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On-line membrane preconcentration for continuous monitoring of trace pharmaceuticals

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Abstract

Membrane pervaporation is presented as a method for on-line concentration and monitoring of trace analytes in a simulated pharmaceutical process stream. Pervaporation involves the selective transport of volatile organics across a membrane and into a gas stream. Experiments were carried out using a polar solvent-permeable Nafion membrane and several model pharmaceutical compounds. Solvent reductions greater than 90% and enrichment factors in excess of 7.9 were observed. Residence time and temperature were found to be important operating parameters. Interaction with membrane bound sulfonic acid residues resulted in the loss of reactive analytes such as 1,2-diphenylhydrazine. The concentrated stream was monitored using HPLC and UV/vis detection. Method detection limits were 0.5–1.2 μg/mL and the relative standard deviation for six repeat injections was 3.9–6.2%.

Keywords: Membrane extraction; Pervaporation; Preconcentration; Chromatography; Sample preparation

1. Introduction

Analytical detection of trace nonvolatile/semi-volatile compounds usually requires extraction and concentration of analytes from the sample matrix [1]. For pharmaceutical preparations containing impurities and degradants, quantitation at low levels is necessary for end-product purity measurements. Even at trace levels, some of the impurities may have toxicological effects, and are under increased scrutiny of the regulatory agencies. Quantitation of these species during the manufacturing processes is the best method for their monitoring and control. However, continuous on-line analysis has been an elusive endeavor in pharmaceutical manufacturing. Typically, samples are collected at various steps in the process, and sent to the laboratory for analysis. The samples undergo various sample preparation steps, such as, extraction and concentration prior to detection. These steps are both labor and time intensive.

Enrichment of semi-volatile analytes may be carried out using liquid–liquid extraction (LLE) or solid-phase extraction (SPE) [2]. In the latter, the analytes are extracted onto a solid sorbent and then eluted with a suitable solvent. LLE is a classical extraction method that uses a liquid solvent for extraction. In both techniques, an additional concentration step may be required to increase the analytical concentration [1], and a solvent exchange may also be necessary for good chromatography.

Evaporative techniques are usually used for analyte concentration [3]. Essentially, it concentrates the sample by selectively removing the solvent. A common procedure is to blow an inert air stream across the sample–air interface. The more volatile solvents are removed, while the less volatile analytes are retained. The rate of evaporation may be increased by heating, thereby, speeding the concentration process. Kuderna–Danish (K–D) concentrators are commercially available, and have been used for this purpose [1]. Rotary evaporators are also common and utilize a water bath as the heat source. On the whole, these are relatively laborious procedures involving multiple handling steps which can

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compound errors during sample handling. They are also slow, and add to the delay between sampling and analysis.

Fully automated on-line extraction and analytical detection is yet to be realized, but several laboratories have made efforts in this field. HPLC coupled with integrated analyte extraction of antibiotics in sputum samples has been reported [4]. Inline LLE and SPE across a membrane along with HPLC detection has been used to determine trace levels of biocides in contaminated soil and water [5,6]. On-line procedures for analytical determinations of biological and environmental samples utilizing GC, MS and LC–MS have been successful [7,8]. In recent years, membranes have been used for on-line extraction [9–14]. It involves selective transport of the analytes across the membrane onto an extractant phase, leading to high enrichment for sensitive measurements. On-line membrane extraction and sensitive detection of volatile/semi-volatile compounds have been the subject of several other studies [10,11,15]. Membrane extraction coupled with gas injection has led to semi-automated on-line extraction, concentration, and detection of volatile compounds utilizing GC [9]. These methods have been developed for on-line, as well as, laboratory analysis procedures [16].

Membrane pervaporation refers to the permeation of volatile organics across a membrane barrier into a gas phase. It has been used for large-scale industrial separations [17]. It has also been used for on-line monitoring of volatile species in aqueous media [10,18]. Recently, we have reported the development of a concentration procedure using pervaporation [16], which served the same purpose as a K–D apparatus or rotary evaporator. In this approach, the sample flows through the lumen of a hollow fiber membrane (HFM), and an inert gas circulates on the permeate side. The selective removal of solvents lead to analyte enrichment. So, pervaporation is the opposite of membrane extraction, where the analytes are selectively transported across the membrane.

