ISSN 1063-455X, Journal of Water Chemistry and Technology, 2014, Vol. 36, No. 6, pp. 273–279. © Allerton Press, Inc., 2014.
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Highly-Effective Liquid Chromatographic Determination of 2,4,6-Trinitrophenol in Surface Waters after Its Selective Solid Phase Extraction

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Received June 17, 2013

Abstract—The paper has proposed a new adsorbent for solid-phase extraction concentration of 2,4,6-trinitriphenol from aqueous objects of the environment followed by its determination in the concentrate by the method of highly performance liquid chromatography (HPLC). Silica modified by N-epoxypropylcarbazol was used as solid-phase extragent, which in the pH 1.5 ± 2.5 interval extracts up to 98% of the matter being analyzed. Sorbent capacity in terms of 2,4,6-trinitrophenol in the Henry area constitutes 5.3 mg/g, distribution coefficients reach values 7.9×10^4 cm^3/g. Nitrophenols are quantitatively eluted in dimethyl sulfoxide, which makes it possible to determine them by the HPLC with a diode array detector at the level 18 μ/dm^3.

DOI: 10.3103/S1063455X14060034

Keywords: high-performance liquid chromatography, complexes with transfer of a charge, silica, 2,4,6-trinitrophenol, solid phase extraction concentration.

INTRODUCTION

Nitroderivatives of aromatic nature are anthropogenic pollutants of the environment. They are widely used as pesticides [1], pyrotechnical means [2], antiseptic preparations [3] and are precursors in the production of some warfare agents [4]. All nitroaromatic compounds (NAC) are highly toxic and getting to an organism inflict damage to the central and peripheral nervous systems, liver, urination tracts, cause irritation of skin and mucus membranes of respiratory tracts [5]. For instance, 2,4,6,-trinitrotoluene manifest its toxicity in drinking water as early as at concentration > 2 $\mu g/dm^3$ [6]. The use of NAC on a great scale, necessity of conducting forensic examinations, projects in the framework of antiterrorist activities and mine clearing as well as their high toxicity require the development of reliable and selective methods of determination of these compounds in water objects of the environment, levels of pollution of territories with industrial and military objects, and ammunition plants. Existing photometric methods based on the reaction of reducing nitrophenols (Yanovsky's reaction [7]), formation of acid salts [8] or complexes with the transfer of the charge [9–11] often do not meet modern requirements of analysis especially due to their low selectivity. Therefore, modern analytical practice widely employ chromatographic methods [12–16], which, however, require carrying out sample preparation for concentration of matter being analyzed and its separation from the main matrix. For this purpose, when determining and identification of trace quantities of NAC in the environment, methods of solid phase extraction (SPE) [12] and micro extraction [17] liquid extraction [14, 15] are used. Modified silica gels with grafted octadecil (SiO_2–C18) [16] and β-cyclodextrine [18] groups are employed as adsorbents for SPE cartridges. Adsorbents used currently for NAC SPE are not selective and cannot secure required separation of the analyte from the matrix. In the first place it concerns polar NOC, for instance, picric acid (2,4,6-trinitrophenol, Pic). In addition, neither of the described SPE methods used for concentration of NAC does not allow us to conduct a visual monitoring of the pollution level of the object being investigated or control the degree of filing the adsorbent with matter being analyzed.

The present study proposed SPE by means of which one may selectively extract from aqueous solutions to the adsorbent phase mono- and polynitrophenols irrespective of their polarity. Efficiency and selectivity of extraction is secured by the formation in the surface layer of complexes with transfer of the charge (CTC) between the molecules of the substance being analyzed and immobilized ligands. The formation of such complexes results in a noticeable change of optical characteristics of the adsorbent (its color and intensity of fluorescence), which makes it possible to do preliminary visual monitoring of the object at the site of sampling [19]. In this study as a selective adsorbent for SPE of nitrophenols from aqueous solution silica was used with groups of N-epoxypropylcarbazol (EPC) immobilized on its surface, EPC possesses π-donor properties and may form CTC with π-acceptor NAC. Capability of SiO_2–EPC to form CTC in the adsorbent phase was studied on an example of Pic, 4-nitropheno and 2,4-dinitrophenol (2, 4 DNP), while for most important substance being analyzed—Pic, the technique of its determination by the HPLC method was developed.

