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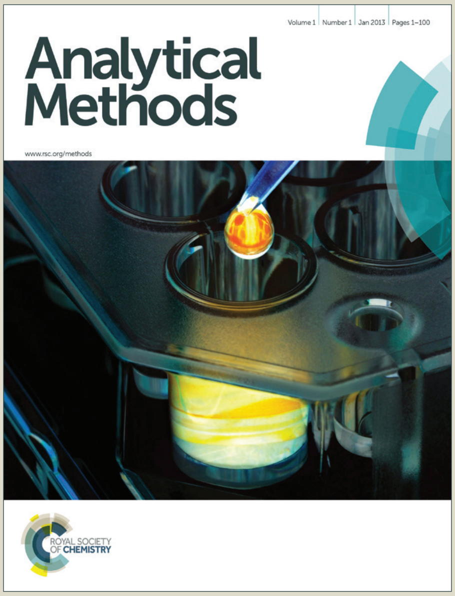

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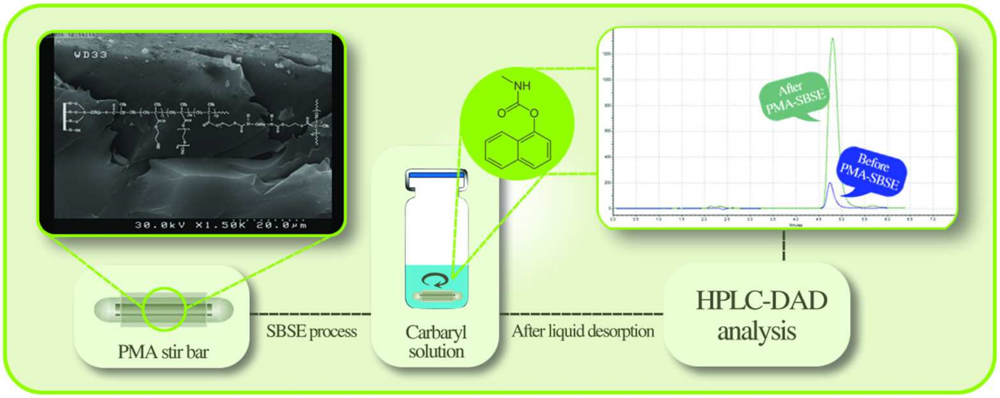

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Paper

Preparation, optimization and application of poly (ethylene glycol) methyl ether methacrylate/urethane methacrylate as a new polar phase for stir bar sorptive extraction

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The aim of this paper is to discuss the determination of carbaryl in water sample using a stir bar sorptive extraction method based on a new polar extraction phase coupled with a liquid desorption high performance liquid chromatography-diode array detection (SBSE-LD-HPLC-DAD). The process was conducted in an extraction medium mainly prepared from poly (ethylene glycol) methyl ether methacrylate (PEG-MMA) and urethane methacrylate (UMA). Polymerization of coating materials was performed under different conditions and final hydrophilicity of the extraction medium was tuned by partial replacement of UMA with 2-hydroxy ethyl methacrylate (HEMA). The home-made polymethacrylate stir bars (PMA) showed good within batch repeatability (RSD < 6.4%) and batch-to-batch reproducibility (RSD < 8.5%). They could be reused at least 35 times for the extraction of carbaryl in water sample with a minimum loss of extraction efficiency. The effects of main parameters including desorption solvent and time, sample ionic strength, extraction time, sample volume and extraction temperature were studied in order to optimize the extraction performance of PMA stir bar. The linear dynamic range for the analysis of carbaryl was obtained in the range of 0.13-10 ng mL^{-1} (R^2=0.9969). Furthermore, for this study, the limit of detection (LOD) and limit of quantification (LOQ) were obtained 0.04 and 0.13 ng mL^{-1}, respectively. Reproducibility of method presented as intra- and inter-day precision was also found with the RSD less than 7.4%. The proposed method was successfully applied to the determination of carbaryl in tap water sample. Moreover, the PMA coating manifested better extraction efficiency for carbaryl than the recently commercialized stir bars (polydimethylsiloxane and Acrylate Twisters®) due to the polar nature of this sorptive phase.

Introduction

Stir bar sorptive extraction method (SBSE) was introduced at the end of the 1990s as an eco-friendly sample preparation method for enrichment of organic compounds from aqueous matrices prior to chromatographic analysis.[1] The coating layer of extraction plays the most important role in the performance of SBSE procedure. Polydimethylsiloxane (PDMS) which is the most widely used commercial sorptive extraction phase is often applicable to the extraction of none and weakly polar compounds. Due to hydrophobic nature of PDMS based stir bars, there is a limitation on the application of such a sorptive material for the extraction of more polar samples. As a result, low affinity and poor sensitivity can be encountered particularly during trace analysis of materials.[2]

To overcome this constraint, new polymeric phases for the SBSE technique with higher affinity for more polar compounds have been proposed. To modify the performance of PDMS stir bar, various research groups utilized sol–gel technique for introduction of polar compounds such as β-cyclodextrin[3], β-cyclodextrin/divinylbenzene[4], polyethylene glycol/poly(vinylalcohol)[5, 6] and tetraethoxysilane[6] into the PDMS network. Sol-gel technology was also used to combine silica and C_{18} as a coating for the preparation of stir bars used in the SBSE method.[7] Monolithic copolymers such as vinylpyridine-ethylene dimethacrylate[8], poly (acrylic acid-ethylene dimethacrylate)[9], vinylpyrrolidone-divinylbenzene[10], poly(2-(methacryloyloxy) ethyl trimethyl ammonium chloride-co-divinylbenzene)[11], poly(vinylphthalimide-co-N,N-methylenebisacrylamide)[13], poly(methacrylic acid-co-divinylbenzene)[14] and poly(poly(ethylene glycol) methacrylate-co-pentaerythritol triacrylate)[15] were also used as diverse coatings of stir bars in order to improve their polarity and consequently their performance for the analysis of polar analytes. Moreover, some SBSE coatings have been developed by polymerization methods which were successfully applied to N-

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nitrosdiphenylamine[16] and carvedilol extracts.[17] Molecular imprinted polymers as sorbent in SBSE method were used too.[18, 19] Crack propagation in polar coatings used for the preparation of stir bars due to stress caused by solvent swelling which is the common shortcoming for most available coatings prepared by sol–gel method.[9] Thus, there is still the need for a polar polymeric material that provides better mechanical and thermal stabilities as well as sensitivity to recover a wide range of polar organic compounds. To address this need, application of poly methacrylate based coatings are considered in the present research. According to some SBSE coatings such as polyurethane foams[20-22] and some properties of methacrylate based monomers, urethane methacrylate (UMA) was selected as the monomer in this study.

