Determination of Phenols in Water Samples using a Supported Liquid Membrane Extraction Probe and Liquid Chromatography with Photodiode Array Detection

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Received 28 September 2006; revised 27 August 2007; accepted 9 October 2007.

ABSTRACT

A simple, selective and inexpensive miniaturized sample preparation method based on a supported liquid membrane extraction probe is described for the extraction and preconcentration in a single step of phenols in water samples. The phenols were extracted from 5 mL aqueous water samples into 0.4 mL aqueous acceptor phase through the organic membrane. The organic membrane consisted of a porous PTFE membrane impregnated with undecane. In order to obtain a selective extraction and enrichment of the phenols, the conditions were kept such that the phenols were non-ionized in the sample and ionized in the acceptor phase. This was achieved by pH adjustments in the sample and acceptor phases. The method was optimized for its extraction time, depth of the probe in the sample and stirring speed. The detection limit ranged from about 4 μg L^{-1} for 2-chlorophenol and 2,4-dichlorophenol to 10 μg L^{-1} for 4-chlorophenol. The resulting enrichment factors were about eight times for 2-chlorophenol and 2,4-dichlorophenol and four times for 4-chlorophenol. The sample preparation method was tested for the determination of phenols in river water samples and landfill leachate. Concentrations of phenols in river water were found to be in the range 4.2 μg L^{-1} for 2-chlorophenol to 50 μg L^{-1} for 4-chlorophenol. In landfill leachate, 4-chlorophenol was detected at a concentration of 80 μg L^{-1}.

KEYWORDS

Supported liquid membrane extraction probe, selectivity, chlorophenols, water samples.

1. Introduction

The presence and levels of phenols in aquatic environments is of concern because of their widespread release as by-products in the production of plastics and dyes, and in the pulp and paper industries.[1] Phenols are also used as part of the raw materials in the production of a large variety of aromatic compounds, such as explosives, fertilizers, paint, paint removers, textiles and drugs.[2,3] They have also been used as disinfectants and insecticides.[2] These processes often lead to wastewater, ground water and surface water contamination. Chlorinated phenols can also form during wastewater treatment, since chlorine is added as a disinfectant. These compounds, especially the chlorinated ones, are toxic even at low μg L^{-1} levels and are also persistent in the environment. Prolonged oral and subcutaneous exposure causes damage to lungs, liver, kidneys and the genito-urinary tract.[4] The US Environmental Protection Agency (EPA) and the European Union have listed phenols as priority pollutants.

The official method for the determination of phenols in aquatic environments by the EPA uses a liquid–liquid extraction technique with dichloromethane. The procedure involves extraction of the sample into an organic phase, drying and concentration of the extract and analysis with GC-MS (EPA method 625).[5] The detection limit of the method is in the single μg L^{-1} range. The liquid–liquid extraction technique is now seen as environmentally unfriendly, since it uses large volumes of organic solvents. It is also regarded as time-consuming and difficult to automate. The solid phase extraction technique is therefore the most popular, as it combines extraction and preconcentration in a single step and can be easily automated. It has been used for the determination of phenols in aquatic environments with detection limits at low μg L^{-1} levels.[6–9] Despite the cited advantages, solid phase extraction still consumes some organic solvents in the conditioning, washing and elution steps. Emphasis has therefore shifted even for the determination of phenols to sample preparation techniques that use little or no organic solvents and simple methods such as solid phase microextraction (SPME)[10,11] and stir bar sorptive extraction (SBSE).[12,13] These are able to detect and quantify phenols in water bodies at trace levels (single μg L^{-1} levels or below).

Another emerging sample preparation technique is based on membrane extractions.[14–16] Some of the various membrane-based extraction configurations have been reviewed by Jonsson and Mathiasson.[17] The main advantage of these techniques is that they give a high degree of selectivity and clean-up, use little or no organic solvents, and can be employed using easily designed and inexpensive configurations which can also be automated. Phenols have been selectively extracted in crude oil using silicone membrane as the separation barrier.[18,19] Schellin *et al.*[14] recently developed a membrane-assisted solvent extraction method for the determination of phenols in water combined with large volume injection gas chromatography. A supported liquid membrane extraction method has also been reported for the determination of phenols in natural waters with electrochemical detection.[20] In all of the above cases of membrane-based extractions for phenols, selective extraction was obtained with little or no consumption of organic solvents.