EXPERIMENTAL

As a carrier for synthesis of the sorbent silica gel by Merck 60 Company (S_{sur}—490 m^2/g, d_{por}—6 nm), on whose surface according to technique of [20] were covalently fixed aminopropyl groups (SiO_2–NH_2).

The solution of 2,4,6-trinitrophenol (recrystalized from 50% alcohol solution) was prepared by dissolving accurate weighted quantities in bidistilled water. For preparing solutions of 0.1 M NCl and NaOH titres were used. In addition, experiments used ethyl alcohol, dimethyl sulfoxide (chemically pure), acetonitrile and methanol.

Acidity of solutions was controlled by means of a pH meter pH-150 MI. Optical density of the solutions was measured on a KFK-2-UKhL 4.2. Spectra of diffusion reflection of tinted sorbents were recorded on a Specord M-40 (Carl Zeiss, Germany): determination of NAC by the HPLC was done on a chromatograph an Agilent 1200 with diode–matrix detector (DMD). IR spectra with Fourier-transformation were recorded in tablets without filling on a spectrometer Nicolet 4700/6700 FT-IR (Thermo).

Technique of Preparing the Adsorbent for SPE Cartridge

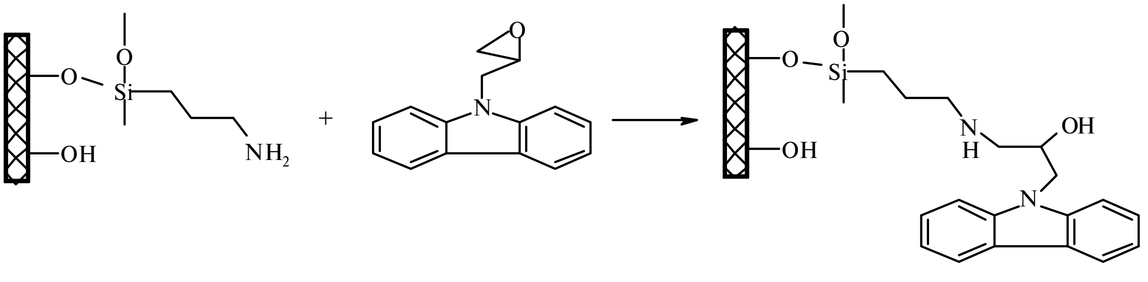
1 g of SiO_2–NH_2 is supplemented by the EPC solution (0.16 g) in toluene. The bend was boiled for 8h and after that solid phase was separated by filtration, transferred to a Soxhlet apparatus and rinsed with toluene until the absence of EPC in the washing solution; then it was dried out at 120°C for 8 h. The result was SiO_2–EPC, which by the data of an elementary analysis contained 460 μmol/g of propylcarbazol groups.

In studying sorption characteristics of SiO_2–EPC under static conditions the weighted quantity of the sorbent (0.1 g) was supplemented by adding 15 cm^3 of aqueous solution Pic containing 0.03–3 mg of the matter being analyzed and then it was stirred for a time sufficient for establishing adsorption equilibrium. The degree of removal (R, %) was investigated depending on the pH of the solution for $C_{\text{Pic}} = (0.4\text{–}10.0) \times 10^{-5}$ M. Distribution coefficients Pic between the sorbent and the solution were calculated by the residual amount of Pic in the solution [8]. Elution of the matter being analyzed in dimethyl sulfoxide (DMSO) was carried out under dynamic condition sampling portions of 1 cm^3.

Determination of Pic was conducted in the mode of gradient elution on an Agilent 1200 chromatographer with DMD. A column Eclipse (4.6 × 250 mm) was used and also movable phases: A—deionized water (pH 7); B—acetonitrile. Gradient program: 0 min (A—95, B—5%); 1 min (A—80, B—20%); 3 min (A—60, B—40%); 5 min (A—25, B—75%); 8 min (A—95, B—5%).

