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On-line microdialysis–high-performance liquid chromatographic determination of aniline and 2-chloroaniline in polymer industrial wastewater

Jen-Fon Jen[a,*], Chun-Tan Chang[a], Thomas C. Yang[b]

[a]*Department of Chemistry, National Chung-Hsing University, Taichung, 40217 Taiwan, ROC*
[b]*Department of Chemistry, National Kao-Hsiung Normal University, Kao-Hsiung, Taiwan, ROC*

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Abstract

Determination of aniline and 2-chloroaniline in polymer industrial wastewater was examined using high-performance liquid chromatography with on-line microdialysis. After dilution, aniline and 2-chloroaniline in the sample were diffused through a cellular dialysis membrane into the perfusion stream under controlled conditions. Conditions for obtaining optimum dialysis efficiency such as flow-rate and polarity modifier in the perfusion stream, pH and added salt in the sample solution, as well as chromatographic conditions were investigated. The results indicate that the dialysis achieved at a sample matrix pH value of 9.5 with 0.1 *M* NaCl addition, and the perfusate at 10-μl/min flow-rate offered optimum dialysis efficiency. The aniline and 2-chloroaniline were well separated in an acceptable time on a reversed-phase C_{18} column eluted with 40% aqueous methanol solution at pH 7.0 and 1.0 ml/min flow-rate. The proposed method provided a very simple procedure to determine aniline and 2-chloroaniline in wastewater. Application was illustrated by the analysis of aniline and 2-chloroaniline in wastewater released from a polymer factory.

Keywords: Water analysis; Aniline; 2-Chloroaniline

1. Introduction

Aniline and 2-chloroaniline are anthropogenic organic compounds widely used in polymer, rubber, pharmaceutical and dye industries [1–3]. Both are suspected carcinogens and are highly toxic to aquatic life [4–6]. The determination of these compounds in wastewater prior to discharge into the environmental is necessary to develop strategies for pollution prevention and minimization. Many analytical techniques are reported in literature for the determination of aromatic amines including aniline and 2-chloroaniline such as gas chromatography (GC) [1,7–9], high-performance liquid chromatography (HPLC) [10–15], capillary zone electrophoresis (CZE) [16] and spectrophotometric method [17,18]. No matter what technique is used, an appropriate pretreatment of wastewater sample is required prior to the analytical measurement.

Current pretreatment methods for organic species in aqueous samples involve liquid–liquid extraction (LLE), solid-phase extraction (SPE) and solid-phase microextraction (SPME) [7,8,13,15,19,20]. LLE is efficient for aqueous hydrophobic species, but not for

*Corresponding author. Tel.: +886-4-2285-3148; fax: +886-4-2286-2547.

E-mail address: jfjen@dragon.nchu.edu.tw (J.-F. Jen).

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polar species such as aniline and 2-chloroaniline. Moreover, using a large quantity of organic solvents in LLE causes pollution problems with the accompanying risk for health, in addition to the formation of emulsions and extensive time-consuming clean-up procedures. With SPE, most disadvantages of LLE can be eliminated or decreased. However, plugging of the cartridges or the disks by the presence of particulate in samples and the problems related to large sample volume still limit the SPE application. In this decade, SPME was developed with advantages of simultaneous extraction and preconcentration, free solvent, more rapid and simple than LLE and SPE. However, the selectivity of extraction is often interfered with by high-molecular-mass species (polymers or oligomers) present in samples. Therefore, a method to extract aniline and 2-chloroaniline in polymer industrial wastewater is needed.

Membrane-based separations have been applied as useful tools in the pretreatment of complicate matrix samples [21]. Compared to the conventional extraction protocol, microdialysis has the advantages of easy operation, rapid isolation of components from sample matrix and free or less-use organic solvents. Therefore, on-line dialysis has been widely used in bioprocess monitoring [22–25]. From the dialysis successfully applied in the sampling of bovine serum, muscle tissue, plasma and for pharmacokinetic studies [26–34], viscous emulsion sample [35], microdialysis has potential to be an alternative to conventional extraction techniques for polymer industrial wastewater.

