stir bar to methanol.

Desorption solvent

Parameters affecting the desorption efficiency are desorbing solvent, its volume and desorption time, which desorbing solvent was studied individually where two remaining factors are investigated using central composite design. The desorption solvent should be able to displace the targeted analytes from the sorbent in a minimum volume. If the hydrophobic interactions cause to the retention of analytes, non-polar solvents would be able to disrupt the forces that bind the analytes to the sorbent. Different organic solvents with variety of functionality and polarity were used to investigate the optimum desorption solvent. First the quenching effect of various solvents including: ethanol, methanol, formamide, acetonitrile, 1,4-dioxane, chloroform and dichloromethane was studied. According to the results shown in Fig. 3a, a significant quench effect was observed when acetonitrile, dichloromethane and chloroform were utilized. Then, for remaining solvents the desorption recoveries were evaluated. The extractions were performed using 10 mL of aqueous sample spiked with NAP at concentration level of 1 µg mL^{-1}, maximum rate of stirrer, room temperature without addition of salt for 30 min. The volume of applied desorption solvent was 1 mL. As shown in Fig. 3b methanol has led to the highest desorption efficiency and was chose as desorption solvent for further experiments.

RSM models development

Generally, a second-order (quadratic) polynomial response surface model (Eq. (1)) can be used to fit the experimental results obtained by CCD. This model, also known as regression equation, describes a polynomial approximation of experimental results with following relationship (if k = 3):

$$\hat{y} = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$$

Eq. (1)

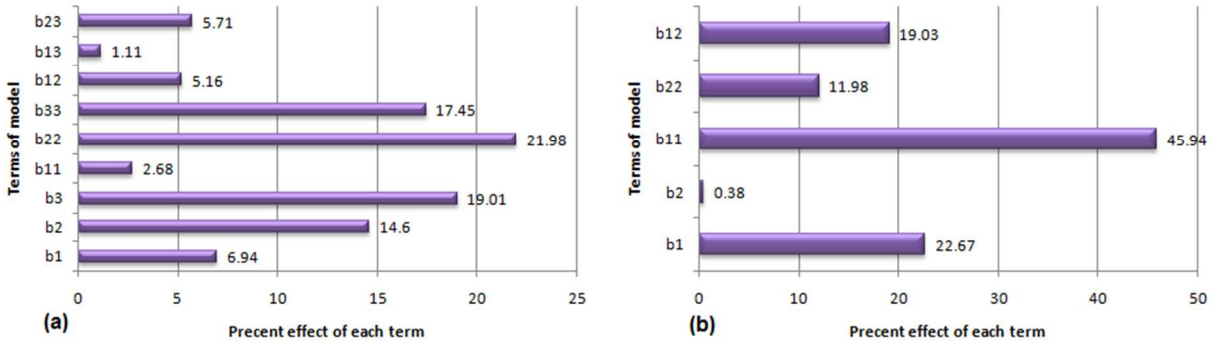

Fig. 5. Percentage effect of each model term obtained using Pareto analysis (a) extraction step (b) desorption step.

where $\hat{y}$ is the predicted response, x_i denotes the coded levels of the factors, b_0 is a constant and b_i, b_{ii} and b_{ij} are the regression coefficients for the linear, quadratic and interaction effects, respectively. These coefficients were calculated by means of ordinary least square.

The regression equation with coded variables for describing the extraction recovery of naproxen from aquatic sample using CNTs/PA stir bar can be presented as follow:

$$y = 80.725 + 22.664x_1 - 32.870x_2 - 37.506x_3 - 14.084x_1^2 + 40.327x_2^2 - 35.934x_3^2 - 19.553x_1x_2 - 9.088x_1x_3 - 20.572x_2x_3$$

Eq. (2)

Where, x_1, x_2 and x_3 are extraction time, salt content and pH respectively. Also, the back extraction or desorption recovery of naproxen based on experimental design results can be modelled as:

$$y = 51.840 - 9.904x_1 + 1.282x_2 + 14.099x_1^2 + 7.199x_2^2 + 9.075x_1x_2$$

Eq. (3)