RESULTS AND DISCUSSION

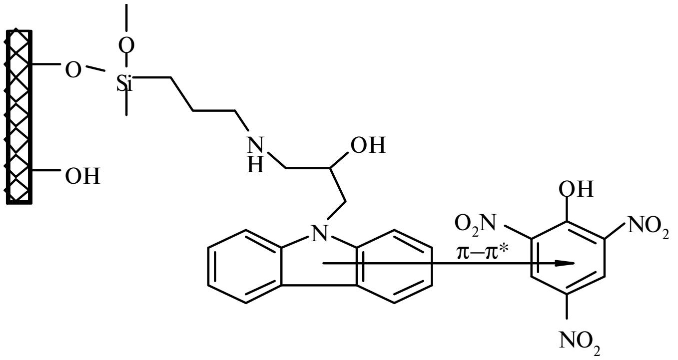
The idea of making an adsorbent for selective extraction of NAC from the solution is based on their ability to form CTC with some polyaromatic compounds. In particular, carbazoles derivatives. For obtaining the adsorbent the ability of EPC to react with alkyl amines was used. If as amine to use silica with aminopropyl groups (SiO_2–NH_2) covalently-fixed on its surface, then its treatment by EPC will result in covalent immobilization of this molecule on the surface with the formation of SiO_2–EPC:



(1)

Immobilization of EPC on silica is confirmed by the data of thermogravimetry, IR-spectroscopy and luminescence. For instance, SiO_2–EPC unlike SiO_2–NH_2 possesses intensive luminescence with the maximum at 406 nm. T is shown that holding of SiO_2—EPC in aqueous solution within pH interval 1–8 does not result in the change of its spectroscopic and adsorption characteristics, from which follows a conclusion about hydraulic stability of the obtained adsorbent within a studied interval of acidity.

Using method of electronic spectroscopy of diffusion reflection (CDO) we investigated interaction SiO_2—EPC with aqueous solutions of different nitrophenols. It was found that when immersing SiO_2—EPC in diluted aqueous solution of nitrophenols the adsorbent is painted orange-red color, which is manifested in CDO in the form of an intensive absorption band with the maximum at 450–500 nm (Fig. 1). Such absorption is an evidence of specific adsorption of nitrophenols on SiO_2—EPC due to formation of CTC by immobilized on the surface of SiO_2—EPC groups of carbazol:



(2)

The efficiency of adsorption of nitrophenols from the aqueous solution under static conditions was investigated on the example of the most polar among all studied NAC—picric acid (pH 1–8). As may be seen from Fig. 2, in the pH range 1.5–3.5 one may observe intensive extraction of Pic by the adsorbent, which at pH 2.1 ± 0.5 constitutes 98 ± 1%.

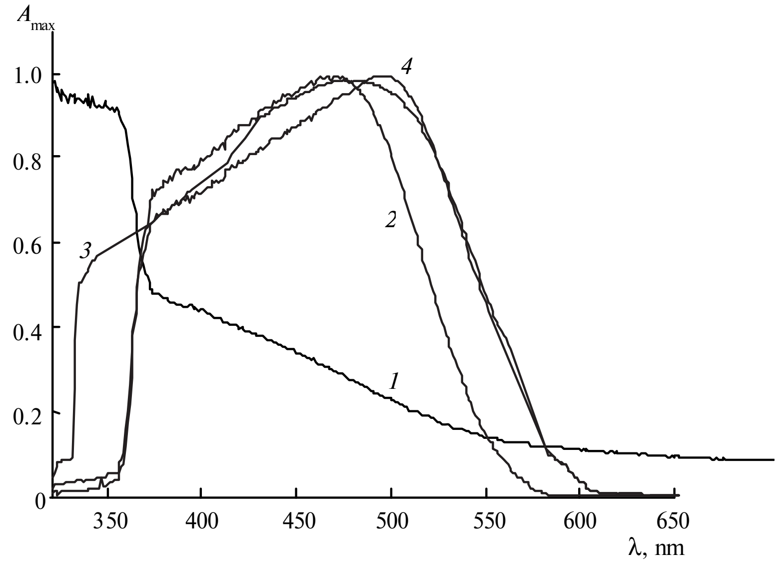


Fig. 1. Normalized spectra of diffusion reflection SiO_2—EPC before (*1*) and after its treatment by solution of 4- nitrophenol (*2*); 2,4-dinitrophenol (*3*) and 2,4,6-trinitrophenol (*4*) at pH 2.

