



Research Article

Simultaneous Determination of 2-Nitrophenol and 4-Nitrophenol in Pharmaceutical Industrial Wastewater by Electromembrane Extraction Coupled with HPLC-UV Analysis

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ABSTRACT

Background: In the present study, an electromembrane extraction (EME) followed by a simple high performance liquid chromatography with ultraviolet detection (HPLC-UV) was developed and validated for simultaneous determination of 2-nitrophenol (2-NP) and 4-nitrophenol (4-NP) in pharmaceutical industrial wastewater sample. Main parameters of electromembrane extraction were evaluated and optimized.

Methods: 1-octanol was immobilized in the pores of a polypropylene hollow fiber as supported liquid membrane. As a driving force, a 100 volt electrical voltage was applied to transfer the analytes from the sample solution (pH, 7.5) through the supported liquid membrane into an acceptor solution (pH, 12).

Results: The best enrichment factors were obtained 36 and 72 for 2-NP and 4-NP, respectively after 15 minutes of extraction. The effect of carbon nanotube, as a solid nanosorbent on EME efficiency, was also evaluated. The proposed method provided the linearity in the range of 10-1000 ng/mL for 2-NP ($R^2 > 0.9997$) and 4-NP ($R^2 > 0.9999$) with repeatability range (% RSD) between 2.6-10.3 % ($n = 3$). The limit of detection was 3 ng/mL and the limit of quantitation was 10 ng/mL.

Conclusion: Finally, the method was applied for the determination of 2-NP and 4-NP in industrial wastewater samples with relative recoveries in the range between 67–76 %. EME improved the sensitivity of HPLC-UV for the determination of trace concentrations of these analytes.

Introduction

During the last two decades, environmental pollutants have made the worrying problems in the human life. The industrialization is the major reason for these problems and contaminations. Most of these materials are toxic and carcinogenic in living organisms and most of them are released in environment as industrial wastes. Nitrophenols are a class of these chemicals which are widely released in environment because of the industrial productions. Nitrophenols are widely released in environment as a waste of plastic factories, pharmaceutical industries (acetaminophen synthesis), leather process, pesticide production, petrochemical industries, dye synthesis and other chemical industries.¹⁻³ Nitrophenols are placed in the list of United States Environmental Protection Agency as priority pollutants.⁴ Therefore, the determination of low level concentrations of these pollutants is very

important in environmental analysis.

There are several analytical methods in literature such as HPLC,⁵⁻¹⁵ μ HPLC,¹⁶ UHPLC,¹⁷ GC and GC-MS,^{18,19} spectrophotometry,²⁰ capillary electrophoresis,^{21,22} and electrochemical methods^{23,24} for the determination of nitrophenols in different sample matrices. Some of the used instruments such as μ HPLC, UHPLC, and GC-MS are expensive methods for environmental analysis. Some of reported HPLC methods require time-consuming and complicated sample preparation process such as liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase microextraction (SPME) and molecularly imprinted polymer assisted solid phase microextraction (MIP-SPME).⁵⁻¹⁵ The requirement of large volume of toxic solvent, long time of sample treatment, use of expensive sorbents and additional sample treatment steps such as filtration, sonication, precipitation, solvent

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evaporation and reconstitution, are the major disadvantages of these complicated sample preparation methods. Also application of large volumes of solvent leads to increase of waste production and is not environmentally friendly. The environmental samples often contain salts, metals, proteins and organic compounds that may interfere with the analyte of interest in instrumental chemical analysis. Therefore application of rapid and easy sample preparation technique using small volumes of toxic solvents (or solventless) is necessary to environmental chemical analysis.

In recent years, miniaturized extraction methods have been developed for the extraction of analytes from different sample matrices.^{25,26} Electromembrane extraction (EME) was recently introduced as an efficient sample preparation technique.²⁷ In this technique, high analyte extraction in a short time is obtained by the migration of charged target analytes from sample solution into an aqueous acceptor solution across a supported liquid membrane (SLM) using an electrical field as a driving force. Ionization of analyte is obtained by pH adjustment, in donor and acceptor solutions. The supported or immobilized liquid membrane is prepared by an impregnated hollow fiber with a very little amount of an organic solvent. After EME, the acceptor solution is collected and subjected to the instrumental analysis without any further treatments. Thus, EME is a good sample preparation approach for extraction of organic compounds from environmental matrices. This process is compatible with analytical equipment (HPLC, GC, etc.) and can improve the detection limit of an analytical method. Furthermore, due to the use of very small volumes of organic solvent, EME is environmentally friendly.²⁶⁻²⁸ To date, EME has been used for the extraction of drugs, dyes, anions, heavy metals, environmental pollutants, amino acids and peptides from different sample matrices.²⁶⁻³³

To the best of our knowledge, EME technique has not been used for simultaneous extraction of 2-nitrophenol (2-NP) and 4-nitrophenol (4-NP) from wastewater samples. In this study, for the first time EME as a micro-extraction technique followed by a simple HPLC-UV method was developed and validated for simultaneous determination of 2-NP and 4-NP from industrial wastewater samples. Major parameters on EME efficiency including composition of SLM, pH of donor and acceptor solutions, voltage, time of extraction and effect of nano-sorbent were evaluated and optimized.