In this paper, with the described advantages, the microdialysis technique on-lined to HPLC is systematically investigated to develop an analytical process to determine aniline and 2-chloroaniline in polymer industrial wastewater.

2. Experimental

2.1. Apparatus

The HPLC used in this work was a Shimadzu LC-9A system (Shimadzu, Kyoto, Japan) and a Waters 484 tunable absorbance detector (Waters, Milford, MA, USA) with a 20-μl-flow cell. The detection wavelength was set at 230 nm. A Supelcosil reversed-phase C_{18} column (25 cm×4.6 mm I.D., 5 μm particle size) (Supelco, Bellefonte, PA, USA) was used for separation. A RP-18 guard column was fitted upstream of the analytical column. A Rheodyne 7010 injector/switching valve (Rohnert Park, CA, USA) with a 20-μl external loop was used as the interface between the microdialysis system and the HPLC system for sample introduction. A Chromatocorder 12 Integrator (SIC, Tokyo, Japan) was used to obtain the chromatogram and perform data calculations. The microdialysis system comprises a baby bee syringe pump, a worker bee controller and a 1-ml syringe (Bioanalytical System, Indiana, USA)

A laboratory-assembled linear cellular membrane probe (cellulose acetate, 5000 Da, I.D. 220 μm, O.D. 310 μm) microdialysis sampling system was prepared and set-up as shown in Fig. 1. The syringe pump containing perfusate was connected to the inlet of the probe with PE tubing. The outlet of the microdialysis probe was connected to sample-loop with PE tubing (I.D. 380 μm, O.D. 1090 μm) for chromatographic determination.

2.2. Chemicals and reagents

Deionized water was produced using a Barnstead Nanopure water system (Thermolyne, Dubuque, IA, USA) for all aqueous solutions. All chemicals and

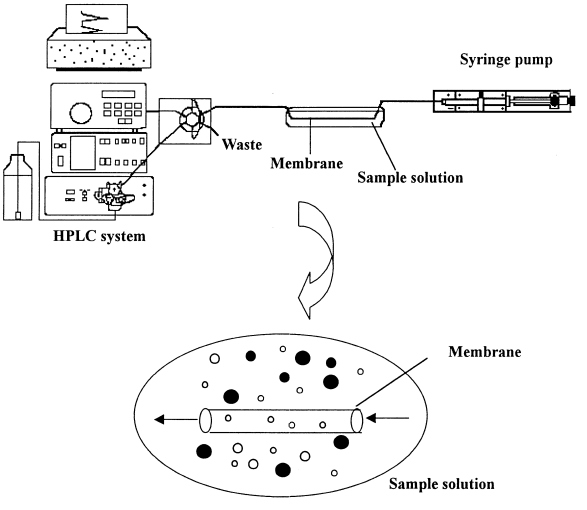

Fig. 1. Assembly of the on-lined microdialysis–HPLC system.

solvents were of ACS reagent grade. Standard stock solutions (1000 μg/ml) of aniline (Riedel-de Haën, Hannover, Germany) and 2-chloroaniline (Acros Organics, Belgium) were prepared by dissolving 0.100 g in 50 ml water and then adding water to adjust the volume to 100 ml. The solutions were stored in brown glass bottles and kept at 4°C. Fresh working solutions were prepared daily by appropriate dilution of the stock solutions. Sulfuric acid and sodium hydroxide and sodium dihydrogen phosphate (Riedel-de Haën) were used for pH adjustments. The HPLC eluent was prepared as 40% methanol in 0.01 *M* phosphate buffer (pH 7.0). The eluent was filtered through a 0.45-μm poly(vinylidenedifluoride) (PVDF) membrane filter and degassed ultrasonically.

2.3. Procedure

Samples were collected and diluted appropriately with 0.10 *M* NaCl solution. After mixing thoroughly, the sample solution was poured into the dialysis cell (50 ml) to achieve the dialysis. The dialysate was collected on-line in a sample loop for HPLC analysis.

3. Results and discussions

In order to optimize the sampling and analytical conditions, factors that affect the dialysis efficiency, such as the flow-rate, polarity modifier and pH in perfusion stream, the pH and salt in sample solution, as well as the chromatographic conditions were studied thoroughly.