In the optimal range of acidity isotherm of sorption of Pic on SiO_2—EPC (Fig. 3) was studied. It is shown that Pic is intensively extracted from the aqueous solution on SiO_2—EPC up to 5.1 mg/g of the sorbent.

The adsorption isotherm has a convex form and is linearized in Langmuir coordinates ($Q = 28.5$ μmol/g, $b = 0.05$). Distribution coefficients of Pic in the Henri region reach the values 7.9×10^4 cm^3/g, which provides a chance to do 4700-fold absolute concentration of Pic in the phase of the adsorbent from the 0.5 dm^3 solution.

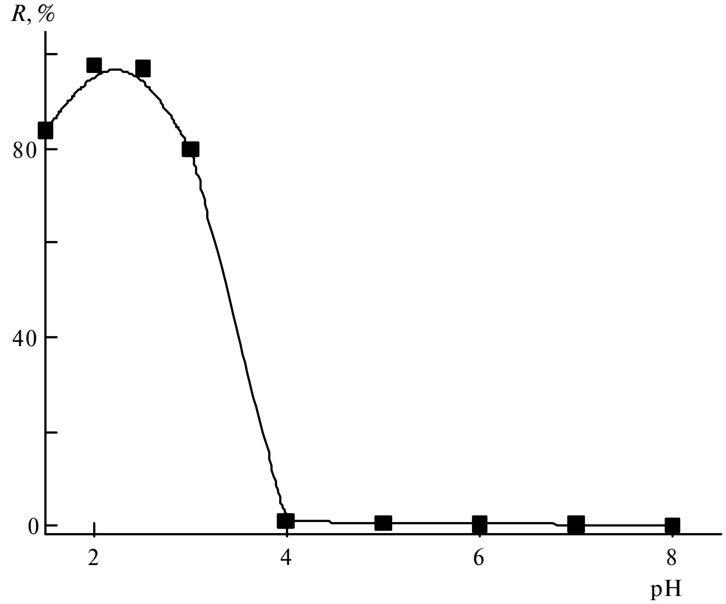


Fig. 2. Relationship between the degree of extraction (*R, %*) of trinitrophenol on SiO_2–EPC and pH of the solution (m—0.1 g, V—15 cm^3, C_{Pic}—3.5×10^{-4} M, τ—60 min.