Methods and Materials

Chemicals and reagents

2-nitrophenol (2-NP) (purity 99%), 4-nitrophenol (4-NP) (purity 99%), methanol, ammonium acetate, trifluoroacetic acid, hydrochloric acid, sodium hydroxide, 2-ethylhexanol, 1-heptanol and 1-octanol were purchased from Merck (Darmstadt, Germany). Accurel PP 300/1200 polypropylene hollow fiber with the inner diameter of 1200 μm , wall thickness of 300 μm , and pore size of 0.2 μm that was purchased from Membrana (Wuppertal,

Germany). Multi-walled carbon nanotubes (outer diameter of 8 nm, a length of $\sim 30 \mu\text{m}$, a specific surface area of $> 500 \text{ m}^2/\text{g}$, and purity $> 95 \%$) were purchased from the Neutrino Company (Tehran, Iran). HPLC grade water was obtained using a Fiestream cyclon system (Leicestershire, UK) and was used to prepare all solutions.

Standard solutions and wastewater samples

The stock standard solutions containing 1 mg/mL of 2-NP and 4-NP were separately prepared in distilled water using ultrasonication and were protected from light and stored at 4 °C. Working standard solutions were prepared by dilution of the stock solution using distilled water. Untreated industrial wastewater was obtained from a pharmaceutical factory (Tehran, Iran). pH of wastewater was adjusted by dropwise addition of NaOH solution prior to extraction.

Electromembrane extraction procedure

The DC power supply EPS-Universal model (Paya Pajohesh Pars, Tehran, Iran) with programmable voltage in the range of 0-400 V, providing currents in the range of 0-0.5 A was used to EME procedure. A homemade glass tube (volume = 7 mL, height = 7 cm and internal diameter = 13 mm) with a screw cap was used as an EME compartment. Platinum wires (diameter 0.2 mm) were used as the electrodes. The porous hollow fiber was an Accurel PP 300/1200 polypropylene (Membrana, Wuppertal, Germany) with an internal diameter of 1200 μm , a wall thickness of 300 μm and pore size of 0.2 μm . Seven mL sample solution was filled in the sample extraction vial. A 35 mm piece of polypropylene hollow fiber was dipped into the organic solvent and was sonicated for 30 s to impregnate the hollow fiber. The excess amount of organic solvent was removed from the inner and outer of hollow fiber by washing with water using a micro-syringe. The lower end of the hollow fiber was closed by thermal and mechanical pressure. Then, the lumen of the fiber was filled with 30 μL of the acceptor solution using a micro-syringe. The hollow fiber, including acceptor solution, was inserted into the sample solution. The negative electrode (cathode) was placed in the sample solution and positive electrode (anode) was placed directly in acceptor solution. Subsequently, the power supply was turned on and the required voltage was applied across the electrodes for a defined time. During all extractions, the sample solution was stirred using a stirrer in 600 rpm. At the end of extraction procedure the acceptor solution was collected by a micro-syringe for analysis using HPLC.

HPLC conditions

Chromatographic separation was performed using a modular HPLC system consisted of Knauer HPLC Pump K-1001, a UV Detector K-2600, a Knauer injection system and a Knauer solvent degasser (Berlin, Germany). Chromatographic data were analyzed using ChromGate[®] software, Version 3.1.7 (Berlin, Germany). A Perfectsil[®]

Target ODS column (3–5 μm , 125 mm \times 4 mm i.d.) from MZ-Analysentechnik (Mainz, Germany) was used for chromatographic separation (temperature = 24 ± 2 °C). A mixture of methanol–ammonium acetate (50 mM) buffer (containing 0.1% v/v trifluoroacetic acid, pH 5) in a 60:40 v/v ratio was used as the mobile phase. The buffer was filtered through 0.45 μm nitrocellulose membrane filter prior to use. The flow rate was 0.8 mL/min and UV detection was performed at 270 nm. The volume of injection was 10 μL .

Evaluation of the Effect of carbon nanotubes

In this study effect of carbon nanotubes (CNTs) was evaluated as a nano-sorbent on membrane performance. Carbon nanotubes were used in two forms (pure CNTs and modified CNTs). Modification was performed by oxidation of the carbon nanotube in order to attach polar functional groups (COOH and/or OH). In brief, 1 g CNTs were dispersed into a 40-ml mixture of concentrated $\text{H}_2\text{SO}_4/\text{HNO}_3$ (3:1, V/V). The solution was sonicated for 30 min and refluxed for 8 hours at 110 °C. This suspension was diluted with deionized water then was centrifuged and rinsed with deionized water for several times. The resulting solution was filtered and dried at 100 °C for 4 h to obtain the modified CNTs.³¹ In order to evaluate the effect of carbon nanotube (CNT) on EME efficiency, pure (unmodified) and modified CNTs were dispersed in SLM solvent in concentration of 1 mg/mL during 20 minutes sonication and then, 35 mm pieces of polypropylene hollow fiber were dipped into these suspensions and the pores of hollow fiber were filled with the mixture during 10 minutes sonication. Then, EME was performed in optimum condition.