3.1. Optimization of chromatographic conditions

The chromatographic conditions were optimized and built-up prior to investigation of microdialysis conditions. Referring to Refs. [10,12,15], a reversed-phase C_{18} column has the potential to resolve aniline and 2-chloroaniline from other species very well and was therefore used. In order to obtain an acceptable resolution of peaks within an appropriate time interval, a quantity of organic modifier is generally added to the eluent and the pH of eluent is also adjusted. From series tests, the optimum separation was achieved by eluting with 40% methanol in 0.01 *M* phosphate buffer solution (pH 7.0) at a 1.0 ml/min flow-rate. In order to obtain the highest detection sensitivity, the wavelength of the detector is best set at or near the λ_{max}. From the UV spectra, aniline and 2-chloroaniline in eluent solution have characteristic $\lambda_{max.}$ values at 203 nm and 209 nm, respectively. Besides, both have other characteristic absorption at 229 nm and 233 nm, respectively. However, detection at lower wavelengths worsens the stability of the baseline. Using a compromise between the detection sensitivity and baseline stability, the detection wavelength was set at 230 nm.

Under these conditions, the chromatogram for standard species of aniline and 2-chloroaniline was as in Fig. 2a, where peaks 1 and 2 relate to aniline and 2-chloroaniline, respectively. It is obvious that both species give sharp and symmetric peaks which are well separated within 12.5 min. The retention times of aniline and 2-chloroaniline are 6.14 min with 0.13% RSD ($n=3$) and 12.19 min with 0.13% RSD ($n=3$), respectively. The reproducibility of quantitative detection for 100 μg/l was 3.86 and

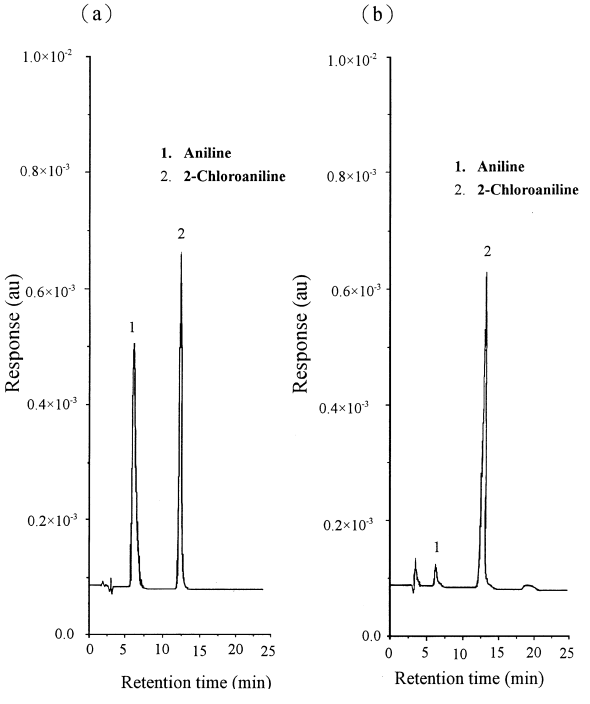

Fig. 2. Chromatograms for standard aniline and 2-chloroaniline. (a) Standard sample solution; (b) authentic wastewater sample.

2.36% RSD for three determinations of aniline and 2-chloroaniline, respectively.

3.2. Effect of sample pH on the dialysis efficiency

As in other extractions, the recovery of species from a dialysis system depends on the sample pH [36–38]. Therefore, the effect of pH on the dialysis of aniline and 2-chloroaniline was studied. Fig. 3 shows the dialysis recoveries of aniline and 2-chloroaniline using varied pH values. The dialysis efficiencies of aniline and 2-chloroaniline are increased with the increase of pH initially and level off after pH 5.0 for 2-chloroaniline and pH 7.0 for aniline. The pK_a values for the deprotonation of protonated aniline and 2-chloroaniline are 4.63 and 2.66, respectively [39], so both aniline and 2-chloroaniline are unlikely to diffuse through the cellular fiber in their protonated forms. This may be caused by the partially negative-charge occurring on the cellulose fiber in the operation pH which might attract the positively-charged species and then retarded the diffusion. If aniline and 2-chloroaniline are in the molecular form, they diffuse through the cellular fiber more easily. To obtain a good dialysis efficiency, the pH of sample matrix for microdialysis is thus recommended at pH>7.0. Because the pH of the wastewater sample after dilution was 9.5, it thus was used for examination.