Carbamates are one of the main classes of pesticides widely used in agricultural applications against insects, fungi and weeds. Carbaryl (1-naphthyl N-methylcarbamate) as a member of this class of pesticides is used due to its broad-spectrum efficacy to control over 100 species of insects. Since carbamates are inhibitors of acetyl-cholinesterase, they are suspected of carcinogenic and mutagenic properties.[23] Furthermore, these compounds are highly soluble in water; thus, lots of them can rapidly penetrate soil in order to reach aquifers and contaminate ground and surface water. The hazards and risks of these water contaminants for human health and also aquatic animals are well established.[24] According to the European Union Directive report (98/83/EC), the maximum allowable concentration of individual and total pesticides in drinking water are 0.1 and 0.5 µg L^{-1}, respectively. Thus, necessity for monitoring and quantifying which belong to their residual amounts at trace levels of these compounds in environmental matrices is obvious.

The most commonly used analytical methods for the determination of carbamate insecticides especially carbaryl, are gas chromatography-mass spectrometry (GC-MS)[25, 26] and high performance liquid chromatography (HPLC) with different detectors such as ultra violet (UV)[27, 28], diode array (DAD)[29], fluorescence (FL)[30], and chemiluminescence (CL)[31]. Due to trace amounts of these contaminants in the samples, extraction and pre-concentration steps prior to chromatography analysis are required to achieve a low detection limit. Various sample preparation methods such as liquid–liquid extraction (LLE)[27], solid-phase extraction (SPE)[32], matrix solid phase dispersion (MSPE)[33], single drop microextraction (SDME)[28, 34] and dispersive liquid-liquid microextraction (DLLME)[25, 35] have been employed for the extraction of carbaryl from different sample matrices.

Introduction of the SBSE method using stir bars coated with polar copolymers consisting of different proportions of poly (ethylene glycol) methyl ether methacrylate (PEG-MMA), urethane methacrylate (UMA) and 2-hydroxy ethyl methacrylate (HEMA) polymerized by either photo or thermal initiated methods was performed in this work. The stabilities of home-made polymethacrylate (PMA) stir bars at aqueous media were investigated, and then their extraction efficiencies for carbaryl as a target analyte were evaluated using liquid desorption and HPLC device equipped with DAD detector (LD-HPLC-DAD). Effective parameters on the SBSE procedure such as desorption solvent and time, ionic strength of sample, extraction time, sample volume and temperature were optimized. Under optimal condition, the proposed method was applied to the determination of carbaryl in tap water. Besides, performance of this novel coating in the extraction of carbaryl was compared with two commercial available SBSE coatings.

Experimental

Chemicals and Reagents

Poly (ethylene glycol) methyl ether methacrylate (PEG-MMA), 2-hydroxy ethyl methacrylate (HEMA) and camphorquinone (CQ) were provided from Sigma-Aldrich (Steinheim, Germany). 3-(trimethoxysilyl)propyl methacrylate (MEMO), benzoyl peroxide (BP), 2,3-butanedione (BD) and trifluoroacetic acid (TFA) were supplied by Merck (Darmstadt, Germany). Urethane methacrylate (UMA) was purchased from Evonik Rohm (Darmstadt, Germany). 1-Methyl-3-octylimidazolium tetrafluoroborate ([Omim] BF4) ionic liquid was obtained from Kimia Exir Chemical Co. (Tehran, Iran). Carbaryl (99.8%) was purchased from Bayer Crop Science (Leverkusen, Germany). Sodium chloride was supplied by LOBA Chemie (Mumbia, India). HPLC-grade methanol (MeOH, 99.9%) and acetonitrile (ACN, 99.9%) were provided from Chem-Lab NV (Zedelgem, Belgium). Absolute ethanol was prepared from Bidestan (Qazvin, Iran). Water used throughout the study was purified using a Milli-Q water purification system (Millipore, St. Quentin, France).

Equipments

A HPLC system (Knauver, Germany) equipped with a pump (Model 1000), a diode array detector (DAD) (Model 2800), a 20 µL loop and Chromgate software (Knauer, Germany) was used in the laboratory experiments. The separation was performed on a C18 column (5 µm, 4.6 mm i.d.× 250 mm, Knauer, Germany). Methanol-water (75:25 v/v) was used as mobile phase at a flow-rate of 1.0 mL min-1and the quantitative experiments were carried out at wavelength of 220 nm.

Morphology of coatings was examined by a scanning electron microscopy (SEM) instrument (Model S4160, Hitachi, Japan). FT-IR was accomplished on a Tensor 27 FTIR instrument (Bruker, Germany).

PDMS (PDMS Twister®, 0.5 mm thickness, 10 mm length and 24 µL volume) and Acrylate (Acrylate Twister®, 0.5 mm thickness, 10 mm length and 25 µL volume) stir bars manufactured by Gerstel were used as the comparison (Germany).

Preparation of PMA coated stir bars

A glass capillary (1.54 mm o.d., 10 mm length) was used as the support of polymeric coating. A glass capillary tube open at both ends was closed using a Bunsen torch after placing a small magnetic bar into it. Before coating, the glass capillary was first dipped in 1.0 M NaOH solution for one hour so as to expose the maximum number of silanol groups on the surface, then cleaned with water and placed in 0.1 M HCl solution for 30 minutes to neutralize the excess NaOH. Afterwards, it was cleaned again, and dried at room temperature. Prior to the coating procedure, the treated bare glass bars were silylated for 3 hours by immersing them into a 10% (v/v) MEMO solution in ethanol at room temperature. Finally, the bars were dried in an oven at 110 °C for one hour.

Preparation of coatings through photo polymerization technique

The home-made PMA coatings were prepared using various polymerization methods. As the first step, preparation of coatings was performed in the presence of MEMO. For this purpose, desired amounts of MEMO (0.3 and 0.15 g) were hydrolyzed thoroughly in the presence of water (0.07 and 0.035 g), absolute ethanol (0.18 and 0.09 g) and TFA (95%, 0.01 mL) for 30 min under stirring the mixture at 100 rpm. Then, different amounts of PEG-MMA, UMA and HEMA as monomers, and CQ and BD as photo initiators were added to the solution of hydrolyzed MEMO. Furthermore, the synthesis and performance of coatings were investigated in the absence of MEMO. In this case, CQ and BD were added into the mixture of UMA, HEMA and PEG-MMA in different ratios (Table 1). For each formulation, the prepared mixture was transferred into a tube and a surface silanated glass bar was immersed into it. Thereafter, the tube was sealed and irradiated under visible light for two hours in order to polymerize free-radicals, then placed in an oven at 50°C for 24 hours.

Preparation of coatings through thermal polymerization technique

For thermal polymerization method, the treatment was considered for the best formulation of photo polymerization method via replacing the photoinitiators with a thermally labile free radical initiator, BP. In this method, given amounts of PEG-MMA, UMA, HEMA and BP (0.01 mol per each mol of double bonds) were mixed. The mixture was transferred to the tube after solving BP and obtaining a homogenous mixture. . Thereafter, the tube was sealed, and then it was kept at 80°C for 24 hours. Details of different formulations were listed in Table 1. Finally, within bar to bar (n= 3) and batch-to-batch reproducibility (n = 5) for the best formulation of the home-made stir bar were investigated.