In this paper, a simple, selective and inexpensive supported

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liquid membrane extraction technique is described for the preconcentration of phenols in water samples. The new design has been previously tested for the extraction of manganese (II) in biological fluids[15] and organotin compounds in natural waters.[16]

2. Experimental

2.1. Chemicals

The following chlorophenol standards were used: 4-chlorophenol (>99%), 2-chlorophenol (>98%) and 2,4-dichlorophenol (>99%) were from Merck-Schuchardt (Darmstadt, Germany). Other chemicals used were trisodium phosphate (99%), proanalysis sulphuric acid (99%) and HPLC grade methanol and acetonitrile, all from Merck-Schuchardt (Darmstadt, Germany).

2.2. Solutions

Stock solutions at 1000 mg L^{-1} were prepared in methanol and were stored in a refrigerator at 4 °C. Fresh stock solutions were prepared every three months. Solutions for membrane extraction were prepared by diluting the stock with water; the methanol concentration did not exceed 0.1%. Calibration standards at the μg L^{-1} levels were prepared and used the same day.

2.3. Chromatographic Conditions

The three chlorophenols were best separated with a mobile phase composition of 60% water and 40% acetonitrile at a flow rate of 1.0 mL min^{-1} with the UV detector set at 280 nm. The injection volume was 100 μL, except in the early stages, where a 20 μL loop was used. A Supelco C_{18} column, with dimensions 25 cm × 4 mm × 5 μm was used. A Waters HPLC system with 515 pump, Waters 996 photodiode array detector and Millenium chromatographic software were used. The mobile phase was continuously degassed using a Perkin Elmer 200 series degasser.

2.4. Supported Liquid Membrane Extraction Probe Unit

The miniaturized supported-liquid membrane extraction probe was made from polypropylene tube with dimensions 13 mm i.d., 16 mm o.d. and 92 mm length. In this configuration, one end of a polypropylene tube was closed with the porous Millipore filter sealed with PTFE tape and soaked with organic solvent (undecane) for 15 min. It was then rinsed with deionized water both inside and outside. The inside of this probe served as the acceptor phase, as shown in Fig. 1.

2.5. Environmental Water Samples

The environmental water samples to test the method were collected from two rivers (Luvuvhu and Mvudi), one stream (Mutshundudi) and leachate from a landfill in and around Thohoyandou, Limpopo Province, South Africa. Thohoyandou is found in the Limpopo valley, in the far northern part of South Africa. The town has one landfill, about 2 years old, one wastewater treatment plant and several perennial rivers. There are no major heavy industries or mining activities in and around the town. The major source of pollution in the area is car emissions and poor waste disposal practices.[21] Two samples were collected at each location at a depth of about 15 cm below the surface. For Mvudi River, two samples were collected, one upstream before the discharge of sewage effluent and the other downstream, after the discharge of the effluent. Altogether, 10 samples were collected. The pH of the water samples was measured in the laboratory using a pH meter which was calibrated at pH 4 and 10 using standard buffers.

2.6. Procedures

2.6.1. *Sample Preparation*

River water samples for optimization of the extraction procedure were first filtered with 0.45 μm Whatman paper. Water samples were then spiked with known concentrations of each phenol. The pH was adjusted to 4.0 using 2 mol L^{-1} sulphuric acid. Then 5 mL of the sample was extracted with stirring, usually for 50 min.

All the various water samples were collected in clean glass containers. The containers were thoroughly washed with soap, soaked in acid and then rinsed with deionized water. The collected samples were then taken into the laboratory where the pH was measured. They were filtered and pH-adjusted as described above. They were then kept in the refrigerator at 4 °C until analysis. Deionized water was used as a blank sample to see if there were any remaining phenols in the membrane between extractions of samples.