On-line interfacing of pervaporation and HPLC is presented here as a continuous monitoring method for the detection of trace level pharmaceutical components in a process stream. Polar solvent-permeable HFM (Nafion) is used for the concentration of a methanol stream containing pharmaceutical compounds. The whole measurement process including concentration and analysis is carried out on-line, and is completed in a matter of minutes. It is also a "user-friendly" procedure for continuous sample analysis that can be used to monitor and control a pharmaceutical process.

2. Experimental

Fig. 1 is a schematic of the HFM interfaced with HPLC. The sample was delivered through the hollow fiber lumen by a HPLC pump (Hewlett-Packard 1050). The permeate side of the HFM had a counter-current nitrogen flow, which removed the permeated solvents. Initial optimization was done by collecting the samples into vials and analyzing by HPLC. In other experiments, the HFM was connected directly to the

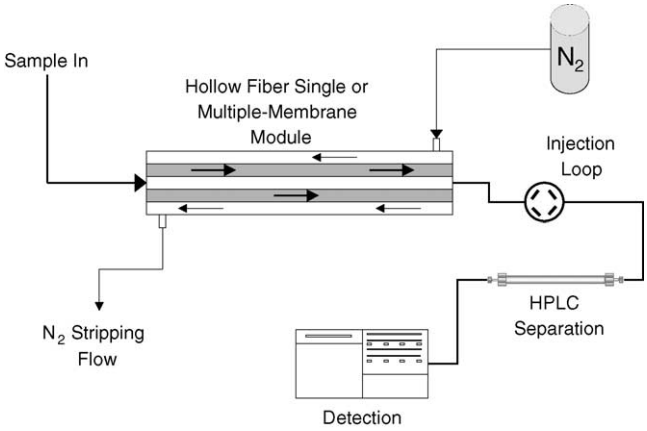

Fig. 1. Schematic of on-line hollow fiber membrane concentration and analysis.

HPLC injector to facilitate on-line analysis. Analysis was done using a Hewlett-Packard 1050 HPLC equipped with a C-18 reverse-phase analytical column (Keystone Aquasil, 5 μm, 100 Å, 4.6 mm × 250 mm) utilizing an isocratic mobile phase at a flow rate of 2 mL/min. A diethylamine phosphate solution (pH 2.5) was made by adding 10.3 mL of diethylamine to 70 mL of water, and adjusting the pH to 2.5 using phosphoric acid. This was then diluted with water to a final volume of 100 mL. The mobile phase was made by adding 3 mL of the pH 2.5 diethylamine phosphate solution to 480 mL of water containing 600 mg KH_2PO_4. The pH was adjusted to pH 4.0 using 0.2N NaOH, and this was added to 520 mL acetonitrile. The UV detection was carried out at 220 nm. MiniChrom v1.61 (SRI Instruments, Torrance, CA) was used for the chromatographic data acquisition and analysis.

The membrane module comprised of a polypropylene casement 30–40 cm in length. A stainless steel "T" (Components and Controls Inc., Carlstadt, NJ) placed at each end of the column coupled the casement and HFM strands, and was sealed using a fast-drying epoxy resin (A1 with activator E, Armstrong Adhesives, Easton, MA). The epoxy-sealed "T" unit prevented intermixing of the lumen and permeate contents, and served as the inlet and outlet for the sample flow through the lumen and the permeate stripping N_2 gas.

The membrane used was a Nafion hollow fiber strand (0.533 mm o.d. × 0.356 mm i.d.; manufacturer and registered trademark—DuPont, Wilmington, DE). Nafion is a copolymer of tetrafluoroethylene (Teflon) and perfluoro-3,6-dioxa-4-methyl-7-octene-sulfonic acid and are permeable to polar solvents [16,19]. Nitrogen gas was obtained from Matheson (Secaucus, NJ). The nitrogen pressure was measured using a battery-operated digital pressure gauge from TIF Instruments (Miami, FL). A fiber-glass insulated electrical heat tape powered by a variable transformer (Staco Energy Products Co., Dayton, OH) was used to heat the HFM. The temperature was measured using a digital thermometer probe from Cole-Palmer (Vernon Hills, IL).