Where x_1 is solvent volume and x_2 is desorption time. The significance and adequacy of the model was evaluated by analysis of variance (ANOVA) and the obtained results are presented in Table 2. The significance of the second-order regression models were determined by the Fisher's variance ratio test (F-test) and lack of fit (LOF) test. According to the results (Table 2), in both processes, the Fisher F-values of regressions, defined as the ratio of mean square of the regression due to the residual error, were much higher than the tabulated F-value (3.68 and 3.79 at 95 % significance for extraction and desorption steps respectively). If the regression model predicts the experimental results suitably, F-value should be higher than the tabulated value. Furthermore, P-values lower than 0.05 (at the significance level of 95%) confirm that the regression model is statistically significant.

Table 3. Estimated model coefficients for extraction and desorption process and corresponding T and P values.

Extraction step					Desorption step				
Coefficient	Coefficient estimate	t- value	P-value	Remark	Coefficient	Coefficient estimate	t- value	P-value	Remark
b_0	80.725	23.365	0.000	-	b_0	51.840	15.720	0.000	-
b_1	22.664	4.945	0.002	S	b_1	-9.904	-3.799	0.007	S
b_2	-32.870	-3.553	0.009	S	b_2	1.282	0.492	0.638	-
b_3	-37.506	-6.467	0.000	HS	b_{11}	14.099	5.043	0.001	S
b_{11}	-14.084	-2.161	0.068	-	b_{22}	7.199	2.575	0.037	S
b_{22}	40.327	3.208	0.015	S	b_{12}	9.075	2.461	0.04	S
b_{33}	-35.934	-5.514	0.001	S					
b_{12}	-19.553	-1.767	0.121	-					
b_{13}	-9.088	-0.821	0.439	-					
b_{23}	-20.572	-1.898	0.099	-					

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In both developed models for extraction step and desorption step, the P-values were 0.000 and 0.004 respectively, which are much lower than 0.05. The other criterion for evaluating the model was LOF test which is performed by comparing the residual with pure error achieved from the replicated design points at the central level of variables. In this case, if the F-value of LOF is lower than tabulated value or the related P-value is greater than 0.05 (at the significance level of 95%), the regression will be adequately significant. Considering the ANOVA results indicated in Table 2, the P-values of LOF test were 0.288 and 0.564, for extraction and back extraction steps respectively, which confirm the insignificant lack of fit.

Additionally, for evaluating the significance of the developed models, the difference between experimental and predicted responses (residuals) can be applied for investigating the adequacy of the model graphically. Residuals are considered as unexplained variations by model and they will occur based on a normal distribution, if the model is a good predictor[41]. Normal probability plots of residuals for extraction and desorption describing models are depicted in Fig. 4. As shown the points in normal plot of residuals for both models, generally form a straight line, which indicate the residuals are randomly distributed.

Effect of variables on extraction efficiency

Determination of the important and effective terms of the developed models was performed considering the corresponding values of Student's t distribution and related P-values (Table 3). The greater T-value and/or the smaller P-value (less than 0.05 at 95 % significance) for a coefficient show the more significant influence of the coefficient[42]. The P-value greater than 0.05 is unmeaning, because it shows that the related coefficient is insignificant in the model. According to the Table 3, the insignificant coefficients in Eqs. (2) and (3) (the coefficient with P-value greater than 0.05) were eliminated and the mentioned equations were rewritten as Eqs. (4) and (5).

$$y = 80.725 + 22.664x_1 - 32.870x_2 - 37.506x_3 + 40.327x_2^2 - 35.934x_3^2$$

Eq. (4)

$$y = 51.840 - 9.904x_1 + 14.099x_1^2 + 7.199x_2^2 + 9.075x_1x_2$$

Eq. (5)

As shown in Eq. (4), the model for extraction step of SBSE consisted of three main effects and two curvature effect. Also there are one main effect, two curvature effect and one interaction effect in the model related to the desorption step of SBSE. In order to determination of significance of each factor, the percentage effect of each term on the response (known as Pareto analysis) can be calculated using the following equation[43]:

$$P_i = \frac{b_i^2}{\sum_{i=1}^{n} b_i^2} \times 100$$

Eq. (6)