2.6.2. *Preparation of the Membrane Units and Extraction Procedures*

The supported liquid membrane (SLM) probe was soaked in a non-polar organic solvent (undecane) for 15 min. It was then flushed with deionized water both inside and outside to remove the excess of organic solvent on the surface. The inside of the

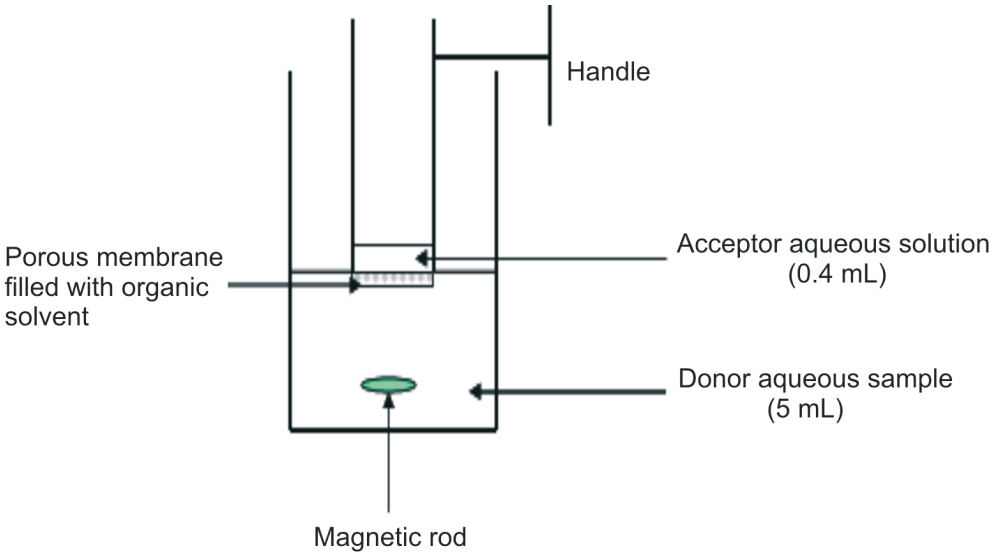

Figure 1 The set-up of the supported liquid membrane extraction probe.

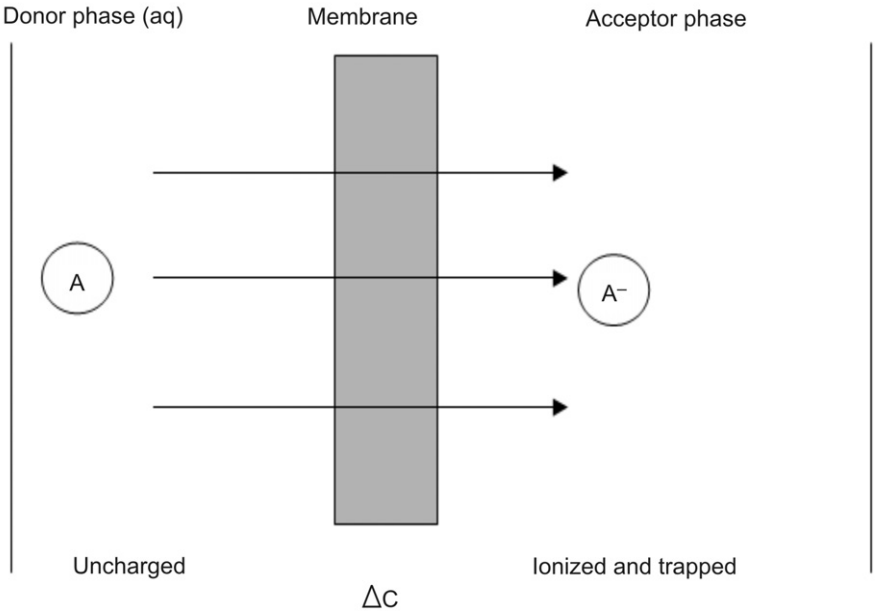

Figure 2 Theory of the extraction process. ΔC denotes the concentration gradient.

probe served as the acceptor phase and was filled with 400 μL of 0.5 mol L^{-1} phosphate buffer. The outside of the membrane probe was immersed in 5 mL of the stirred sample solution. The extraction was allowed to proceed for 50 min. The probe was then removed from the sample solution and the acceptor phase was collected in a polypropylene vial. The pH was adjusted by adding 100 μL of 2 mol L^{-1} sulphuric acid. The extracts were analysed immediately or closed and stored at 4 °C until analysed. Before the next extraction was performed, the inside of the probe was flushed with about three 5 mL portions of fresh acceptor solution and the outside with deionized water. Thorough washing between extractions is very important to make sure that there are no carry-over effects in the membrane to the next sample extraction.