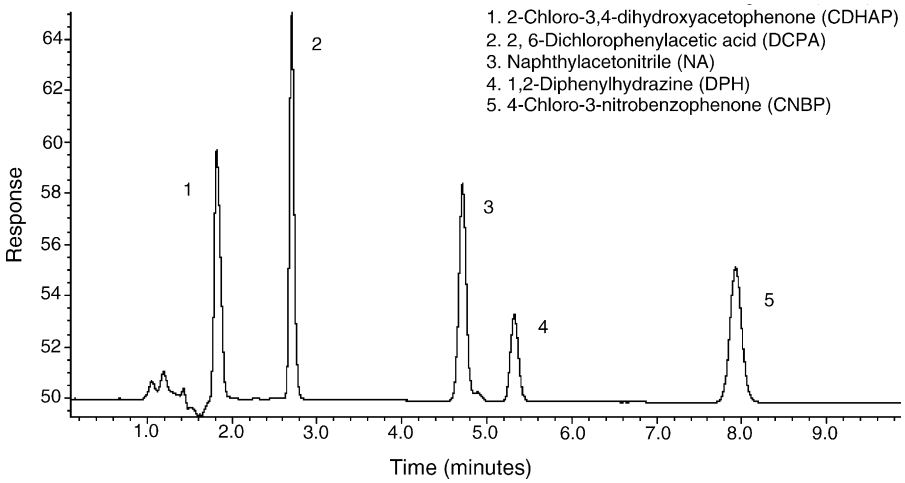

Fig. 2. Typical HPLC chromatogram for the separation of CDHAP, DCPA, NA, DPH, and CNBP.

All chemicals and solvents used in the experiments were of analytical grade or better from Supelco, Inc. (Supelco Park, PA) and Sigma-Aldrich (Milwaukee, WI). Five model reagents were tested. Fig. 2 shows a typical chromatographic separation of these analytes. The analytes chosen were as follows: 2,6-dichlorophenylacetic acid (DCPA), used in the synthesis of Guanfacine, an anti-hypertensive; naphthylacetonitrile (NA) is a reagent for the manufacture of Naphazoline, a decongestant; 4-chloro-3-nitrobenzophenone (CNBP) is a halo-ketone used for the synthesis of Mebendazole, an anthelmintic; 1,2-diphenylhydrazine (DPH) is used in the manufacture of phenylbutazone, an anti-inflammatory; and 2-chloro-3′,4′-dihydroxyacetophenone (CDHAP) is a reagent used as a precursor for the synthesis of Dipivefrin, an ocular andrenergic, and for Isoproterenol, a bronchodilator.

3. Results and discussion

In the membrane extraction/recovery procedure, recovery (R) is defined as

$$R = \left(\frac{C_o V_o}{C_i V_i}\right)$$

where C_o is the outlet analyte concentration, V_o the outlet volume, C_i the inlet concentration and V_i the inlet volume.

Enrichment factor (EF) is defined as

$$\mathrm{EF} = \frac{C_o}{C_i}$$

Residence time (t_R) is defined as

$$t_R = \frac{V}{F}$$

where V is the internal (lumen) volume of the hollow fiber and F the flow rate in minutes.

The steady-state membrane permeation flux (rate of diffusion) is described by Fick's first law:

$$J = -D\left(\frac{\delta c}{\delta x}\right) \quad (1)$$

where D is the diffusion coefficient of the penetrant across the membrane, and $(\delta c/\delta x)$ the concentration gradient across the membrane. At steady state, the sample is introduced continuously and the detection is done under equilibrium conditions.