Calculation of enrichment factor and relative recovery

The enrichment factor (EF) is defined as the ratio of the final analyte concentration in the acceptor solution (C_a) to the initial concentration of analyte in the donor solution (C_d). EF was calculated using Equation 1:

$$EF = \frac{C_a}{C_d} \quad \text{Eq. (1)}$$

The relative recovery (RR) was calculated for industrial wastewater sample using Equation 2:

$$RR = \frac{C_f - C_r}{C_s} \times 100 \quad \text{Eq. (2)}$$

Where, C_f is the concentration of analyte found after addition of known amount of standard into the real sample, C_r is the concentration of analyte in real sample, and C_s is the concentration of known amount of standard, spiked into the real sample.

Results

In order to obtain the maximum extraction efficiency, the effective parameters of the EME procedure including membrane composition, pH of donor and acceptor solutions, applied voltage, extraction time and carbon nanotube effect were evaluated and optimized.

Supported liquid membrane composition

Membrane composition may affect the diffusion coefficient of analyte of interest and therefore may have an impact on the extraction recovery. Solvent such as 1-octanol, 1-heptanol and 2-ethylhexanol were evaluated as SLM composition. The results are shown in Figure 1.

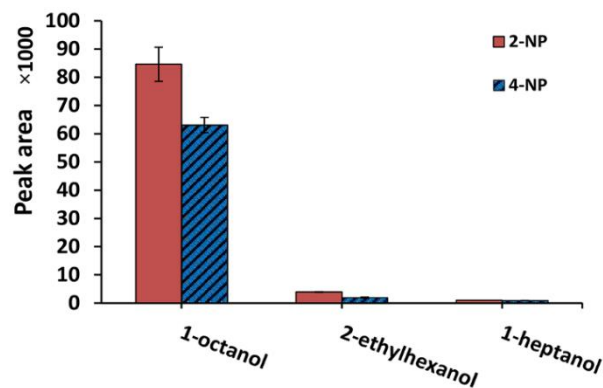


Figure 1. Effect of SLM composition on EME efficiency (extraction condition; concentration in donor: 1 $\mu\text{g/mL}$, pH of donor: 12, pH of acceptor: 12, time of extraction: 10 min and voltage: 30 V).

Applied voltage

The extraction of nitrophenols was evaluated using applied voltage in the range 30 to 200 V. The results are shown in Figure 2. Consequently 100 V was selected as the optimum voltage for further experiments.

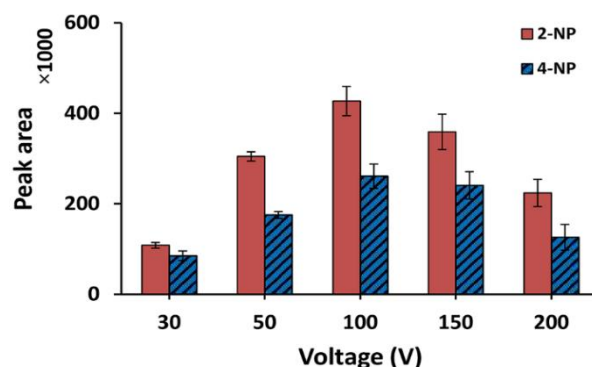


Figure 2. Effect of voltage on EME efficiency (extraction condition; concentration in donor: 1 $\mu\text{g/mL}$, pH of donor: 12, pH of acceptor: 12, time of extraction: 10 min and SLM: 1-octanol).

pH of donor and acceptor solutions

In an EME procedure the analyte should be in the ionic form in the donor solution to ensure maximum extraction efficiency. In the first step, the effect of pH in the donor solution was evaluated whilst retaining the pH of the acceptor solution at 12.0. As shown in Figure 3A, the best pH for the donor solution was 7.5. In the second step, the effect of different pH values (2.0–11.0) in the acceptor solution was also evaluated whilst maintaining donor solution pH at 7.5. As shown in Figure 3B the maximum extraction efficiency was observed at a pH of 12.0 for acceptor solution. Consequently a pH of 7.5 and 12.0 were selected for the donor and acceptor solutions, respectively.