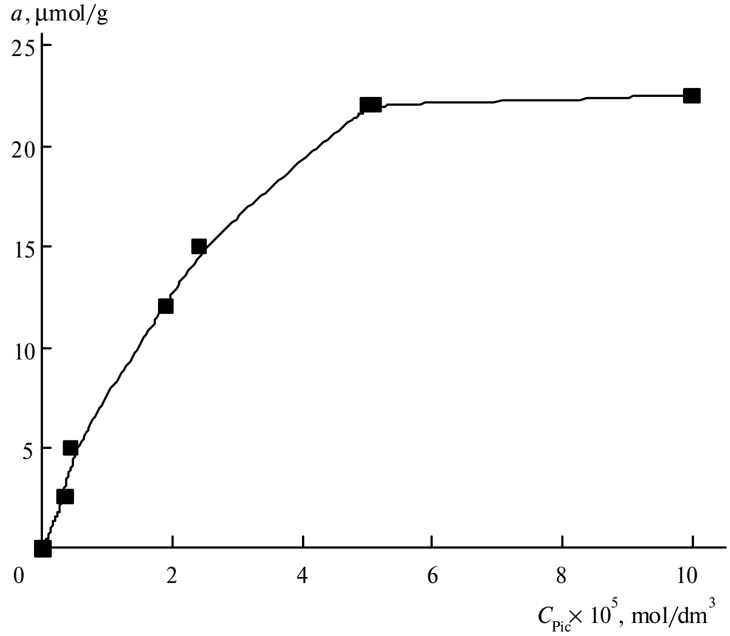
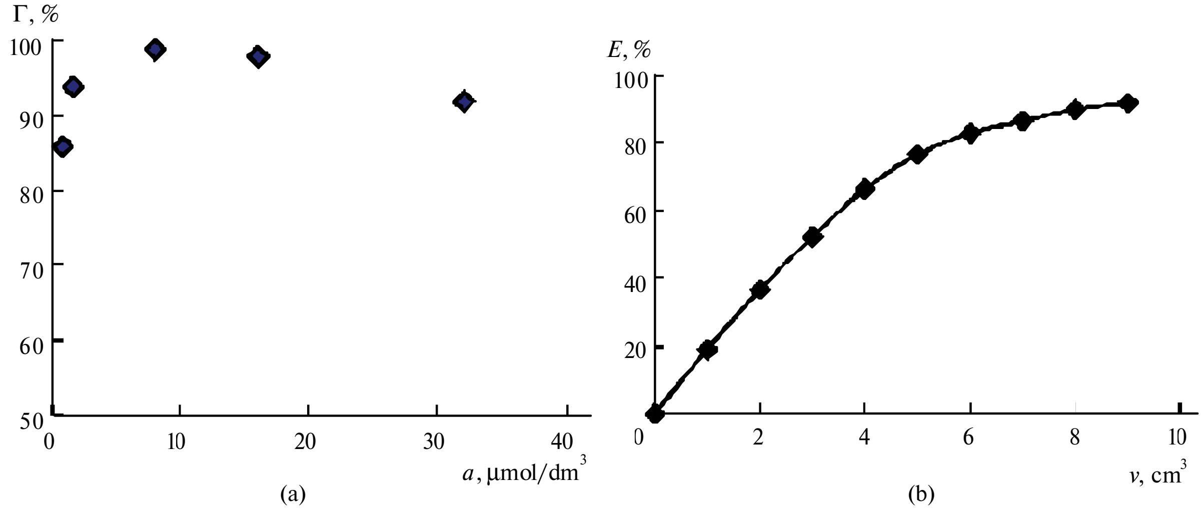


Fig. 3. Sorption isotherm of 2,4,6-trinitrophenol on SiO_2–EPC (m—0.1 g, V—15 cm^3).

High affinity of the adsorbent to nitrophenols makes it possible to use SiO_2–EPC for extracting nitrophenols from diluted solutions under the static mode. It was established that when using 0.1 g of SiO_2–EPC we achieve quantitative extraction of picric acid within the interval of concentration $(0.6–30) \times 10^{-6}$ mol/dm^3 (Fig. 4a).

With the aim of chromatographic determination of NAC in the concentrate it was necessary to optimize conditions for elution of adsorbents with SiO_2–EPC. With the account of the chosen method of analysis, the whole rage of solvents as eluent compatible with the HPLC-determination was investigated. It was found that usual solvents do not lead to desorption of Pic and only when using dimethyl sulfoxide (DMSO) we managed to achieve more than 90% elution of Pic into the concentrate (see Fig. 4b). When using 8 cm^3 of this eluent and SiO_2–EPC as SPE may be achieved more than 100-fold absolute concentration of Pic at the total yield of at least 79%.



(a) (b)

Fig. 4. Relationship between the degree of extraction of 2,4-trinitiphenol in the phase of the adsorbent (a) and eluent (b) and its concentration in the aqueous solutions (a) and the volume of dimethyl sulfoxide (b). (m(SiO_2–EPC) = 0.1 g, pH 2).

Under optimal conditions analytical concentration of Pic and 2,4-dinitrophenol from aqueous solutions was carried out and determined analytical characteristics of the techniques of their HPLC-determination using diode–matrix detection. For calculation of detection limit (DL) and quantitative determination (QD) of picric acid and 2,4-DNP 3S and 10S criteria were used. A linear enlargement of the area of the chromatographic peak of the substance being analyzed at an increase of its concentration in the solution was observed at the wavelength of detection λ = 360 nm, which was chosen as the working one. Results of the calculation of analytical parameters are given in Table 1.