2.6.3. *Preparation of Calibration Curves*

External standards used to quantify extracted samples were prepared from the stock solutions in deionized water. Peak areas were used for quantification. Standard solutions in the μg L^{-1} range were prepared daily while those in the mg L^{-1} range were stored in a refrigerator for about one week.

3. Results and Discussion

3.1. Theory of the Extraction Process

The detailed theory of the SLM extraction technique has been discussed previously.[22–24] In order for phenols to dissolve in the non-polar organic solvent impregnated in the membrane from the sample, they have to be non-ionized at the sample pH. The compounds then diffuse through the membrane into the acceptor phase. Once in the acceptor phase, they are ionized and trapped. The concentration of non-ionized phenols in the acceptor phase is thus kept zero. This maintains a difference in concentration gradient between the donor and acceptor phases. In this way the concentrations of the compounds in the acceptor solution can be increased much higher than in the original sample without experiencing a plateau or maximum and are limited by the sample volume and/or the extraction time. This also gives selective enrichment since only compounds that are ionized at the pH of the acceptor phase are enriched.[25] Compounds that are ionized at the pH of the sample solution do not dissolve into the membrane since they have low partition coefficients. Larger molecules cannot pass through the pores of the membrane and are thus excluded. Generally it is possible to predict in what form a compound will exist at a certain pH by knowing its pK_a. This also helps to set the sample and acceptor pHs. From the developed theory,[22,23] acidic compounds (like phenols) need acceptor pHs that are 3.3 units above their pK_a values for them to be ionized fully and the sample pHs must be 2 units below their pK_a values for the compounds to dissolve into the membrane. These conditions are summarized in Table 1, while the extraction process is shown in Fig. 2.

Two important parameters measured in the supported liquid membrane extraction technique are the extraction efficiency, E, and the enrichment factor, E_n. The extraction efficiency is defined as the fraction of analyte in the extracted sample that is found in the acceptor phase and is given by equation (1) below,[22–24]

$$E = C_A V_A / C_D V_D \,, \quad (1)$$

where C_A is the concentration in the collected acceptor fraction and C_D is the concentration in the extracted sample. V_A is the collected acceptor volume, while V_D is the volume of the sample that has been extracted. The extraction efficiency is also a measure of mass transfer between the donor and acceptor phases and is constant under specified extraction conditions.

The enrichment factor is the ratio of the concentration found in the acceptor phase to that in the original sample. This determines the detection limit of the method. It is given by equation (2) below.[22–24]

$$E_n = C_A / C_D \,. \quad (2)$$

Both the extraction efficiency and the enrichment factor are constant under specified extraction conditions.

3.2. Optimization of the Supported Liquid Membrane Extraction Probe (SLMP)

3.2.1. *Effect of Extraction Time*

The effect of extraction time on the amount of phenol extracted is shown in Fig. 3a. The experiment was conducted under the following conditions: a constant stirring setting of 2 on the