Conditioning of prepared stir bars

After completion of the polymerization step, the tube was cracked carefully. Prior to the first usage, the coated stir bars were placed into a vial containing distillated water for four hours, then placed in a vial containing acetonitrile and sonicated for 20 minutes to remove the residual monomers and initiators followed by a drying step using a lint-free tissue. Among the successive extractions, the used stir bars were ultrasonically cleaned in 1.5 mL of methanol for 15 minutes followed by a drying step using a lint-free tissue.

SBSE procedure

The SBSE procedure was carried out by introducing the prepared PMA stir bar into an aqueous sample containing specific amount of NaCl. In the extraction step, this solution was stirred at different times under controlled temperature and constant speed (900 rpm). For the desorption step, the stir bar was taken out using a clean tweezers, gently dried with a tissue and placed in a 1.5 mL vial containing 350 μL of desorption solvent ensuring the total immersion. Desorption was performed by ultrasonic treatment during a selected time. After the desorption step, 20 μL of desorption solution was injected into the HPLC system.

In order to optimize the proposed extraction method, several parameters which could influence the extraction efficiency of PMA stir bar for carbaryl as a target analyte were investigated. In the desorption step, triplicate assays were performed to test the desorption solvents (acetonitrile, methanol and methanolic solution of [Omim] BF4) and desorption time (5, 10 and 20 min). The occurrence of carry-over was tested by performing a consecutive second desorption. In the extraction step, the effects of ionic strength on sample solution (adding 0, 10, 20, 25 and 30 %w/v NaCl salt), extraction time (1, 1.5, 2 and 5 hours), sample volume (10, 25, 50 and 100 mL) and temperature of sample solution (20, 35 and 50°C) were studied. Each experiment was repeated three times.

Method validation

After optimization of the extraction and desorption conditions in PMA-SBSE, the method was tested for validation. Calibration curve was investigated with different standard solutions of carbaryl over a concentration range from 0.04 to 10 ng mL^{-1}. Its linearity was evaluated by the least-squares regression method which was used to calculate the regression coefficient (R^2) value, y-intercept, and slope of the regression line. The limit of detection (LOD) and limit of quantification (LOQ) values were calculated based on the standard deviation of the blank response (σ) and the slope (s) of carbaryl calibration curve according to the following equations (1 and 2):

$$LOD=3\ (\sigma/s) \qquad (1)$$
$$LOQ=10\ (\sigma/s) \qquad (2)$$

Accuracy and precision of the proposed method were determined using three control solutions at three concentration levels (0.5, 5 and 8 ng mL^{-1}) for carbaryl and established by the recovery (R %) and its relative standard deviation (RSD %), respectively. Intra- and inter-day analyses were performed using three replicate extractions of each control solution on three days. Ultimately, the applicability of this procedure on real samples was tested by analyzing carbaryl in tap water.

Results and discussions

Preparation of PMA coating as SBSE sorbent

In this work, novel polar PMA based coatings were synthesized using two different polymerization methods including photo polymerization and thermal polymerization. In order to obtain an adequate PMA coated stir bar several formulations were firstly prepared by photo polymerization method and some important factors such as ability of swelling in water and polar solvent, mechanical stability and high capacity of analyte absorption were considered at the same time. In order to obtain a coating which is expected to swell in water and polar solvent as it is mechanically stable with high capacity for analyte absorption, it was preferred to use PEG-MMA not only to establish more hydrogen bonding in order to increase hydrophilicity but also to increase free volume between chains. In fact PEG with two methyl methacrylate groups in its chain would be trapped in the polymer network which enhanced the chance of analyte penetration into the coating. In addition, UMA was used to obtain appropriate mechanical stability. The primary results confirmed that introducing another ingredient such as HEMA can be effective to improve the polarity of the coatings. Thus, various ratios of UMA and HEMA were applied to prepare PMA coatings with different polarities. Due to the presence of hydroxyl groups in HEMA, both internal and external hydrogen bonding interactions were enhanced and as a result, the strength and polarity of coating were also increased. Moreover, the chance of interaction between glass substrate and coating and also their adhesiveness were enhanced. So, the stability and strength of coating were amplified.

Testing of prepared coatings showed that the formulations obtained using PEG-MMA> 0.3 g or HEMA> 0.5 g were mechanically unstable along the conditioning step. Therefore, they failed and were removed from the list and only a few formulations which were able to extract the target analyte, were discussed (Table 1). To study the performance of each prepared PMA coating, the SBSE procedure was carried out by introducing the stir bars into 25 mL of carbaryl solution (0.005 mg mL^{-1}). The obtained area of carbaryl after using the SBSE method, was shown in Fig. 1. Preparation of coatings by photo polymerization was investigated either with or without the presence of MEMO in formulations. As it can be seen, the existence of MEMO in formulations demonstrated no specific improvement in the extraction efficiency of carbaryl. Also, the extraction efficiencies of prepared coatings were enhanced when UMA and HEMA were used at 1:1 weight ratio. The highest extraction efficiency was obtained using photo polymerization method in F6. But, all coatings which were prepared using photo polymerization methods (F_1 to F_6) failed because of their cracking during the extraction procedure after one or two cycles. This can be due to the stress induced by solvent swelling which causes physical and chemical bonds breaking.[34]

To investigate possible effects of the polymerization method on performance of coated stir bar, thermal polymerization of methacrylate monomers were also considered in the present study. Since the F_6 coating exhibited better extraction efficiency compared to other formulations, another coating (F_7) with the same formulation was prepared by thermal polymerization using BP as an initiator. Compared to other coatings, F_7 simultaneously manifested acceptable stability and extraction efficiency. Thus, this coating was chosen as the final phase of the extractor (PMA). Thickness and volume of PMA coating were 0.38 mm and 30 µL, respectively.

Characterization of PMA coating as SBSE sorbent

In order to investigate and confirm the PMA coating, FTIR analysis was performed for F_7 formulation as the best coating before and after thermal polymerization. In the spectrum of the prepared mixture before polymerization, a peak was appeared at 3374 cm^{-1} which was related to the stretching vibrations of -OH and -NH bands. The adsorption observed at 1722 cm^{-1} was related to the stretching vibration of ester and acrylate carbonyl group. In 1635 cm^{-1} absorption of C=C acrylate group and in 1530 cm^{-1} the stretching vibration band of C-N were observed. The stretching vibration of C-O-C at 1000-1300 cm^{-1} and the bending vibration of C=C for acrylate group at 816 cm^{-1} were also recorded. After the polymerization step, the absorption of C=C for acrylate group at 1635 and 816 cm^{-1} were observed to reduce. The decrease in peak intensity at 3348 cm^{-1} was due to the elimination of water from the mixture, during the polymerization. Quantitative analysis of chemical reactions were followed by monitoring the changes in the appeared peak for C=C acrylate bond at 1635 cm^{-1} (The spectra are shown in Electronic Supplementary Information).[35] Based on the peak area observed in the spectra of coating before and after polymerization, the double bond conversion was calculated at 60.4%. A schematic presentation of stir bar pretreatment steps and proposed structure of prepared PMA coating are shown in Fig. 2a and b, respectively.