As shown in Eq. (1), Fick's first law defines the permeation flux, and for a hollow fiber membrane:

$$\left(\frac{\delta c}{\delta x}\right) = \left(\frac{C_p - KC_M}{L}\right) \quad (2)$$

where C_p is the concentration of the permeant on the permeate side, C_M the concentration of the permeant on the lumen side, K the partition coefficient between the membrane and permeant, and L the membrane thickness. The stripping gas flow removes essentially all of the permeate rapidly as it diffuses through the membrane, thus, C_p approaches zero. The upstream concentration of the sample permeant is described by KC_M in Eq. (2). Simplifying Eqs. (1) and (2) yields a third equation:

$$J = \frac{DKC_M}{L} \quad (3)$$

3.1. Solvent removal

Fig. 3 shows a typical methanol pervaporation as a function of residence time (calculated based on inlet flow rate) in the Nafion HFM module using DCPA as the analyte. The pressure of the permeate strip gas was maintained at 10 psi (68,948 Pa). It was observed that a higher residence time increased the enrichment factor (EF), as there was more time for solvent permeation. The same effect was mirrored by solvent loss, which also increased with residence time. The analyte recovery remained constant at between 70 and 80% irrespec-

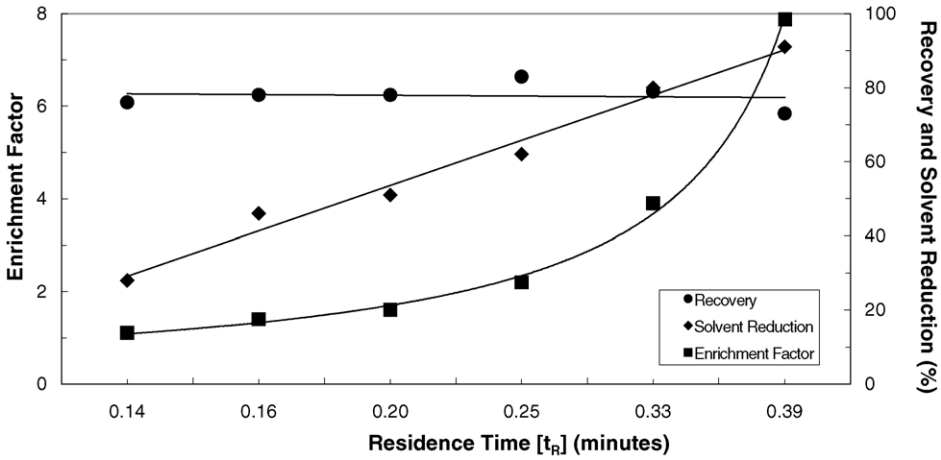

Fig. 3. Preconcentration of 2,6-dichlorophenylacetic acid from a methanol extract. Recovery, solvent reduction and enrichment factor are plotted as a function of residence time. Calculated residence times are based on inlet flow rate. Experimental conditions were: initial volume, 2 mL; initial concentration, 10 ppm; counter-current N_2 flow, 10 psi; temperature, 20 °C.

tive of the flow rates. All the model compounds, except DPH, exhibited an overall increase in EF as a function of residence time. DPH-methanol pervaporation patterns are shown in Fig. 4. It showed nominal increase in the EF only at the highest residence time. Described in a previous paper [16], Nafion membrane is a polytetrafluoroethylene structure containing sulfonic acid residue terminating on fluorocarbon sidechains which exhibit selectivity for primary and secondary amines. DPH was lost due to relatively high permeability through the membrane. All other analytes tested exhibited a low permeability, while the solvent permeated through.

3.2. *Effect of temperature*

Pervaporation is known to be a temperature dependent process [20,21]. Diffusion through the membrane follows an Arrhenius-type relationship as the diffusion coefficients increase with temperature. However, solubility or partition coefficient in the membrane decreases with the temperature. Since pervaporation depends on both these phenomena, temperature was expected to be an important process variable. Fig. 5 shows recovery, solvent loss and EF for CNBP in methanol as a function of the membrane temperature. Sample flow rate and the permeate stripping gas were kept constant as the temperature was varied. It is clearly seen that as the temperature increased, solvent loss and the EF increased. Higher temperature also led to higher flux which facilitated higher sample throughput by the faster analysis. However, temperature cannot be increased indefinitely and eventually the decrease in partition coefficient would decrease enrichment factor.