Where P_i is the percentage effect of each factor and b_i is the corresponding coefficient. According to the results, shown in the Fig. 5a, the most effective term is related to the quadratic effect of salt content (b_{22}, 21.98 %). The main effect of extraction time (b_1, 6.94 %), salt content (b_2, 14.6%), pH (b_3, 19.01 %) and curvature effect of pH (b_{33}, 17.45 %) are also important. Extraction time is one of important parameters influencing SBSE which the effect of this factor can be indicated using the Pareto analysis and also response surface plot (Fig. 6). As shown in Fig. 6 extraction time has a positive effect on extraction recovery and increasing the extraction time causes to the increase of the extraction recovery. Salt content is shown as effective factor with negative sign which indicates the reduction of extraction recovery with increase of salt content. In general, inert salts such as sodium chloride are added during SBSE in order to modify the ionic strength of the sample solution. It has been observed that for hydrophobic analytes (log $K_{o,w}$>3) the addition of an inert salt does not improve, but even reduces, the extraction efficiency[44-46]. On the contrary, for polar analytes the response increases with the addition of inert salts[44,46]. To explain the decreased response obtained for non-polar solutes after salt addition, various hypotheses are given. According to some reports[45] the salt addition causes an "oil effect" that promotes the movement of non-polar compounds to the water surface, minimizing the interaction with PDMS-coated stir-bar. Similarly, other authors attribute such a decrease for hydrophobic analytes as a result of the increase of viscosity, which slows down the extraction kinetics of the compounds[44]. Since log $K_{o,w}$ = 3.18 for naproxen, it can be considered as a hydrophobic analyte and the addition of salt concentration leads to a decrease of extraction recovery.

Sample pH is an important variable during SBSE for those analytes with acidic or basic properties and, in that case, pH should be adjusted in order to obtain the solute partially or totally in the non-ionic form leading to the maximum extraction efficiency[47]. According to the Eq. 4 and response surface plots (Fig. 6), it is observed that pH is significant with negative sign. Naproxen is an acidic compound due to the presence of carboxylic moiety in its structure (pK_a = 4.15), then it is expected to show higher extraction recovery in lower sample pH.

In the case of desorption condition, the factors of solvent volume and desorption time were studied using CCD and the results are depicted in Fig. 7. According to the results shown in the Fig. 5b, the most effective term is related to the quadratic effect of solvent volume (b_{11}, 45.94 %). The main effect of solvent volume (b_1, 22.67 %), curvature effect of desorption time (b_{22}, 11.98 %) and interaction effect of solvent volume and desorption time (b_{12}, 19.03 %) are also important.