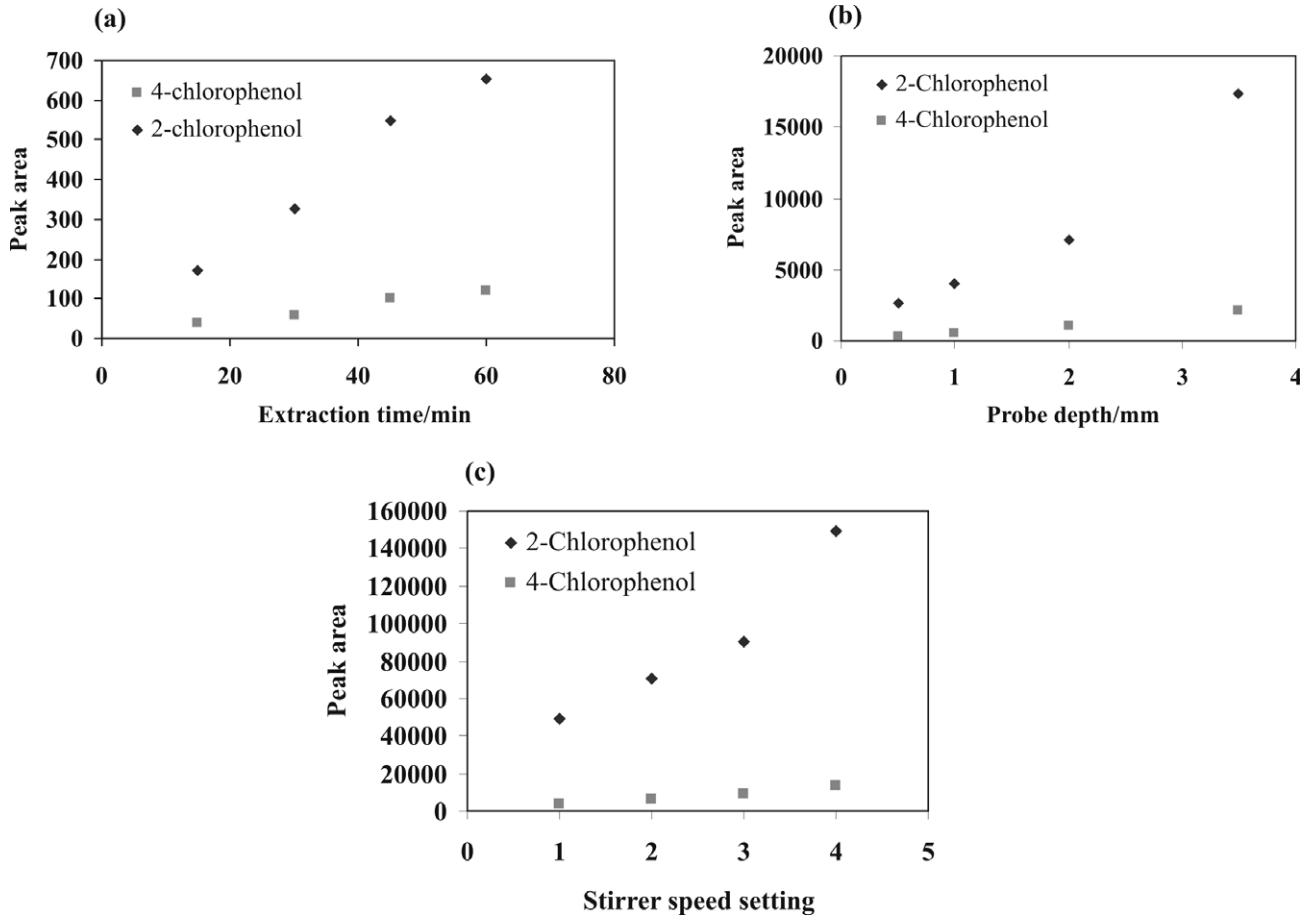

Figure 3 (**a**) Optimization of the extraction time results; (**b**) optimization of the probe depth results; (**c**) optimization of the stirring speed results.

magnetic stirrer and a probe depth of 0.5 mm into the sample solution. The amount of phenol extracted increased with the extraction time. This trend can be explained by the fact that longer extraction times allow for the donor solution to be in contact with the acceptor solvent for longer periods, which enables a greater degree of diffusion of the non-polar analytes from the donor to the acceptor phase through the hydrophobic membrane. This phenomenon has been observed in other applications of the SLMP.[15,16] Ideally, since the same sample is extracted all the time and analytes are trapped in the acceptor phase, in the end all phenols will end up in the acceptor phase. It is seen from Fig. 3a that for 2-chlorophenol, the increase is much steeper, indicating faster mass transfer between the sample and the acceptor phase. For 4-chlorophenol, the mass transfer was slower than for 2-chlorophenol. This could be due to incomplete trapping or partial ionization in the acceptor phase.[24] From Table 1, it is seen that the ideal conditions for trapping 4-chlorophenol were not fully met in the acceptor phase. The fraction of 4-chlorophenol not ionized at the interface between the membrane and the acceptor solution could have influenced the overall mass transfer across the system. An extraction time of 50 min was chosen as the optimum since it was a trade-off between sample extraction throughput and amount extracted. It is also possible to do parallel extractions to increase the sample throughput.