Fig. 3 demonstrates SEM micrograph of PMA coating. It can be seen that the surface of the coating is uniform, homogeneous and dense. To investigate water absorption ability of PMA coating, the bars were weighted after complete drying. They were then placed in water for 5 hours and their weights were recorded every hour after removal from the water. The highest water absorption percent for this type of coating was recorded at 6.3%. Compared to the same urethane acrylate systems, this level of absorption indicated significant improvement.[38] Increase in water absorption was due to the introduction of HEMA and PEG-MMA into the polymer network.

Finally, the contact angle measurement was done in order to evaluate hydrophilicity/hydrophobicity of the surface coating. In addition, this parameter exhibits the surface wettability. If the angle that the drop makes with the surface is less than 90°, the surface is considered to be polar and hydrophilic. If the angle is greater (>90°), the surface is assumed to be more hydrophobic. The contact angle of water drop with the surface of F_7 coating was measured 65° by ImageJ 1.43u (National institute of Health, USA) software and as expected, it confirmed the polarity and hydrophilic behaviour of the surface. Also, water absorption diagram of PMA coating confirmed its hydrophilic behaviour (The diagram is shown in Electronic Supplementary Information).

Lifetime and preparation reproducibility of PMA coated stir bar

The optimum coating developed in the present study (PMA) can be simply regenerated by implementing a clean-up step between runs using the procedure described in the experimental section. The lifetime and preparation repeatability of the coating were investigated using a carbaryl aqueous solution with concentration of 5 ng mL-1 as the sample and the chromatographic peak areas as the signal responses. The robustness of the F7 stir bar was confirmed using over 35 extractions with a minimum loss of extraction efficiency and RSD of less than 7.2%. In order to investigate reproducibility of the method, three bars were prepared in the same batch and identically prepared bars among five different batches (one bar from each batch) were tested for the extraction of carbaryl from the sample. Good and reasonable reproducibility was obtained, not only within each batch (RSD <6.4%, n = 3) but also between batches (RSD < 8.5%, n = 5).

Optimization of PMA-SBSE procedure

In order to achieve the highest extraction efficiency of the novel polar PMA stir bar for carbaryl as the target analyte, factors affecting desorption and extraction steps of the SBSE process were individually investigated and systematic assays were performed to optimize the effective parameters. In the entire study, each experiment was replicated three times and standard deviations were shown in the related graphs.

Performing a complete desorption step depends on various parameters such as desorption solvent type and process time. Selected solvents must have enough power to promote the best stripping of the target analyte from the polymeric phases in the first desorption step. During the present study, 350 µL acetonitrile, methanol and methanolic solution of 0.1 M hydrophobic ionic liquid ([Omim] BF4) were tested as the desorption solvents. Since in the desorption step, a minimum amount of solvent is used to grantee entire immersion of stir bar in solvent, 350 µL of desorption solvent were chosen in these experiments. The sample volume, extraction time and temperature were also maintained constant at 25 mL, 2 hour, and 25°C, respectively. In order to compare desorption efficiencies of different solvents and carry-over of PMA sorbent, desorption step for each solvent was consecutively performed two times. The obtained data (see Fig. 4a) demonstrated that the best desorption efficiency (expressed as higher peak area at the first desorption step) and the least carry-over (expressed as lower peak area at the second desorption step) were obtained when methanol was used as the desorption solvent. Moreover, the usage of ionic liquid in desorption solvent did not show any improvement in the results.[15] Based on these results and better performance of methanol with the selected mobile phase, this solvent chosen as the desorption solvent for the subsequent experiments.

The effect of desorption time on desorption efficiency of PMA coating for carbaryl was also studied using ultrasonic agitation of the stir bar in methanol for 5, 10 and 20 min. The experimental results (summarized in Fig. 4b) showed that desorption equilibrium was achieved after 10 min and further increase in desorption time did not improve desorption efficiency and carry-over. Consequently, selected time for the rest of the investigations was 10 min as the optimal desorption time.

In optimization step of the extraction conditions, several factors were evaluated including sample ionic strength, extraction time, sample volume and temperature. These parameters influence the mass transfer of the analyte during the extraction process.

In SBSE, any increase in the ionic strength of the sample solution favors the extraction of polar organic analytes into the stir bar coating[7] due to the salting-out effect. To evaluate the impact of ionic strength on the extraction efficiency of carbaryl by PMA-SBSE, extractions were performed for aqueous solutions of the samples with NaCl concentrations varying from 0 to 30% w/v. As it can be seen in Fig. 5a, the extraction efficiency reached a maximum level by adding 25% NaCl. However, no increase was observed in the extraction efficiency with the higher percentage of salt (i. e. 30%) due to the salt saturation phenomena. Therefore, 25% w/v of NaCl was chosen as the best result for further investigations.

The theory of SBSE similar to SPME is based on the distribution equilibrium of analyte between the stir bar coating and the sample solution.[1] In order to estimate the most suitable equilibrium time, experiments were performed in three different volumes (10, 25 and 50 mL) for 1, 1.5, 2 and 5 hours to obtain the proper extraction time in various phase ratios (β). In all of the solutions, the amount of carbaryl was considered constant (0.125 mg). The extraction time profiles (for three volumes) are illustrated in Fig. 5b. Results indicated that in all used sample volumes, the extraction efficiency increased when the extraction time increased to 2 hour and no obvious significant change was seen afterwards. Since a compromise between the extraction time and efficiency was necessary, the minimum process time (2 hour) was selected as the extraction time in for following experiments.

In order to achieve the appropriate sample volume in the extraction process, solutions with different volumes (10, 25, 50 and 100 mL) and the same concentration (0.005 mg mL^{-1}) were prepared. The results (see Fig. 5c) indicated that with the sample volume of 50 mL, the highest extraction efficiency was obtained. Further increase in the sample volume up to 100 mL caused a decrease in the extraction efficiency due to the incomplete equilibrium time and unsuitable agitation.[12] Consequently, the volume of 50 mL was chosen as the optimum sample volume for the remaining studies.

One of the effective factors in the extraction processes is the temperature of the sample solution. To achieve the proper temperature, various extraction temperatures (20, 35 and 50 °C) were tested. According to the extraction temperature profile shown in Fig. 5d, it can be clearly observed that the extraction efficiencies are improved with the increase in the extraction temperatures from 20 to 35 °C. As the temperature increases up to 50 °C, a decline in the extraction efficiency is evident. This can be due to the fact that at higher temperatures, the distribution coefficient of the analyte between the coating and water is reduced and the extraction efficiency is also decreased.[39] Thus, the selected optimum temperature for the extraction step was 35 °C.

To summarize the parametric studies in this experimental study, the optimal conditions for the extraction of carbaryl by PMA-SBSE are as follows: 50 mL of sample solution containing 25% w/v NaCl stirred at 900 rpm for 2 hours at 35 °C. After the extraction, the analyte was desorbed in 350 µL methanol by ultrasonic agitation of the stir bar for 10 min.