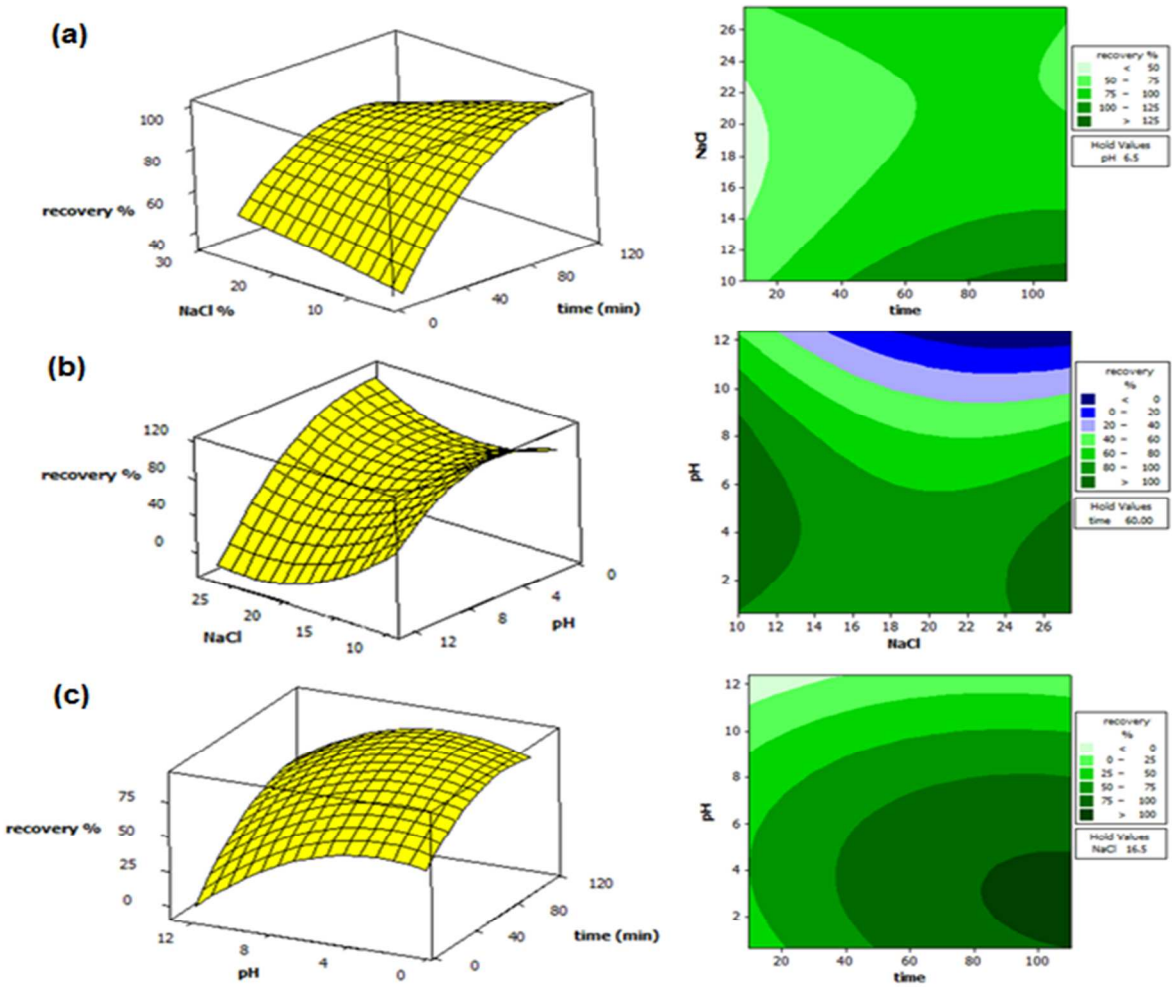

Fig. 6. Response surface and counter plots of predicted NAP extraction recovery as a function of (a) extraction time and NaCl content (b) NaCl content and pH (c) extraction time and pH, keeping other variables at central point levels.

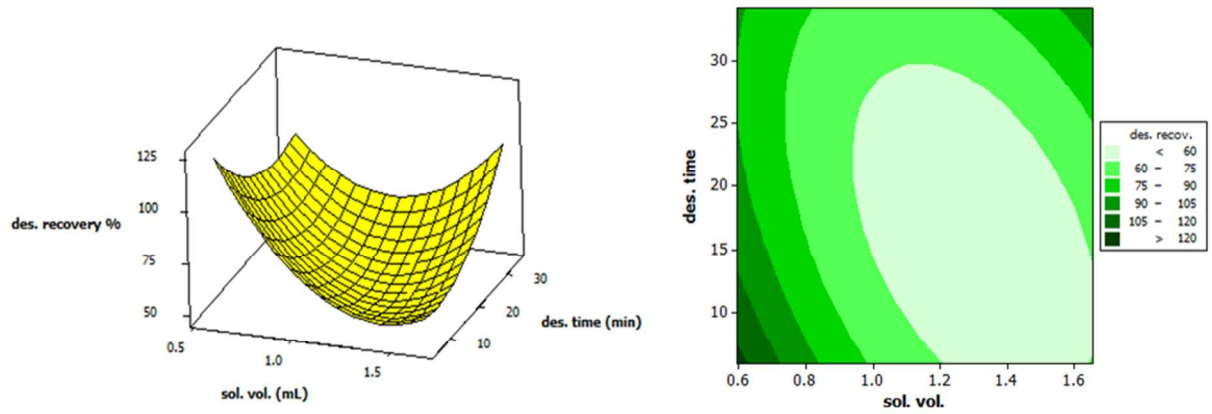

Fig. 7. Response surface and counter plots of predicted NAP desorption recovery as a function of solvent volume and desorption time.