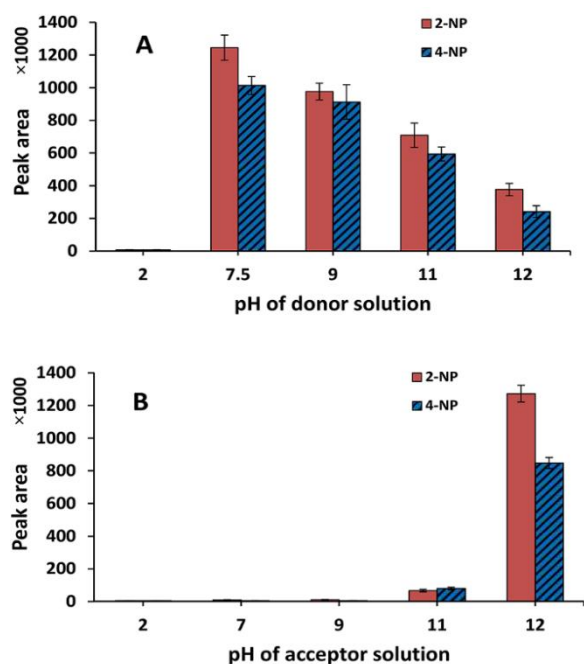


Figure 3. Effect of (A) pH of donor and (B) acceptor solution on EME efficiency (extraction condition; concentration in donor: 1 $\mu\text{g/mL}$, time of extraction: 10 min, SLM: 1-octanol and voltage: 100 V).

Extraction time

Duration of extraction is the other important parameter which should be evaluated to obtain the maximum extraction efficiency. As shown in Figure 4, extraction was increased in the first 15 min.

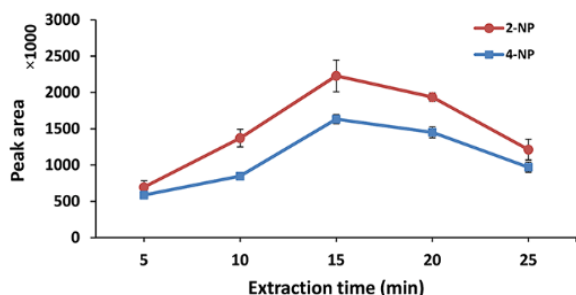


Figure 4. Effect of extraction time on EME efficiency (extraction condition; concentration in donor: 1 $\mu\text{g/mL}$, pH of donor: 7.5, pH of acceptor: 12, SLM: 1-octanol and voltage: 100 V).

Effect of carbon nanotube

Carbon nanotubes were recently used as the effective sorbents in different analytical techniques.²⁹ In this study effect of carbon nanotubes (CNTs) was evaluated as a nano-sorbent on membrane performance. Carbon nanotubes were used in two forms (pure CNTs and modified CNTs). As illustrated in Figure 5, CNTs led to a decrease in extraction efficiency.

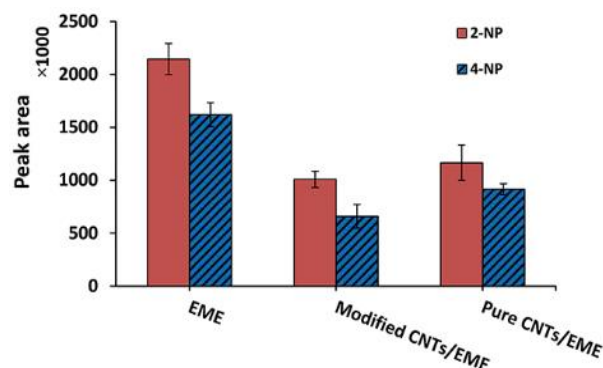


Figure 5. Effect of carbon nanotubes on EME efficiency (extraction condition; concentration in donor: 1 $\mu\text{g/mL}$, pH of donor: 7.5, pH of acceptor: 12, SLM: 1-octanol, time of extraction: 15 min and voltage: 100 V).

Analytical method validation and wastewater sample analysis

The proposed EME-HPLC-UV method validation was performed under optimum conditions by determining the linearity, limit of detection (LOD), limit of quantitation (LOQ), repeatability (RSD%), accuracy (relative error%) and enrichment factor. Analytical characteristics are summarized in Table 1. As can be seen, the enrichment factors (EF) for 2-NP and 4-NP were 36 and 72, respectively. LOD and LOQ were estimated according to a signal-to-noise ratio of 3 and 10, respectively. The calibration standard solutions were prepared using five concentrations of each analyte in water and the solutions were extracted under the optimized condition and then analyzed by HPLC. The linearity was tested over the range 10-1000 ng/mL. The repeatability and relative error were determined at three different concentration levels. As shown in Table 2, the relative standard deviations (RSDs) are in the range between 2.6 % and 10.3 %. The pH of industrial wastewater sample adjusted to 7.5 before EME process.

Table 2. Accuracy and precision data of the proposed EME-HPLC-UV method ($n = 3$).

Nominal concentration ^a	Analyte	Measured concentration ^a (Mean \pm SD)	RSD ^b (%)	RE ^c (%)
50	2-NP	45.2 \pm 2.9	6.4	-9.5
	4-NP	51.9 \pm 1.7	3.3	+3.8
100	2-NP	97.2 \pm 9.7	9.9	-2.7
	4-NP	96.3 \pm 9.9	10.3	-3.7
1000	2-NP	994.6 \pm 43.5	4.4	-0.5
	4-NP	997.4 \pm 26.4	2.6	-0.3

^a ng/mL.

^b Relative standard deviation.

^c Relative error.

Table 1. Analytical characteristics of proposed EME-HPLC-UV method.