Comparison with commercial stir bars

The extraction efficiency of home-made PMA stir bar for carbaryl as a polar target analyte (Log Ko/w= 2.36) was compared with two commercial PDMS and Acrylate stir bars under the same conditions. The typical chromatograms are shown in Fig. 6. The extraction mechanism of acrylate and methacrylate coatings is mostly based on the absorption. In this type of coatings, the analytes migrate in and out of the absorbent phase. The analytes are attracted to the coating primarily according to their polarities and the mass transfer or retention of the analytes is based on the thickness of the coating.[40, 41] The theory of SBSE was applied to estimate the partition coefficient values (K) of different extraction phases for carbaryl. According to this theory[1], partition coefficient of the analytes between the aqueous matrix and sorbent phase can be estimated by the following equation (3):

$$K_{(sorbentphase/w)} = C_{sorbentphase}/C_w = m_{sorbentphase}/m_w \times V_w/V_{sorbentphase} \quad (3)$$

where the sorbent phase can be PMA, PDMS and Acrylate and w is water. The volume of aqueous phase was 50 mL (V_W) and volumes of different sorbent phases ($V_{sorbent\ phase}$) were 30, 24 and 25 µL for PMA, PDMS and Acrylate, respectively. The values of K_{PMA}, K_{PDMS} and $K_{Acrylate}$ for carbaryl were acquired 123, 48 and 58, respectively. Due to the high extraction efficiency and K value for PMA-SBSE, great enhancement of the peak area can be obtained, which indicates that a lower detection limit will be achieved. In any case, it is evident that the PMA stir bar possesses the highest extraction efficiency for carbaryl of two commercially available stir bars. This performance can be related to the existence of high enough polar and hydrophilic characteristics of this coating due to the presence of HEMA and PEG-MMA in the structure of the coating and similar urethane functional groups in both PMA coating and carbaryl.

Method validation and application of PMA-SBSE-HPLC method

Under the optimum extraction conditions of proposed PMA-SBSE-HPLC, a calibration curve was obtained through the extraction of spiked ultra pure water samples with carbaryl (0.02 to 10 ng mL^{-1}). The data of linear dynamic range, correlation coefficient, LOD, LOQ, intra- and inter-day accuracy and precision and also achieved recoveries in real sample are listed in Table 2.

The linearity range of the method was observed from 0.13 to 10 ng mL^{-1} based on the verification of calibration curve. The values of the slope, intercept and regression coefficient (R^2) for linear equation were 3823 (with standard error 151), 97136 (with standard error 758), and 0.9969, respectively. Besides, the regression of linear equation was verified using analysis of variance (ANOVA). The obtained results in ANOVA table showed that the regression was significant at 95% confidence level. Furthermore, the limit of detection (LOD) and limit of quantification (LOQ) were calculated 0.04 and 0.13 ng mL^{-1}, respectively based on the equations 1 and 2.

The intra- and inter-day accuracy and precision were evaluated at three concentration levels of carbaryl (0.5, 5 and 8 ng mL^{-1}) according to the procedure described in Section 2.6. The results which are summarized in Table 2 demonstrated the excellent recovery values (96.4-99.9) for the assay with RSD values less than 7.4%. Based on these results, it was asserted that the proposed method had achieved acceptable accuracy and precision.

The performance of this method was investigated by the analysis of carbaryl in tap water. Carbaryl was not detected in tap water sample using the PMA-SBSE-HPLC method. Hence, this sample was fortified at 0.5, 5 and 8 ng mL^{-1} of carbaryl and analyzed three times in each concentration level using the proposed method. It was observed that reasonable recoveries in the range of 98.2-99.9 with RSD values less than 7% were reached at all concentration levels (Table 2).

Finally, a comparative study of the proposed method with other reported methods for analysis of carbaryl in water was performed and the linear range and LODs were also listed in Table 3. Compared to the existing methods involving DLLME, the LOD for the carbaryl obtained by the proposed method in this study was lower than other methods with the same kind of detector (UV or DAD)[40, 41], but it was comparable to fluorescence detector.[40] Additionally, only DLLME-GC-MS/MS method[21] provided the lowest LOD for carbaryl. Moreover, the SBSE method for the extraction of carbaryl had shown better LOD than SDME-GC-MS/MS without a derivatization step and also UASEME-HPLC-DAD.[32,41]

Conclusions

In this study, a new PMA-SBSE method combined with a HPLC-DAD system was successfully applied to extract and analyze carbaryl at trace level in water sample. Several formulations of UMA, HEMA, and PEG-MMA with different curing methods such as thermal or photo polymerization were evaluated. PMA-SBSE coating manifested excellent extraction efficiency, selectivity, and tendency towards carbaryl compared with PDMS and Acrylate (two commercial SBSE coatings). This was due to suitable polarity, hydrophilicity and swelling ability of PMA coating in water samples. Moreover, the results for the parametric study of this proposed method in a series of systematic laboratory experiments demonstrated linearity, accuracy, precision and also a very small limit of detection for this method.

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Analytical methods

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Figure legend

Fig. 1. Extraction efficiency of prepared polymethacrylate (PMA) coatings using different polymerization methods.

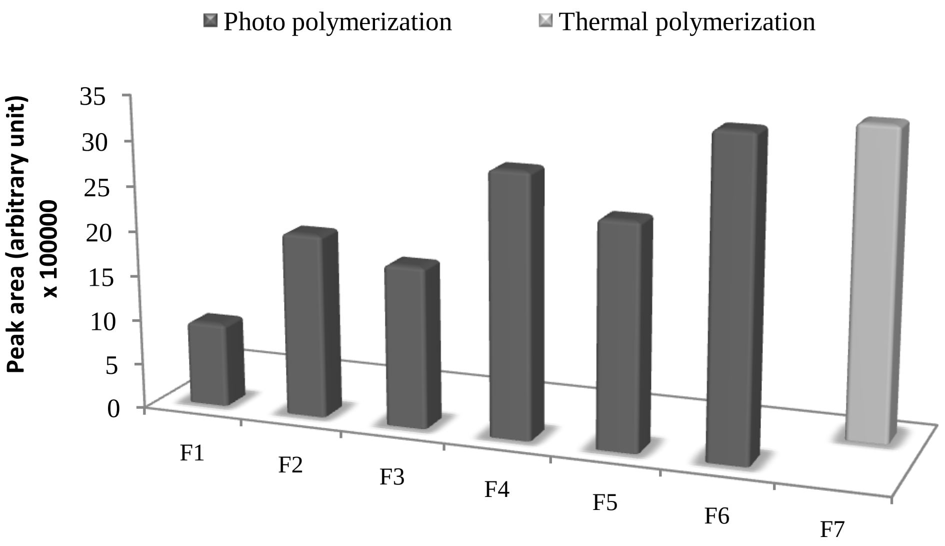
Fig. 2. a) Pretreatment steps of the glass capillary surface before coating and b) chemical structure of prepared polymethacrylate (PMA) coating with the silanated glass surface.

Fig. 3. Scanning electron micrograph of polymethacrylate (PMA) stir bar surface.