Solvent volume has negative sign, showing decrease of fluorescence signal due to the decrease of naproxen concentration, but desorption time has positive sign which indicate the increase of desorption recovery with increase of the time. Finally the optimum conditions for the extraction and desorption step were determined using the Minitab software from the models developed by CCD to maximize the extraction and desorption efficiency. The optimum values for extraction time, salt content and pH are 50 min, 5.56 % and 1.4 respectively. For desorption step the optimum value for solvent volume is 0.6 mL

and for desorption time is 10 min.

Method validation

Based on the method development observed above, the optimum value for effective factors were selected and applied for the determination of naproxen in biological samples. Double distilled water spiked with NAP was used to evaluate the precision of the measurements, the limit of detection and the dynamic range of the method. The linearity of the method was studied by preparing the calibration curve for NAP. The obtained calibration graph is linear in the concentration range of 7-1000 ng mL^{-1}. The regression coefficient for NAP was rather satisfactory (R^2>0.994). Limit of detection based on $3S_b/m$ definition (where m is slope of the calibration curve and S_b is the standard deviation for four blank measurements) was 2 ng mL^{-1}.

The method precision (RSD %) with three replicates was 3.4 %. The presented method reproducibility was evaluated by preparing eight stir bars using unit solvent and performing eight extractions from the aqueous solution applying different stir bars. The obtained RSD % was 3.0 % at 1 µg mL^{-1}. The potential for carryover of analytes was investigated by desorption of washed stir bar after extraction of a spiked sample solution at level of 1 µg mL^{-1}. According to the results, there was no carryover effect.

Table 4 compares the results of this method with those obtained by other relevant reports for the determination of naproxen[30-35]. As compared in Table 4, the present method has lower detection limit and comparable linear range in compared with HPLC and pure spectrofluorometric methods for determination of naproxen. The major advantages of the current method are the method simplicity, speed and sensitivity toward naproxen.

Analysis of real samples

As most microextraction techniques, efficiency of extraction by SBSE can be affected by the composition of the matrix. High levels of dissolved or suspended organic matters and biomolecules contained in biological samples may compete with the analytes for the adsorption/absorption on/into stir bar or may disrupt analytes from extraction to the stir bar, thus the extraction yield might changes from sample to sample. Therefore, possible matrix effects were investigated by comparing the responses obtained for Milli-Q water and biological samples.

To evaluate the matrix effect and applicability of the developed method for real samples, extraction and analysis was performed on urine, plasma and tap water samples. In order to identify the influence of the matrix, the extraction recovery of NAP from real samples were compared with the extraction recovery of NAP from double distilled water sample. First 10 mL of each real sample were extracted under optimum condition without spiking in order to evaluate the presence of analyte in the real samples. Next the double distilled water sample, urine, plasma and tap water samples were spiked with 1 µg mL^{-1} of NAP and analyzed. Finally, relative recovery (RR) was calculated from the extraction recovery of the NAP in the spiked distilled water and real samples. Relative recovery percentages were obtained to be 99 %, 96 % and 95 % for urine, plasma and tap water samples respectively indicating negligible matrix effects for the urine, plasma and tap water samples. The developed method precision for determination of NAP in real water samples was calculated with three replicates. The obtained RSD % was 3.0 %, 3.7 % and 5.2 % for urine, plasma and tap water samples respectively.

Conclusions

The presented method is novel, simple and fast method for preparation of stir bars. The introduced stir bar can be a candidate to solve the drawbacks of commercial PDMS stir bars, due to the polarity of the coating. CNTs/PA coating show high mechanical stability and long lifetime, which can be applied more than 30 times for extraction of NAP. Moreover the CNTs doping in the bulk of PA can increase the specific surface area and therefore leads to higher extraction capability. Analytical data, confirm that CNTs/PA-based sorbent is a suitable candidate as a SBSE sorbent for extracting organic compounds from biological sample. Furthermore, compared to other commercial SBSE designs, this technique is inexpensive, simple and can be used for the determination of organic compounds with sufficient sensitivity and reproducibility.