3.3. Effect of salt addition in sample matrix on the dialysis

A salting-out effect is often applied to improve the recovery in conventional extraction processes. In the studies, varied amounts of NaCl were added into the sample solution to investigate the effect on diffusion efficiency. Fig. 4 demonstrates the recovery of dialysis increases with the NaCl addition and goes to flatness after 0.10 *M* addition. The enhancement of recovery for 2-chloroaniline was slightly higher than that for aniline due to the higher hydrophobicity of 2-chloroaniline. Thus, 0.10 *M* of NaCl was added to the sample solution in the studies. Because the recovery of dialysis can be up to 90% if no NaCl is added, if the aniline and 2-chloroaniline in the sample were high enough to detection, the addition of NaCl is recommended to simplify the procedure. A simple on-line monitoring procedure for aniline and 2-chloroaniline in wastewater can thus be set-up easily.

3.4. Effect of polarity modifier (methanol) in the perfusion stream on the dialysis efficiency

The aniline and 2-chloroaniline diffuse through the cellular membrane as neutral types. Based on ‘‘like dissolves in like’’, diffusion efficiency of species should be somehow influenced by the polarity of perfusate. Therefore, a polarity modifier was added

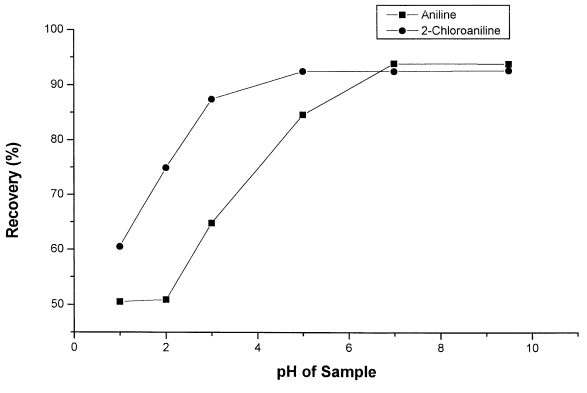

Fig. 3. Effect of sample pH on the dialysis efficiency of aniline and 2-chloroaniline.

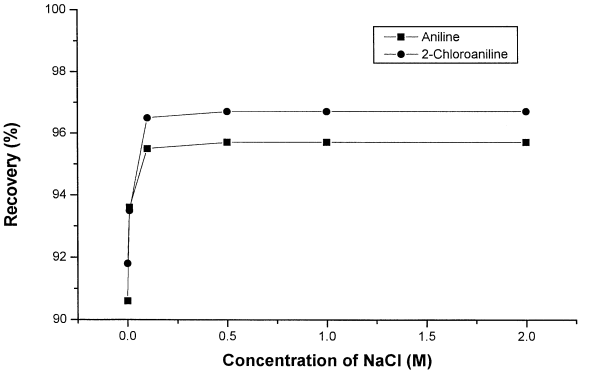

Fig. 4. Effect of addition of salt on the dialysis of aniline and 2-chloroaniline.

to the perfusate and its effect on diffusion efficiency was investigated. Because the cellular membrane is more stable in methanol than in acetonitrile, methanol was selected as the polarity modifier. Tests for the effect of methanol in the perfusion stream on diffusion recovery indicate that the recovery of aniline and 2-chloroaniline increases with the quantity of methanol and levels off at 20%. Thus, 20% methanol can be recommended to add into perfusion stream to obtain a better recovery. However, since the effect of adding methanol is not significant (only <4%), it can be omitted to simplify the process.