Fig. 4. Effect of (a) desorption solvent and (b) desorption time on the extraction efficiency of carbaryl (expressed as peak area) using polymethacrylate (PMA) coating.

Fig. 5. Effect of (a) sample ionic strength, (b) extraction time, (c) sample volume and d) extraction temperature on the extraction efficiency of carbaryl (expressed as peak area) using polymethaceylate (PMA) coating.

Fig. 6. a) Comparison of extraction efficiency of proposed coating (PMA) and two commercial coatings (PDMS and Acrylate) for extraction of carbaryl at 5 µg mL^{-1} concentration level from water sample at the same condition. b) Chromatograms of real sample before and after spiking of carbaryl at 8 ng mL^{-1} level.



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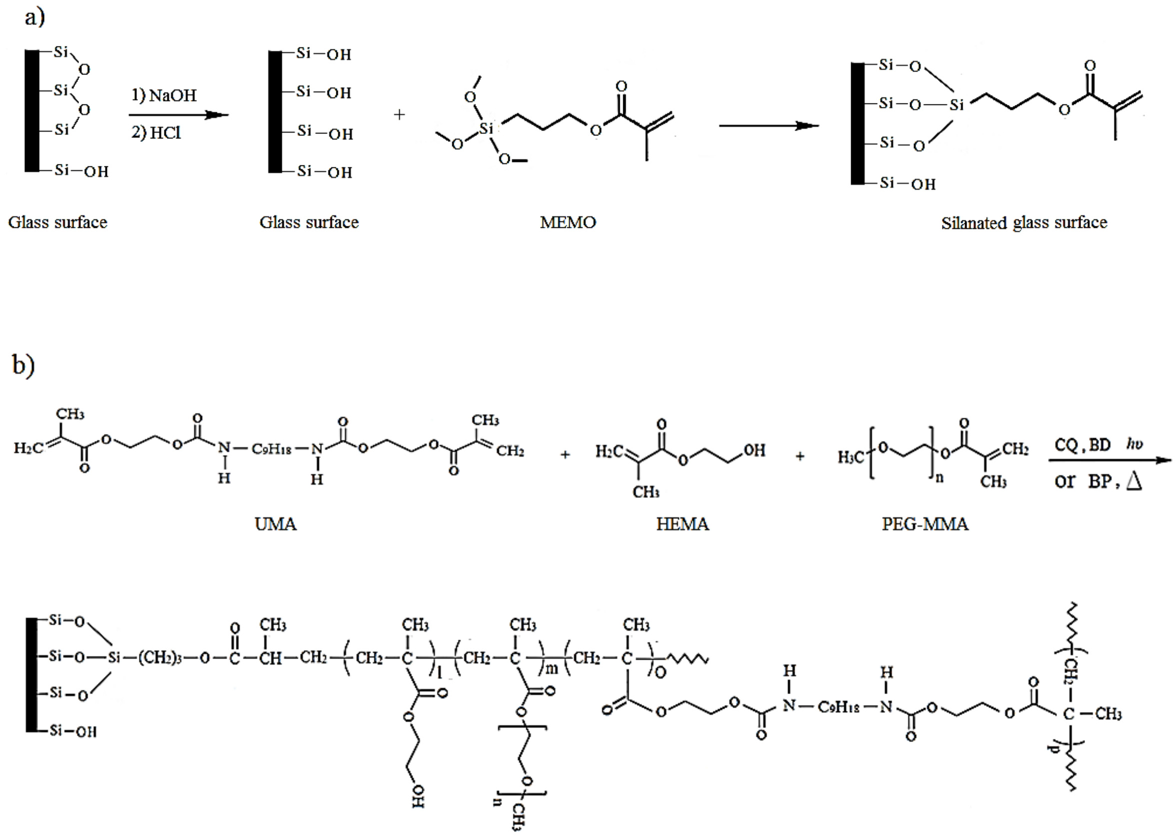


Fig. 2. a) Pretreatment steps of the glass capillary surface before coating and b) chemical structure of prepared polymethacrylate (PMA) coating with the silanated glass surface.

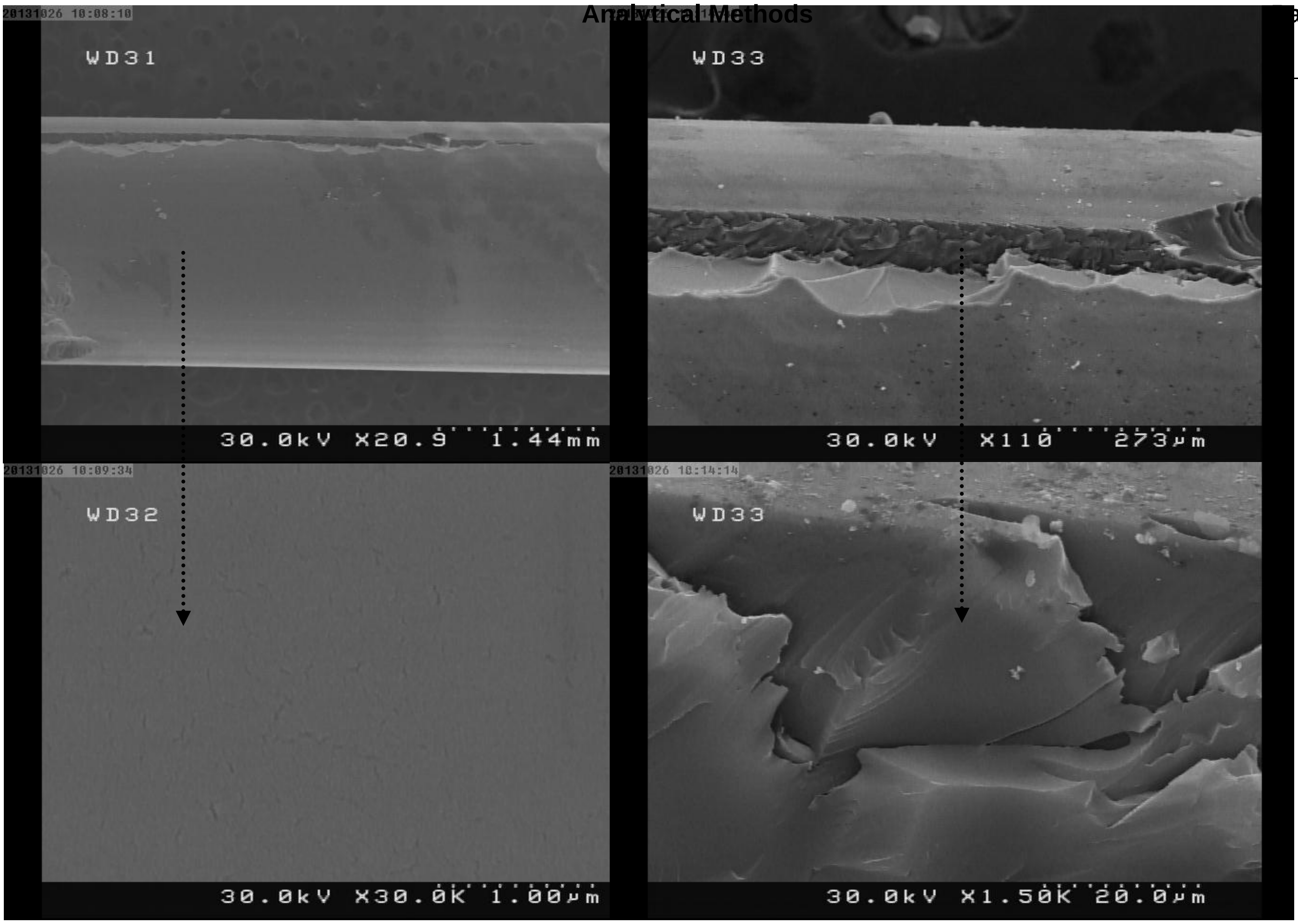
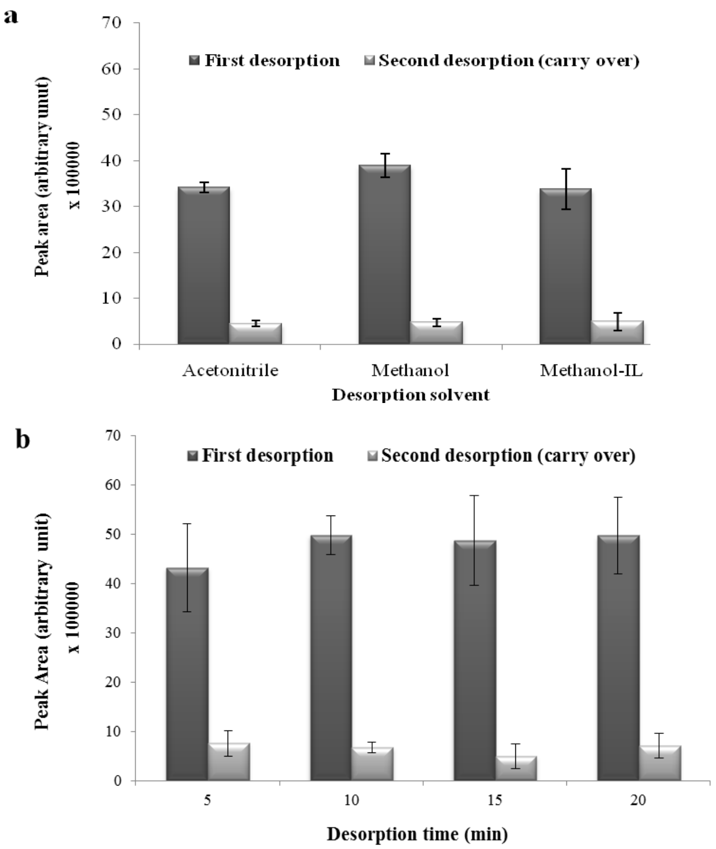