Analyte	Linearity (ng/mL)	Regression equation	R ²	LOD ^a (ng/mL)	LOQ ^b (ng/mL)	EF ^c
2-NP	10-1000	Y = 1501 X + 1433	0.9997	3	10	36
4-NP	10-1000	Y = 1141 X + 8235	0.9999	3	10	72

^a Limit of detection. ^b Limit of quantitation. ^c Enrichment factors were calculated at a concentration level of 50 ng/mL for each analyte.

2-NP and 4-NP were not detected in the investigated wastewater sample. In the next experiment, the analytes were spiked to wastewater in final concentration level of 100 ng/mL and the extraction was carried out under optimum condition. Concentrations of 2-NP and 4-NP in wastewater samples were calculated by the obtained calibration equation for each analyte. The relative recoveries were calculated using equation 2. As summarized in Table 3, the relative recoveries were obtained between 67 and 76 % for untreated industrial wastewater samples. The results of RSDs % based on three replicated determinations were less than 5.8 %. Figure 6 shows the chromatograms related to pure water and wastewater samples after EME process.

Table 3. Results obtained for the analysis of industrial wastewater sample by the proposed EME-HPLC-UV method (n = 3).

Analyte	C _{real} ^a sample ^a	C _{added} ^a	C _{found} ^a (mean ± SD)	RSD (%)	RR ^b (%)
2-NP	ND ^c	100	66.9 ± 3.6	5.4	67
4-NP	ND	100	76.1 ± 4.4	5.8	76

^a ng/mL.

^bRelative recovery.

^cNot detected

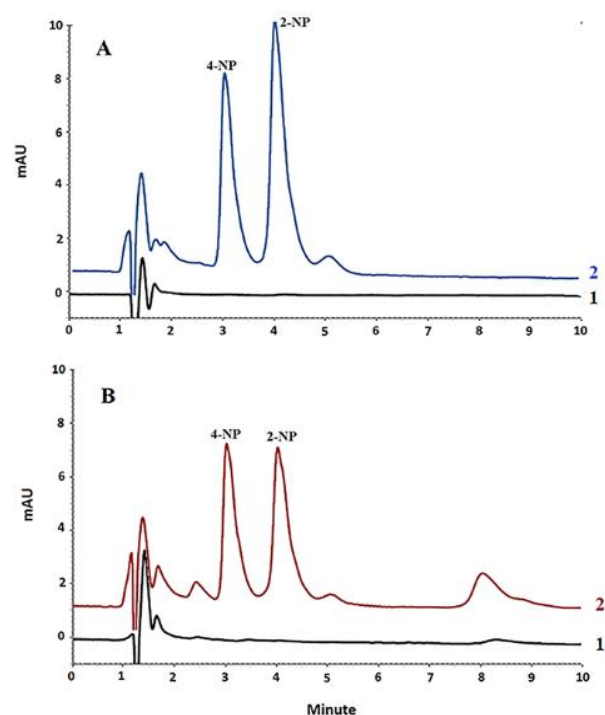


Figure 6. Typical chromatograms of (A) water and (B) industrial wastewater after EME procedure (1, blank and 2, spiked samples).

Discussion

Electromembrane extraction efficiency

Nitrophenols are weak acids and acidic compounds as previous reports are compatible with aliphatic alcohols as SLM in EME.^{26,28} The results indicated that 1-octanol was the best solvent that gave the most efficient extraction. The potential difference between compartments is the main driving force for the migration of the analytes across

liquid membranes. The applied voltage is one of the most important parameters that affects the flux of analytes and must be optimized.²⁶⁻²⁸ As shown in Figure 2 the extractabilities of 2-NP and 4-NP were increased by increasing the voltage up to 100 V without a further increase in extraction when voltages >100 V was used. In this situation, the current in the system was increased and resulted in electrolysis and bubble formation. These conditions made the system unstable and decreased the efficiency of analyte/proton exchange and led to decrease in extraction recovery. The pH of donor and acceptor solutions can impact the ionic balance in the system. For effective extraction by EME technique, the analytes should be ionized in solution. It was revealed that the total ionic concentration in the donor solution to that in the acceptor solution has a direct impact on the flux across the membrane.³⁴ The pKa values of 2-NP and 4-NP are 7.2 and 7.1, respectively and both of them are weak acids. Therefore, in a solution of pH 7.5 more than 50 % of the molecules in donor solution are likely to be ionized and an increase in pH would result in greater ionization of the molecules but extraction of analytes were decreased. It seems that increasing of donor pH (>7.5) caused to increase the total ionic concentration in donor solution, and consequently, led to increase of ionic balance in the system. Therefore, the decrease in extraction was observed in higher pHs. As shown in Figure 3B the maximum extraction efficiency was observed at a pH of 12.0 for acceptor solution. In this condition, the EME system had the minimum ionic balance and minimum back diffusion from the acceptor solution. Time of extraction is the other parameter that was evaluated. After 15 minutes, a decrease in extraction was observed. The saturation of the analyte in the acceptor solution and bubble formation in the donor solution are the main reasons for this phenomenon. Back diffusion from the acceptor to the donor solution and loss of organic solvent in the SLM as a result of heat generation in the system are also possible reasons for decrease in extraction recoveries during long time extraction.²⁶⁻²⁸