Table 1. Parameters of equations of the graduation graph and values of limits of detection and quantity determination of picric acid and 2,4-DNP

Substance, mg/dm^3	λ, nm	a	b	DL	QD*	DL*	QD*
				mg/dm^3		μg/dm^3	
Pic	360	0.24 ± 1.5	12 ± 0.2	0.4	1.3	5	18
2,4-DNP	360	0.24 ± 0.26	1.56 ± 0.01	0.5	1.7	7	24

* DL and QD—detection limits and quantitative determination of matter being analyzed with the account of the concentration from 500 cm^3 of the solution and elution of 4 cm^3 of DMSO.

The influence of the main salt components of water on the correctness of determination of Pic in aqueous matrices is shown in Table 2, from which it may be seen that at concentrations of the matter being analyzed close to QD, cations of metals being studied, more likely, do not affect the quality of analysis, whereas the presence of chloride–anions reduce the total yield of Pic. This is an evidence of the necessity of using an "introduced–found" approach when analyzing water with an unknown mineral composition.

Table 2. Impact of foreign matter on correctness of determination of picric acid by the HPLC method after its sorption concentration on SiO_2–EPC (n = 3; P = 0.95)

Compound	Concentration ratio	Found Pic, μg	S_r, %
–	–	19.3	2
NaCl	1 : 100	15.8	21
$NaNO_3$	1 : 100	19.7	2
Na_2SO_4	1 : 100	18.4	1
$FeCl_3$	1 : 100	14.6	27
$CaCl_2$	1 : 100	15.3	24

Note. Introduce Pic—22.3 μg; m—0.1 g; V_1 (volume of natural water sample)—100 cm^3; V_2 (eluate volume)—4 cm^3; v—1 cm^3/min.

The appraisal of the proposed method of SPE concentration of picric acid was conducted on surface waters by the “introduced–found” method. For investigations river, lake and ground waters were used (Table 3).

Table 3. Results of determination of picric acid in natural waters by the “ introduced–found” method on SiO_2–EPC ($n = 3$; $P = 0.95$)

Type of water	Introduced, μg	Found, μg	S_r, %
River water, Gorlovka	0	0.0 ± 0.01	–
	20.1	20.0 ± 0.2	1.1
Ground water, Gorlovka	0	2.6 ± 0.8	–
	20.1	22.8 ± 0.2	0.92
Lake water, Kiev	0	0.0 ± 0.02	–
	20.1	19.9 ± 0.3	1.3

Note. m—0.1 g; V_1—100, V_2—4 cm^3; v—1 cm^3/min.

Technique of Determination of the Content of Picric Acid

Lake or river water (0.1–0.5 dm^3) containing up to 2.3 mg of Pic, then it was filtered, brought to pH 2.1 ± 0.1 and passed it through a SPE column filled with SiO_2–EPC (m—0.1 g, d—5 mm, h—10 mm) at a rate of 1.0 cm^3/min. The change in the color of the sorbent in the column was an indication of the presence of nitrophenols in the water. After the passage of whole volume of the sample the column was washed out with water (10 cm^3), dried out by passing air through the cartridge and eluted the matter being analyzed 4–6 cm^3 of DMSO at a rate 1.0 cm^3/min. When determining the content of picric acid in the concentrate an aliquot 1 mm^3 was taken and analyzed by the HPLC method on an Agilent 1200 with DMD liquid chromatograph. In this case an Eclipse column was used (4.6 × 250 mm, particle size—5 μm); movable phases: A—deionized water at pH 7; B—acetonitrile. The gradient program: 0 min (A—95%, B—5%); 1 min (A—80, B—20%); 3 min (A— 60, B—40%); 5 min (A—25, B—75%); 8 min (A—95, B—5%); flow rate of the movable phase—1.0 cm^3/min^{-1}; $\lambda = 360$ nm. The concentration of picric acid determined by the “introduced–found” method from the dependence of the area of the peak of this acid on the content of the latter.

CONCLUSIONS

As can be seen from the given data the proposed approach makes it possible to concentrate selectively from surface water both well (Pic) and badly water soluble nitrophenols at the level of MAC. The change of the adsorbent color in the cartridge for SPE indicates possible pollution of water by nitrophenols. The degree of water pollution is determined by the HPLC method after elution of analyzed matter to DMSO.

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Translated by A. Koziy