Fig. 3. Scanning electron micrograph of polymethacrylate (PMA) stir bar surface.



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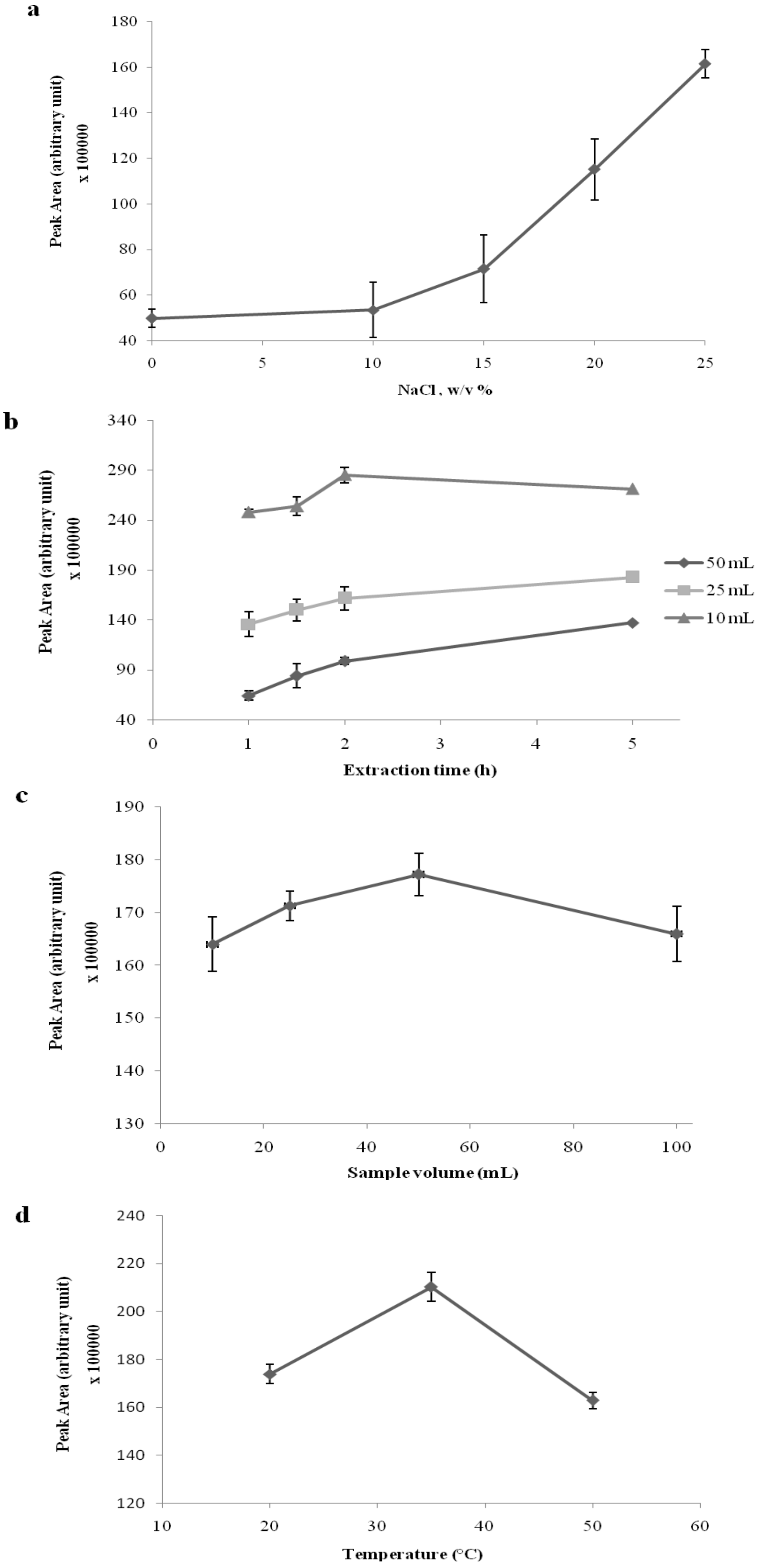