Table 4. Comparison of the developed method with other reports for determination of naproxen.

Method	*Sample matrix*	*LOD (ng mL−1)*	*Linear range (ng mL−1)*	*Reference*
HPLC–ECL	Human urine	16	40–2000	[30]
CIM–SSF	Pharmaceuticals	110	500–20,000	[32]
MECC–LIF	Waste-water	70	100–2000	[33]
SPMTE–HPLC-UV	Human plasma	8	10–10,000	[31]
MIPMCNTs–FL	Human urine	2	4–40	[34]
CS–PPy–MNPs–FL	Human urine and plasma	15	40–1000	[35]
CNTs/PA-SBSE-FL	Human urine and plasma	2	7-1000	Current method

ECL: electrogenerated chemiluminescence.
CIM: complex imprinted membrane; SSF: solid surface fluorescence.
MECC: micellar electrokinetic capillary chromatography; LIF: laser induced fluorescence.
SPMTE: solid phase membrane tip extraction.
MIPMCNTs: molecular imprinted polymer based on suspension polymerization on magnetic multi-walled carbon nanotubes; FL: fluorescence spectrometry.

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Notes and references

[a] *Department of Chemistry, Faculty of Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran, Fax: +98 41 34327541; Tel: +98 41 34327500 , E-mail: ayazi@azaruniv.edu*

[b] *Department of Chemistry, Institute for Advanced Studies in Basic Sciences (IASBS),Zanjan, Iran, Fax: +98 24 33153232; Tel: +98 24 33153101, Email: rafighi@iasbs.ac.ir*