3.2.2. *Effect of Probe Depth*

The effect of probe depth on the extent of extraction of phenols is shown in Fig. 3b. It shows that the maximum amount of phenol was extracted when the membrane-bound probe was inserted 3.5 mm into the donor solution. Generally, the deeper into the donor solution that the probe is immersed, the higher is the volume of the sample coming into contact with the membrane. This results in an increase in the amount extracted. Probe depths higher than 3.5 mm were not tested because in a previous study,[16] it was shown that beyond 3.5 mm, the amount extracted starts to decrease due to the smaller volume of sample that comes into contact with the membrane. There is therefore an upper limit to which the probe depth in the sample can be increased. Thereafter the amount of compound extracted starts to decrease because the working membrane area is only at the bottom of the probe.

3.2.3. *Effect of Agitation Rate*

The effect of agitation rate on the SLMP of the phenols is shown in Fig. 3c. This parameter was investigated using a

Table 1 Ideal sample and acceptor pHs for the SLMP extraction conditions.

Compound	pK_a	log P	Sample pH (ideal)	Acceptor pH (ideal)
2-chlorophenol	8.55	2.15	8.5–2 = 6.5	8.5 + 3.3 = 11.8
4-chlorophenol	9.43	2.41	9.43–2 = 7.43	9.43 + 3.3 = 12.73
2,4-dichlorophenol	7.6	3.23	7.6–2 = 5.43	7.6 + 3.3 = 10.9

Table 2 Extraction efficiency after varying the concentration extracted in spiked river water.

Concentration extracted/μg L^{-1}	% extraction efficiency		
	2-chlorophenol	4-chlorophenol	2,4-dichlorophenol
100	70 (5)	24 (11)	62 (6)
30	65 (7)	25 (8)	57 (10)
15	64 (8)	21 (12)	54 (8)
7	70 (10)	not determined	56 (11)

Table 3 Comparison of detection limits in μg L^{-1} after 50 min extraction time.

	Compound		
	2-chlorophenol	4-chlorophenol	2,4-dichlorophenol
Direct injection (deionized water)	20	25	30
After SLMP (river water)	3.5	10	4

magnetic stirrer equipped with six stirrer settings. It was observed that, generally, an increase in stirring rate results in an increase in the amount extracted. This may be explained in terms of contact time between the analyte and the hydrophobic membrane, and the pressure exerted on the membrane. Increasing the rate of agitation enables more effective mixing of the donor solution and contact with the membrane surface. Stirring enables fresh portions of sample solution to come into contact with the donor side of the membrane surface. This enhances the extent to which diffusion can occur, and hence increases the concentration of phenols extracted in the acceptor phase. At a very high stirring rate the amount extracted does not depend on the transport to the membrane only, but also on the kinetics of the extraction. This may explain why, for 4-chlorophenol, the increase was not very pronounced, especially since the conditions for full trapping in the acceptor phase were not fully met.

3.2.4. *Extraction Efficiency, Concentration Extracted and Detection Limit*

The extraction efficiency was constant in the range 7 to 30 μg L^{-1} investigated, as shown in Table 2. The results are an average of three replicate extractions. The extraction efficiency was calculated after extraction of river water blank and river water spiked with the appropriate concentrations of the phenols. For accurate quantification, the extraction efficiency should not be influenced by the sample matrix and concentration. This is important since in the potential application of the developed extraction method, the concentrations of phenols are unknown. In other applications of the supported liquid membrane extraction technique,[25,26] it has been found that the extraction process is independent of sample concentration and matrices found in water bodies. Table 3 shows a comparison of the detection limits obtained by direct injection and after SLMP extraction. The detection limit of the method was calculated from the signal to noise ratio of 3:1 after extraction of 15 μg L^{-1} spiked phenol compounds in river water. The detection limit ranged from about 4 μg L^{-1} for 2-chlorophenol and 2,4-dichlorophenol to 10 μg L^{-1} for 4-chlorophenol. The resulting enrichment factors were 8 times for 2-chlorophenol and 2,4-dichlorophenol and 4 times for 4-chlorophenol. Although the detection limits were not very low, a number of environmental water samples could be identified and quantified for phenols when the extraction method was tested on real samples. One way to lower the detection limit is to use a much smaller acceptor volume, say 150 μL. Alternatively, sequential extraction can be performed in which the same acceptor solution is kept but two 5 mL portions of the same sample are extracted one after the other. This increases the sample volume extracted from 5 mL to 10 mL.