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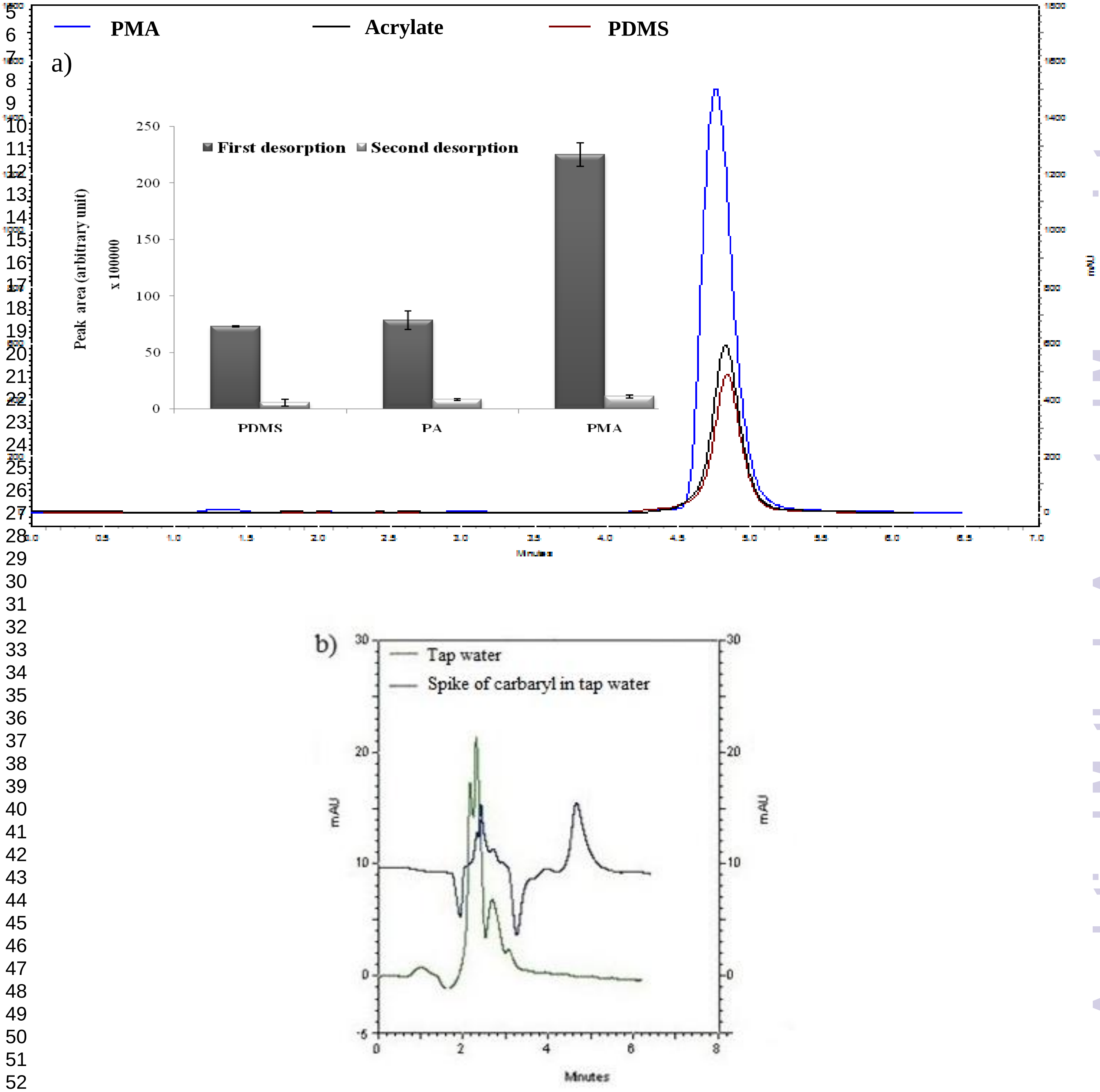

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Table 1. Composition of formulations used to prepare polymethacrylate (PMA) coatings by different polymerization methods.

Coating	MEMO[a] (g)	UMA[b] (g)	HEMA[c] (g)	PEG-MMA[d] (g)	Initiator (g)		
Photo polymerization							
					CQ[e]	BD[f]	BP[g]
F_1	0.30	0.70	0.35	0.30	0.00410	0.01650	-
F_2	0.30	0.35	0.35	0.30	0.00325	0.01300	-
F_3	0.15	0.35	0.35	0.30	0.00287	0.01150	-
F_4	0.15	0.35	0.50	0.30	0.00325	0.013	-
F_5	-	0.70	0.35	0.30	0.00340	0.01350	-
F_6	-	0.35	0.35	0.30	0.00250	0.01000	-
Thermal polymerization							
F_7	-	0.35	0.35	0.30	-	-	0.01160

[a]3-(trimethoxysilyl)propyl methacrylate, [b]Urethane methacrylate, [c]2-hydroxy ethyl methacrylate, [d]Poly(ethylene glycol) methyl ether methacrylate, [e]camphorquinone, [f]2,3-butanedione and [g]benzoyl peroxide

Table 2. Validation parameters for quantitative analysis of carbaryl using proposed PMA-SBSE-HPLC-DAD method.

Linearity range (ng mL^{-1})	r^2	LOD [a] (ng mL^{-1})	LOQ [b] (ng mL^{-1})	Spiked amount (ng mL^{-1})	Intra-day		Inter-day		Tap water	
					R [c]%	RSD [d]%	R%	RSD%	R%	RSD%
0.13-10	0.9969	0.04	0.13	0.5	96.4	5.8	96.7	6.8	98.2	5.2
				5	98.3	5.6	99.0	7.4	99.9	5.8
				8	99.6	4.4	99.9	4.7	99.0	7.0

[a] Limit of Detection, [b] Limit of Qualification, [c] Recovery and [d] Relative Standard Deviation

Table 3. Comparison of PEG-MMA/UMA-SBSE-HPLC-PDA method with other reported methods for analysis of carbaryl in aqueous samples

Sample	**Extraction method**	**Analysis method**	**Linear range ($ng\ mL^{-1}$)**	**LOD ($ng\ mL^{-1}$)**	**Ref**
Water and wastewater	DLLME[b]	GC–MS/MS	0.1-40	0.001-0.031	25
Water	SDME[a]	GC-MS/MS	0.2-10	0.08 and 0.003 without and with derivatization respectively	34
Water	DLLME	HPLC-DAD[d]	5-1000	0.1	42
Water	DLLME	HPLC-UV	1-1000	0.1	43
Water and fruit juice	DLLME	HPLC-FLD[c]	0.1–1000	0.0123	44
Water	UASEME[e]	HPLC-DAD	0.3–200	0.1	45
Tap water	PMA-SBSE	HPLC-DAD	0.13-10	0.04	Present study

[a] Single drop micro extraction, [b] Dispersive liquid liquid micro extraction, [c] High performance liquid chromatography- fluorescence detector, [d] High performance liquid chromatography- diod array detector, [e] ultrasound-assisted surfactant-enhanced emulsification microextraction, [f] Multi-walled carbon nanotubes-Dispersive solid phase extraction.