Several reports introduced the application of carbon nanotube (CNT) in EME procedure.^{29,31} In our experiments, CNTs led to a decrease in extraction efficiency more than likely due to CNTs acting as physical barriers that impede analyte migration. In this situation, mass transfer was reduced by the addition of CNTs to the SLM because of trapping of target analyte in the SLM. In case of modified CNTs, the inhibition of extraction was more than pure CNTs, due to the interaction between functional groups of modified CNTs (OH, COOH) and hydrophilic groups (OH) of 4-NP and 2-NP. Carbon nanotubes also exhibit electrical conductivity and resulted in a reduced resistance of the SLM and consequently a reduced electrical field. Furthermore, analytes may be adsorbed onto the sorbents and the desorption force (applied voltage) may not be sufficient to release the analyte into the acceptor solution. These phenomena are likely to lead to decrease in extraction efficiency.³¹

Table 4. Comparison of the proposed method to the other reported methods for the determination of 2-NP and/or 4-NP in different sample matrices.

Method ^a	Analyte	Matrix	LOD (ng/mL)	LOQ (ng/mL)	Sample treatment ^b	Estimated Analysis Time	Relative Recovery %	Ref.
MS-USAEME-SFO-UHPLC-DAD	4-NP	Lake water	0.6	2.5	Yes	-	101	[17]
UAE-DLLME-GC-MS	2-NP, 4-NP	Soil	0.6, 0.8 ^c	2.1, 2.6 ^c	Yes	> 22 min	101, 105	[19]
HF-LPME-CE-MS	2-NP, 4-NP	Atmosphere	2, 0.01 ^d	-	Yes	> 75 min	21, 64	[21]
SMS-LLME-HPLC-UV	2-NP, 4-NP	River water, wastewater	0.58, 0.26	2, 2	Yes	> 35 min	90-98	[10]
BA μ E-LD-HPLC-DAD	4-NP	water	0.3	0.8	Yes	~ 17 h	102	[12]
EMM- μ HPLC-UV	2-NP	Fresh water, River	0.1	0.3-1.5	No	> 25 min	85-114	[16]
EME-HPLC-UV	2-NP, 4-NP	Industrial wastewater	3, 3	10, 10	No	~ 20 min	67, 76	This work

^a MS-USAEME-SFO: manual shaking-enhanced ultrasound-assisted emulsification microextraction based on solidification of floating organic, UHPLC: ultra high performance liquid chromatography, DAD: diode array detector, UAE: ultrasound assisted extraction, DLLME: dispersive liquid-liquid microextraction, GC-MS: gas chromatography/Mass spectroscopy, HF-LPME: hollow fiber liquid phase microextraction, CE-MS: capillary electrophoresis/mass spectroscopy, SMS-LLME: supramolecular solvent liquid-liquid microextraction, HPLC: high performance liquid chromatography, UV: ultraviolet, BA μ E-LD: Bar adsorptive microextraction combined with liquid desorption, EMM: electro-mediated microextraction.

^b further sample treatment steps such as filtration, sonication, precipitation, centrifuge and solvent evaporation. ^c ng/g. ^d ng/m³.

Performance of analytical method

The proposed EME-HPLC-UV method was applied to the determination of nitrophenols in industrial wastewater samples to establish the applicability of the method. The relative recoveries were obtained between 67 and 76 % for untreated industrial wastewater samples. Industrial wastewaters often contain salts, metals and other organic compounds. Presence of these materials could be the reason of lower relative recovery of the wastewater in comparison to pure water analysis. Table 4 shows a comparison of the proposed method to the other techniques for the determination of 2-NP and 4-NP in different sample matrices. The results suggest that the proposed method for simultaneous determination of 2-NP and 4-NP in wastewater samples is relevant and applicable. This method is simple and cheap in comparison to expensive methods such as GC-MS, UHPLC, and μ HPLC. Some of the reported HPLC methods need to time-consuming and complicated sample preparation process such as liquid-liquid extraction, solid phase extraction, solid phase microextraction and molecularly imprinted polymer assisted SPME.^{7,10-12} Some of these methods require large volume of toxic solvent, long duration of sample treatment. Some of them require the expensive sorbents and need to further complicated steps such as filtration, sonication, precipitation and solvent evaporation for analysis.^{5,11,17} The presented EME-HPLC-UV method could be performed directly on wastewater samples without any other sample preparation steps. The short extraction and analysis time, consumption of small volumes of organic solvent (~15 μ L) and a cheap and simple analytical technique (HPLC-UV), make the method applicable for simultaneous determination of 2-NP and 4-NP in wastewater samples.