3.5. Effect of perfusion flow-rate

A high perfusion flow-rate would decrease the detection sensitivity due to the dilution effect and the arisen pressure that decrease the diffusion tendency from the sample aliquot [35]. In order to obtain an acceptable diffusion recovery in an acceptable time, the influences of perfusion flow-rate on diffusion efficiency of aniline and 2-chloroaniline were examined. The results are shown in Fig. 5. The higher the flow-rate of perfusion, the lower the recovery is obtained. Although a low perfusion flow-rate increases the diffusion recovery, it takes time to collect perfusate enough to clear the eluent in the sample loop and be injected into the chromatographic system. Because 12.5 min is required for one analytical run, the flow-rate of 10-μl/min is enough to obtain five times the volume of the sample loop. Therefore, the flow-rate of perfusion was selected as 10 μl/min. As to the effect of diffusate pH, no significant change of recovery was found from pH 2.0 to 8.0.

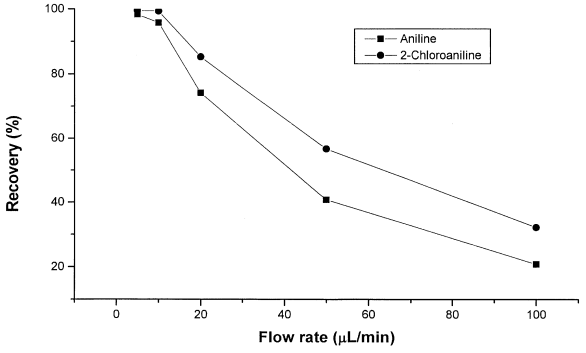

Fig. 5. Effect of perfusion flow-rate on the diffusion recovery.

3.6. Calibration plots of aniline and 2-chloroaniline injected directly and via dialysis

In order to realize the diffusion efficiency in various concentrations and the application for quantitative determination, calibration plots were built-up by injecting standard solutions of aniline and 2-chloroaniline directly or the dialysates collected from on-line dialysis of standard solutions. The calibration plots of aniline standard solution were with equations of $y = 89222x + 1529$ and $y = 84972x - 1636$ in the range of 0.1–10 μg/ml for injected directly and via on-line microdialysis, respectively. For the calibration plots of 2-chloroaniline, they were $y = 74035x + 1142$ and $y = 72990x + 1098$ in the range of 0.1–10 μg/ml for injected directly and via on-line microdialysis, respectively. The linear correlation coefficients were all above 0.999. The detection limits were calculated from the three times of the standard deviation, which were 10 μg/l in all cases for aniline and 2-chloroaniline. The repeatability was examined with three replicated injections of 0.1 μg/ml aniline and 2-chloroaniline. The RSD values for retention times and peak areas were all within 0.13% and 3.86%, respectively. The sample for direct injection is considered to have 100% recovery in concentration from dialysis. Because the slope of calibration plot refers to the detection sensitivity, thus the average diffusion recoveries through the dialysis membrane can be obtained from the slope ratio of linear regression equations (injection via on-line dialysis to direct injection). They are 95 and 99% for aniline and 2-chloroaniline, respectively. This shows that under the optimal dialysis conditions both aniline and 2-chloroaniline have similar detection sensitivity to that by direct injection.

3.7. Analysis of aniline and 2-chloroaniline in authentic sample

In order to test the applicability of the proposed

method, an authentic sample collected from a polymer factory was examined. After being diluted and on-lined dialyzed under the optimum conditions, the diffusate was analyzed for its content of aniline and 2-chloroaniline. The chromatogram is shown in Fig. 2b. The peaks of aniline and 2-chloroaniline were well separated from other species. To identify these species, not only were the retention behaviors compared with the standards, but they were also reconfirmed using an UV–Vis spectrophotometer, following fraction collection. Peaks 1 and 2 are aniline and 2-chloroaniline, respectively. The concentrations of aniline and 2-chloroaniline in wastewater were 13 mg/l (3.40% RSD) and 230 mg/l (3.51% RSD), respectively. In the spiked test, the recoveries were >96% with 2.74 and 2.67% RSD for three determinations of aniline and 2-chloroaniline, respectively.

4. Conclusion

This paper investigated the potential of using microdialysis on-lined to HPLC–UV to analyze aniline and 2-chloroaniline in wastewater. The optimal conditions for on-line microdialysis were determined. The results show that the proposed method can be an alternative to conventional extraction steps to determine aniline and 2-chloroaniline in polymer industrial wastewater with the advantages of easy operation, high recovery, less time–expense, and less use of organic solvent.

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