The key to enrichment was solvent loss through the membrane. Fig. 6 shows the solvent loss during the concentration procedure and its effect on EF. Table 1 presents typical results

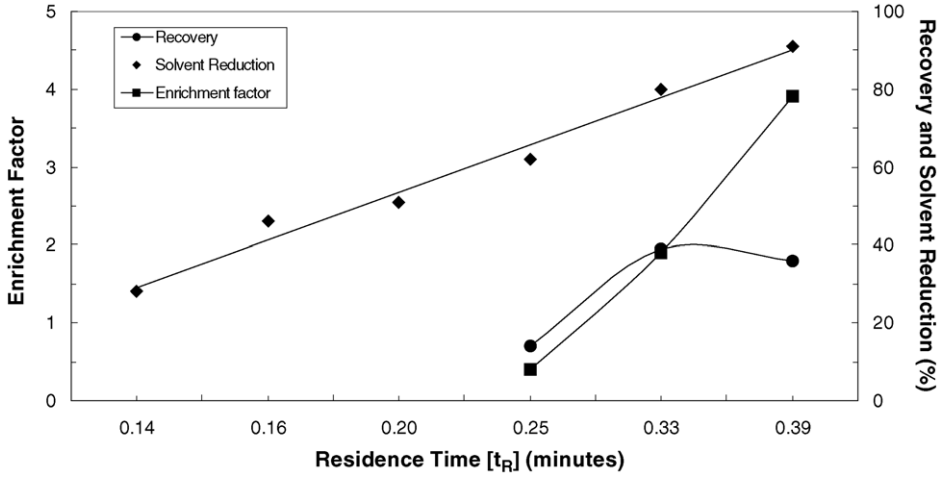

Fig. 4. Preconcentration 1,2-diphenylhydrazine from a methanol extract. Recovery, solvent reduction and enrichment factor are plotted as a function of residence time. Calculated residence times are based on inlet flow rate. Experimental conditions were: initial volume, 2 mL; initial concentration, 10 ppm; counter-current N_2 flow, 10 psi; temperature, 20 °C.

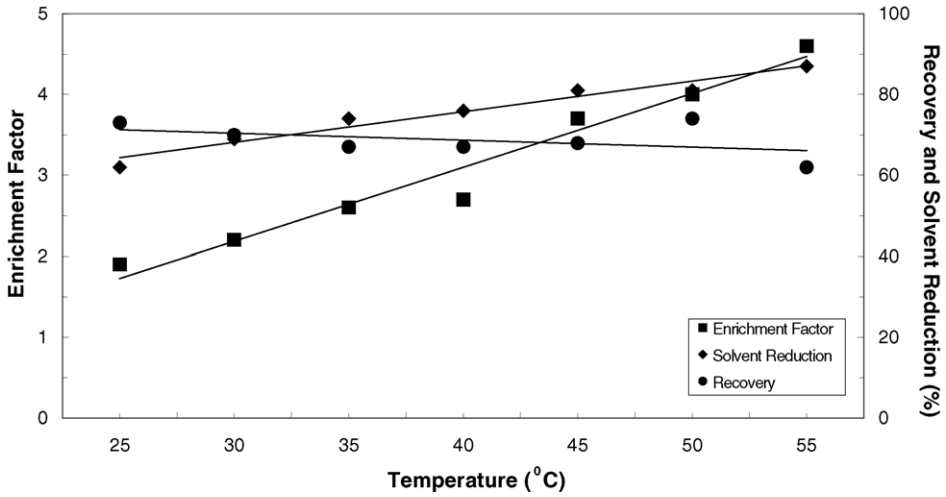

Fig. 5. Preconcentration of 2-chloro-3′,4′-dihydroxyacetophenone from a methanol extract. Recovery, solvent reduction and enrichment factor are plotted as a function of temperature. Experimental conditions were: initial volume, 2 mL; initial concentration, 10 ppm; counter-current N_2 flow, 10 psi.