Conclusion

An efficient and simple EME-HPLC-UV method has been reported for simultaneous determination of 2-NP and 4-NP in industrial wastewater samples. Some parameters including SLM composition, applied voltage, pH of donor

and acceptor solution and time of extraction were evaluated and optimized. The effect of carbon nanotubes as a solid nano-sorbent on membrane performance in EME procedure was also evaluated. Acceptable results were obtained for analysis of wastewater sample after a short time extraction (15 min). The data indicated that the proposed EME-HPLC-UV method was a simple and rapid analytical method for the determination of nitrophenols. Because of the consumption of very small volumes of solvent, the proposed method is environmentally friendly.

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Conflict of interests

The authors claim that there is no conflict of interest.

References

- Achamo T, Yadav OP. Removal of 4-Nitrophenol from Water Using Ag-NP-Tridoped TiO₂ by Photocatalytic Oxidation Technique. *Anal Chem Insights*. 2016;11:29-34. doi:10.4137/ACI.S31508
- Assi N, Mohammadi A, Sadr Manuchehri Q, Walker RB. Synthesis and characterization of ZnO nanoparticle synthesized by a microwave-assisted combustion method and catalytic activity for the removal of ortho-nitrophenol. *Desalination Water Treat*. 2015;54(7):1939-48. doi:10.1080/19443994.2014.891083
- U.S. Environmental Protection Agency. <https://www.epa.gov/sites/production/files/2015-09/documents/priority-pollutant-list-epa.pdf>
- U.S. Environmental Protection Agency. Ambient Water Quality Criteria for Nitrophenols. <https://nepis.epa.gov/Exec/ZyPURL.cgi?Dockey=9100H5QK.TXT>
- Realini PA. Determination of priority pollutant phenols in water by HPLC. *J Chromatogr Sci*. 1981;19(3):124-9. doi: 10.1093/chromsci/19.3.124

6. Galeano-Díaz T, Guiberteau-Cabanillas A, Mora-Díez N, Parrilla-Vázquez P, Salinas-López F. Rapid and sensitive determination of 4-nitrophenol, 3-methyl-4-nitrophenol, 4, 6-dinitro-o-cresol, parathion-methyl, fenitrothion, and parathion-ethyl by liquid chromatography with electrochemical detection. *J Agric Food Chem.* 2000;48(10):4508-13. doi:10.1021/jf000118z
7. Zarejousheghani M, Möder M, Borsdorf H. A new strategy for synthesis of an in-tube molecularly imprinted polymer-solid phase microextraction device: selective off-line extraction of 4-nitrophenol as an example of priority pollutants from environmental water samples. *Anal Chim Acta.* 2013;798:48-55. doi:10.1016/j.aca.2013.08.038
8. González A, Avivar J, Cerdà V. Determination of priority phenolic pollutants exploiting an in-syringe dispersive liquid-liquid microextraction-multisyringe chromatography system. *Anal Bioanal Chem.* 2015;407(7):2013-22. doi:10.1007/s00216-015-8464-1
9. Hou F, Deng T, Jiang X. Dispersive liquid-liquid microextraction of phenolic compounds using solidified floating organic droplets, and their determination by HPLC. *Microchim Acta.* 2013;180(5-6):341-6. doi:10.1007/s00604-012-0937-8
10. Yang Q, Chen X, Jiang X. Liquid-liquid microextraction of nitrophenols using supramolecular solvent and their determination by HPLC with UV detection. *Chromatographia.* 2013;76(23-24):1641-7. doi:10.1007/s10337-013-2554-z
11. Moradi M, Yamini Y, Seidi S, Ghambarian M, Esrafil A. Ultrasound-assisted emulsification microextraction using low density solvent for analysis of toxic nitrophenols in natural waters. *Int J Environ Anal Chem.* 2013;93(2):199-212. doi:10.1080/03067319.2011.603080
12. Neng NR, Nogueira JM. Determination of phenol compounds in surface water matrices by bar adsorptive microextraction-high performance liquid chromatography-diode array detection. *Molecules.* 2014;19(7):9369-79. doi:10.3390/molecules19079369
13. Sun JN, Chen J, Shi YP. Multiple functional ionic liquids based dispersive liquid-liquid microextraction combined with high performance chromatography for the determination of phenolic compounds in water samples. *Talanta.* 2014;125:329-35. doi:10.1016/j.talanta.2014.03.013
14. Ji XN, Ai SS, Tang J, Qu H, Bian DJ. Determination of Nitrophenol Isomers in Water. *Adv Mat Res.* 2015;1083:80-3. doi:10.4028/www.scientific.net/AMR.1083.80
15. Zhang Y, Mei M, Huang X, Yuan D. Extraction of trace nitrophenols in environmental water samples using boronate affinity sorbent. *Anal Chim Acta.* 2015;899:75-84. doi:10.1016/j.aca.2015.10.004
16. Chong YT, Ariffin MM, Tahir NM, Loh SH. A green solvent holder in electro-mediated microextraction for the extraction of phenols in water. *Talanta.* 2018;176:558-64. doi:10.1016/j.talanta.2017.08.068
17. Chung RJ, Leong MI, Huang SD. Determination of nitrophenols using ultrahigh pressure liquid chromatography and a new manual shaking-enhanced, ultrasound-assisted emulsification microextraction method based on solidification of a floating organic droplet. *J Chromatogr A.* 2012;1246:55-61. doi:10.1016/j.chroma.2012.02.025
18. Herterich R. Gas chromatographic determination of nitrophenols in atmospheric liquid water and airborne particulates. *J Chromatogr A.* 1991;549:313-24. doi:10.1016/S0021-9673(00)91442-0
19. Cacho JI, Campillo N, Viñas P, Hernández-Córdoba M. Dispersive liquid-liquid microextraction for the determination of nitrophenols in soils by microvial insert large volume injection-gas chromatography-mass spectrometry. *J Chromatogr A.* 2016;1456:27-33. doi:10.1016/j.chroma.2016.05.098
20. Niazi A, Yazdanipour A. Spectrophotometric simultaneous determination of nitrophenol isomers by orthogonal signal correction and partial least squares. *J Hazard Mater.* 2007;146(1-2):421-7. doi:10.1016/j.jhazmat.2007.03.063
21. Teich M, van Pinxteren D, Herrmann H. Determination of nitrophenolic compounds from atmospheric particles using hollow-fiber liquid-phase microextraction and capillary electrophoresis/mass spectrometry analysis. *Electrophoresis.* 2014;35(9):1353-61. doi:10.1002/elps.201300448
22. Guo X, Wang Z, Zhou S. The separation and determination of nitrophenol isomers by high-performance capillary zone electrophoresis. *Talanta.* 2004;64(1):135-9. doi:10.1016/j.talanta.2004.01.020
23. Zhang T, Lang Q, Yang D, Li L, Zeng L, Zheng C, et al. Simultaneous voltammetric determination of nitrophenol isomers at ordered mesoporous carbon modified electrode. *Electrochim Acta.* 2013;106:127-34. doi:10.1016/j.electacta.2013.05.055
24. Luo LQ, Zou XL, Ding YP, Wu QS. Derivative voltammetric direct simultaneous determination of nitrophenol isomers at a carbon nanotube modified electrode. *Sens Actuators B Chem.* 2008;135(1):61-5. doi:10.1016/j.snb.2008.07.019
25. Jouyban A, Abbaspour M, Farajzadeh MA, Khoubnasabjafari M. Determination of 2-octanone in biological samples using liquid-liquid microextractions followed by gas chromatography-flame ionization detection. *Pharm Sci.* 2017;23(2):121-8. doi:10.1517/PS.2017.18
26. Krishna Marothu V, Gorrepati M, Vusa R. Electromembrane extraction—a novel extraction technique for pharmaceutical, chemical, clinical and environmental analysis. *J Chromatogr Sci.* 2013;51(7):619-31. doi:10.1093/chromsci/bmt041
27. Pedersen-Bjergaard S, Rasmussen KE. Electrokinetic migration across artificial liquid membranes: new