3.3. Application to Real Water Samples

The extraction method was tested on river water samples from various areas around Thohoyandou. In a landfill leachate, only 4-chlorophenol was detected at a concentration of 80 μg L^{-1}. This compound was also the only one detected in Mvudi River before and after the discharge of municipal wastewater effluent. The concentration was however higher after the discharge (20 μg L^{-1}) than before (14 μg L^{-1}), suggesting that some of the concentration could be coming from the wastewater effluent. In Luvuvhu River (the largest river in the area), all three chlorophenols were detected at 4.2 μg L^{-1} for 2-chlorophenol, 50 μg L^{-1} for 4-chlorophenol and 6.2 μg L^{-1} for 2,4-chlorophenol. In Mutshundudi stream, no target chlorophenol was detected. The UV spectra generated from the photodiode array detector for the standards and the samples were used to reconfirm the identification, apart from the retention time alone. Fig. 4 shows a comparison of the UV spectra from standard 4-chlorophenol and from the landfill leachate sample as a typical example. The pH of river water samples ranged from 7.33 to 7.55, while that of landfill leachate was 6.40. There was therefore no significant difference in pH values for river samples. The major source of these compounds in water bodies around Thohoyandou may be attributed to poor waste management practices. In a previous study,[21] sewage leaking in many parts of the distribution system to the treatment plant was found to be the major source of heavy metal pollution. In this study, it was also found that there is illegal solid waste dumping in areas not far from the rivers. These could be a major source of phenolic compound pollution. The landfill leachate is also not collected for possible safe disposal. During the rainy season, this could leach out to the surrounding water bodies as run-off, especially to Mvudi River, which is nearer to it. The results of the concentration of chlorophenols found in this study in some cases are higher than those found by other researchers in various water bodies.[6,13] Montero *et al.*[13] investigated the concentration of lake and ground water samples using stir-bar sorptive extraction-thermal desorption gas chromatography-mass spectrometry and found concentrations of around 40 μg L^{-1} for 2-chlorophenol.