1 E. Baltussen, P. Sandra, F. David, C. Cramers, *J. Microcolumn Sep.* 1999, 11, 737.
2 E. Pérez-Carrera, V.M.L. Len, A.G. Parra, E. Gonzalez-Mazo, *J. Chromatogr.* A 2007, 1170, 82.
3 P. Serodio, J.M.F. Nogueira, *Anal. Chim. Acta* 2004, 517, 21.
4 J. Sanchez-Avila, J. Quintana, F. Ventura, R. Tauler, C.M. Duarte, S. Lacorte, *Mar. Poll. Bull.* 2010, 60, 103.
5 N. Fontanals, R.M. Marcé, F. Borrull, *J. Chromatogr. A* 2007, 1152, 14.
6 X. Huang, N. Qiu, D. Yuan, Q. Lin, *J. Chromatogr. A* 2009, 1216, 4354.
7 X. Huang, J. Lin, D. Yuan, R. Hu, *J. Chromatogr. A* 2009, 121, 3508.
8 N.R. Neng, M.L. Pinto, J. Pires, P.M. Marcos, J.M.F. Nogueira, *J. Chromatogr. A* 2007, 1171, 8.
9 A.R.M. Silva, F.C.M. Portugal, J.M.F. Nogueira, *J. Chromatogr. A* 2008, 1209, 10.
10 C. Yu, Z. Yao, B. Hu, *Anal. Chim. Acta* 2009, 641, 75.
11 H. Bagheri, Z. Ayazi, A. Aghakhani, *Anal. Chim. Acta* 2011, 683, 212.
12 R.Q. Long, R.T. Yang, J. Am. Chem. Soc. 2001,123, 2058.
13 Y.Q. Cai, G.B. Jiang, J.F. Liu, Q.X. Zhou, *Anal. Chem.* 2003, 75, 2517.
14 Q.X. Zhou, W.D. Wang, J.P. Xiao, J.H. Wang, G.G. Liu, Q.Z. Shi, G.L. Guo, *Microchim. Acta* 2006, 152, 215.
15 Y.Q. Cai, Y.E. Cai, S.F. Mou, Y.Q. Lu, *J. Chromatogr. A* 2005, 1081, 245.
16 G. Liu, J. Wang, Y. Zhu, X. Zhang, *Anal. Lett.* 2004, 37, 3085.
17 S.F. Xiao, Z.H. Wang, G.A. Luo, Chin. *J. Anal. Chem.* 2005, 33, 261.
18 Q.X. Zhou, W.D. Wang, J.P. Xiao, *Anal. Chim. Acta* 2006, 559, 200.
19 Q.X. Zhou, Y.J. Ding, J.P. Xia, *Chromatographia* 2007, 65, 25.
20 Q.X. Zhou, J.P. Xiao, W.D. Wang, *Microchim. Acta* 2007, 157, 93.
21 J.X. Wang, D.Q. Jiang, Z.Y. Gu, X.P. Yan, *J. Chromatogr. A* 2006, 1137, 8.
22 M. Biesaga, K. Pyrzynska, *J. Sep. Sci.* 2006, 29, 2241.
23 H. Bagheri, Z. Ayazi, H. Sistani, *Microchim Acta* 2011, 174, 295.
24 H. Bagheri, Z. Ayazi, A. Es-haghi, A. Aghakhani, *J. Chromatogr. A* 2012, 1222, 13.
25 E. Ballesteros, *J. Chromatogr. A* 2000, 869, 101.
26 C.S. Boynton, C.F. Dick, G.H. Mayor, *J. Clin. Pharmacol.* 1988, 28, 512.
27 United States Pharmacopeia National Formulary, USP 26, NF 21, United States Pharmacopeia National Formulary, Rockville, 2003, pp. 1273.
28 J.R.Vane, R.M. Botting, *Inflamm. Res.* 1995, 44, 1.
29 K.S. Galliard-Grigioni, W.H.Reinhart, *Eur. J. Pharmacol.* 2009, 609, 96.
30 Y. Sun, Z. Zhang, Z. Xi, Z. Shi, *Talanta* 2009,79, 676.
31 S. Kamaruzaman, M.M. Sanagi, S. Endud, W.A.W. Ibrahim, N. Yahaya, *J. Chromatogr. B* 2013, 940, 59.
32 H. Lian, Y. Hu, G. Li, *Talanta* 2013, 116, 460.
33 A. Sebok, A. Vasanits-Zsigrai, G. Palko, G. Zaray, I. Molnar-Perl, *Talanta* 2008, 76, 642.
34 T. Madrakian, M. Ahmadi, A. Afkhami, M. Soleimani, *Analyst* 2013, 138, 4542.
35 H. Bagheri, A. Roostaie, M.Y. Baktash, *Anal. Chim. Acta* 2014, 816, 1.
36 Y.S. Song, J.R. Youn, *Carbon* 2005, 43, 1378.
37 J. Chen, M.A. Hamon, H. Hu, Y. Chen, A.M. Rao, P.C. Eklund, *Science* 1998, 282, 95.
38 O. Breuer, U. Sundararaj, *Polym. Compos.* 2004, 25, 630.
39 Z. Yang, X. Chen, C. Chen, W. Li, H. Zhang, L. Xu, *Polym. Compos.* 2007, 28, 36.
40 Z. Chan, F. Miao, Z. Xiao, H. Juan, Z. Hongbing, *Mater. Lett.* 2007, 61, 644.
41 L.A. Sarabia, M.C. Ortiz, Response surface methodology, in: S.D. Brown, R. Tauler, B. Walczak (Eds.), Comprehensive Chemometrics, Elsevier, Oxford, 2009, pp. 345–390.
42 A.I. Khuri, J.A. Cornell, Response Surface: Design and Analysis, Dekker, New York, 1987.
43 P.D. Haaland, Experimental Design in Biotechnology, Marcel Dekker, New York, 1989
44 J.B. Quintana, R. Rodil, S. Muniategui-Lorenzo, P. Lopez-Mahia, D. Prada- Rodriguez, *J. Chromatogr. A* 2007, 1174, 27.
45 V.G. Zuin, L. Montero, C. Bauer, P. Popp, *J. Chromatogr. A* 2005, 1091, 2.
46 N. Ochiai, K. Sasamoto, H. Kanda, S. Nakamura, *J. Chromatogr. A* 2006, 1130, 83.
47 L.P. Melo, A.M. Nogueira, F.M. Lancas, M.E.C. Queiroz, *Anal. Chim. Acta* 2009, 633, 57.