- concept for rapid sample preparation of biological fluids. *J Chromatogr A*. 2006;1109(2):183-90. doi:10.1016/j.chroma.2006.01.025
28. Huang C, Seip KF, Gjelstad A, Pedersen-Bjergaard S. Electromembrane extraction for pharmaceutical and biomedical analysis—Quo vadis. *J Pharm Biomed Anal*. 2015;113:97-107. doi:10.1016/j.jpba.2015.01.038
29. Hasheminasab KS, Fakhari AR. Development and application of carbon nanotubes assisted electromembrane extraction (CNTs/EME) for the determination of buprenorphine as a model of basic drugs from urine samples. *Anal Chim Acta*. 2013;767:75-80. doi:10.1016/j.aca.2012.12.046
30. Nojavan S, Bidarmanesh T, Mohammadi A, Yaripour S. Electromembrane extraction of gonadotropin-releasing hormone agonists from plasma and wastewater samples. *Electrophoresis*. 2016;37(5-6):826-33. doi:10.1002/elps.201500555
31. Yaripour S, Mohammadi A, Nojavan S. Electromembrane extraction of tartrazine from food samples: Effects of nano-sorbents on membrane performance. *J Sep Sci*. 2016;39(13):2642-51. doi:10.1002/jssc.201600071
32. Yaripour S, Mohammadi A, Esfanjani I, Walker RB, Nojavan S. Quantitation of zolpidem in biological fluids by electro-driven microextraction combined with HPLC-UV analysis. *EXCLI J*. 2018;17:349-61. doi:10.17179/excli2018-1140
33. Yaripour S, Zaheri M, Mohammadi A. An electromembrane extraction—HPLC-UV analysis for the determination of valproic acid in human plasma. *J Chin Chem Soc*. 2018;65(8):989–994. doi:10.1002/jccs.201700397
34. Gjelstad A, Rasmussen KE, Pedersen-Bjergaard S. Simulation of flux during electro-membrane extraction based on the Nernst–Planck equation. *J Chromatogr A*. 2007;1174(1-2):104-11. doi:10.1016/j.chroma.2007.08.057