Figure 5 shows a standard chromatogram and one after the

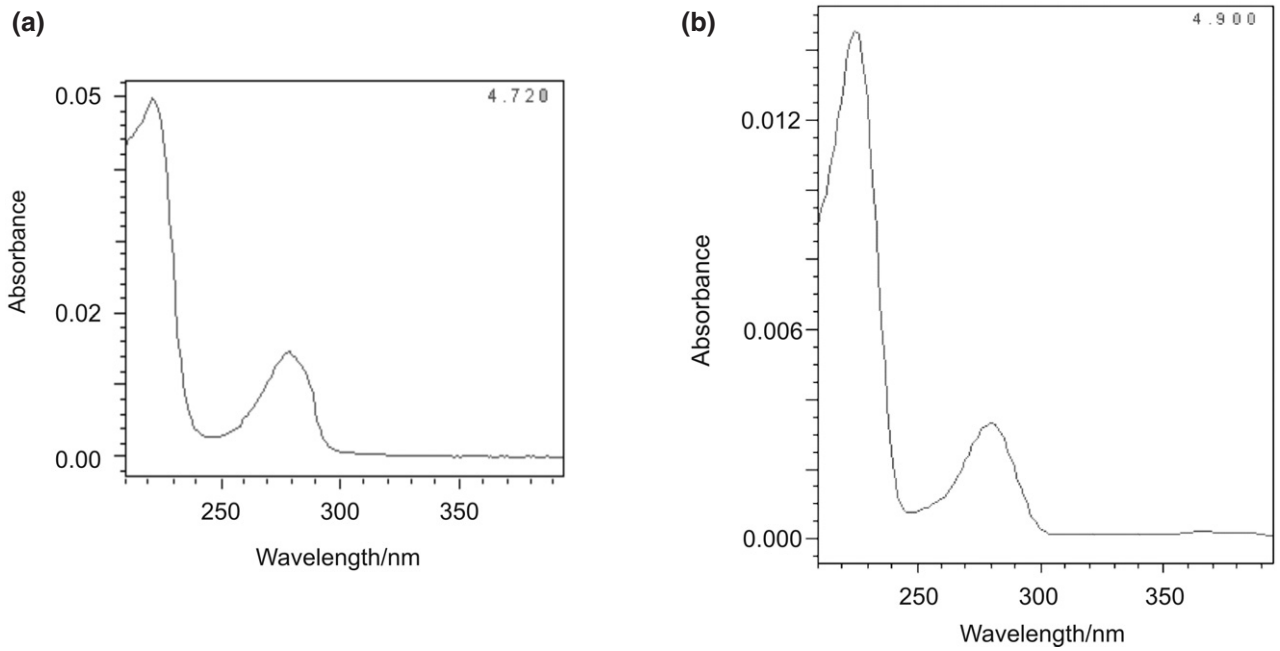

Figure 4 Comparison of UV spectra of 4-chlorophenol obtained from (**a**) landfill leachate sample and (**b**) standard mixture.

extraction of a landfill leachate sample. The landfill leachate chromatogram is quite clean except for the early part, which is due to the phosphate buffer. This demonstrates the selectivity of the sample preparation method. The landfill leachate before extraction was generally black in colour but the extract obtained in the acceptor was as clear as deionized water. Many interfering sample matrices found in the sample were thus not extracted in the acceptor phase.

4. Conclusions

It is possible to use the supported liquid membrane extraction probe for the extraction of phenolic compounds from water. The proposed sample preparation method is simple and inexpensive. The SLMP can be easily home made.

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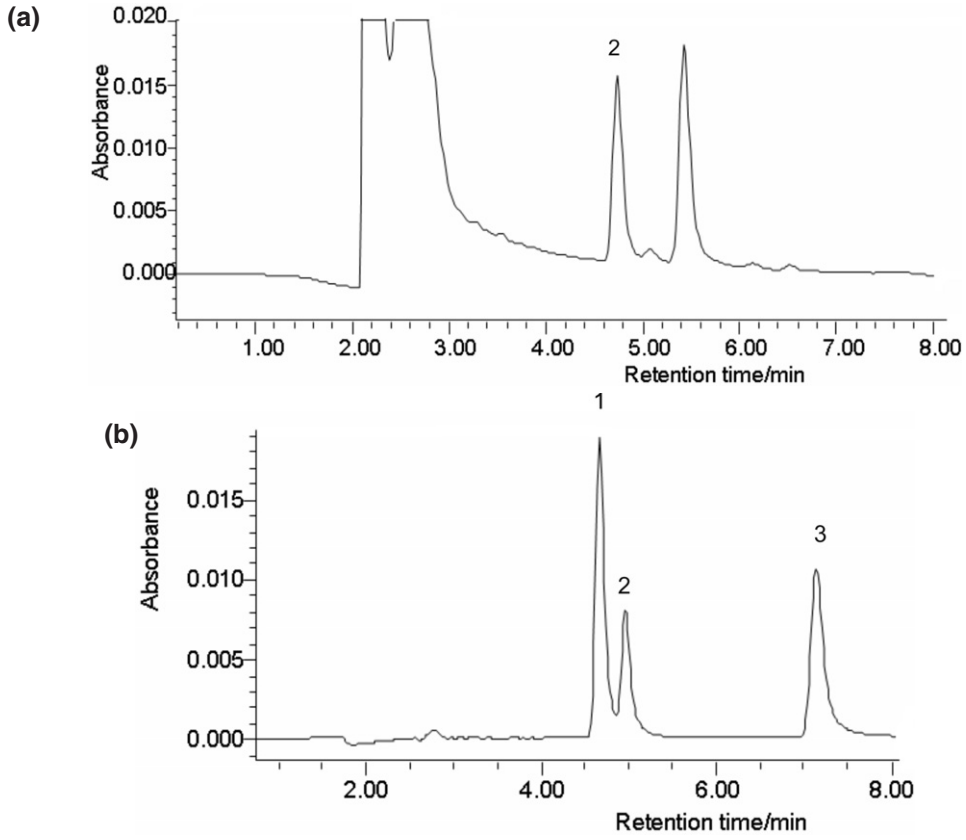

Figure 5 Comparison of chromatograms of the extraction of chlorophenols (**a**) in a landfill leachate and (**b**) obtained from direct injection of 0.1 mg L^{-1} standard mixture. Absorbance measured at 280 nm.

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