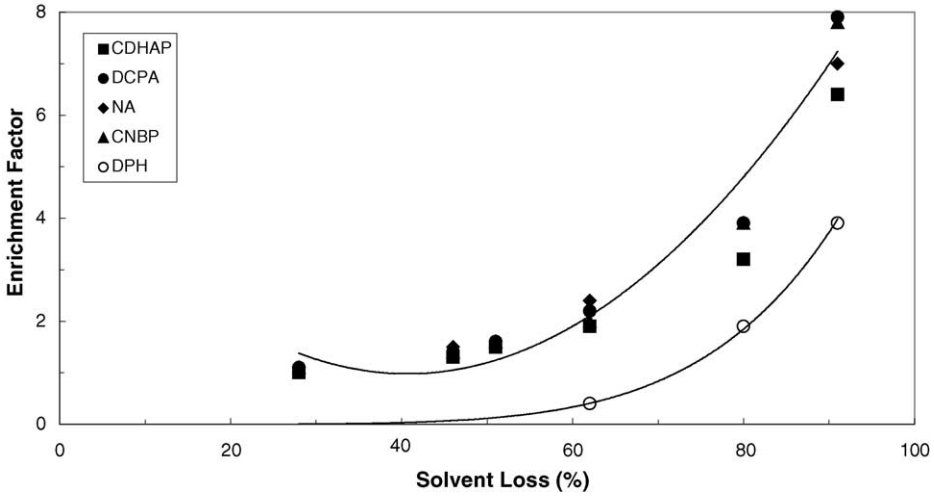

Fig. 6. Enrichment factor as a function of solvent loss during analyte concentration using the Nafion module. CDHAP, DCPA, NA, CNBP and DPH were the analytes. Experimental conditions were: initial volume, 2 mL; initial concentration, 10 ppm; counter-current N_2 flow, 10 psi.

Table 1
Analyte recovery, enrichment factors and solvent volume reduction for Nafion HFM concentration treatment

Analyte	log $K_{O/W}$	Solvent reduction (%)	Percent recovery (%)	Enrichment factor	# of HPLC injections	R.S.D. (%)
2,6-Dichlorophenylacetic acid	2.72	91	73	7.9	6	5.2
Naphthylacetonitrile	2.74	91	74	7.0	6	5.0
2-Chloro-3′,4′-dihydroxyacetophenone	0.97	91	60	6.4	6	3.9
4-Chloro-3-nitrobenzophenone	3.61	91	72	7.8	6	6.2
1,2-Diphenylhydrazine	3.06	91	36	3.9	6	4.6

Notes: Experimental conditions were as follows: initial volume, 3 mL; initial flow rate, 0.2 mL/min; initial concentration for each analyte, 10 ppm; counter-current N_2 flow, 10 psi; operating temperature, 55 °C. Percent recovery is the quantity of analyte recovered from the initial amount added. R.S.D. is relative standard deviation for the six replicate injections.

from a concentration of DCPA, NA, CDHAP, CNBP, and NA using the Nafion concentrator. Samples were run using the optimal configuration of flow rate and N_2 pressure. At optimum solvent loss conditions (>90% reduction), high EF and recovery ($\geq$60%) were obtained for all the compounds. The only exception was DPH which had a strong affinity for the Nafion and was lost through the membrane. A different membrane would be recommended for DPH and other amines.

By interfacing a membrane concentrator directly to the HPLC injector, fast analytical detection could be carried out limited only by the time required for HPLC separation. The sample was concentrated by continuously passing through the membrane lumen, and flowed into the sample loop of the HPLC injector. Injections were made at fixed intervals of time, and corresponding to each injection, a chromatogram was obtained. The longest retention time was 8.5 min for CNBP. At the optimum conditions, 90% solvent reduction was achieved, and typical injection volumes were 10–20 μL. For DCPA, CDHAP, and CNBP, the method detection limits were 0.5, 1.0, and 1.2 μg/mL, respectively. The relative standard deviation for six repeat injections were between 3.9 and 6.2%.

4. Conclusions

Pervaporation through a Nafion hollow fiber membrane was used to investigate concentration of trace model pharmaceutical grade analytes. This paper demonstrates the capability of solvent pervaporation as a rapid method for concentrating the analytes for on-line quantitative detection. A polar solvent-permeable membrane was demonstrated here for methanol, which is commonly used in pharmaceutical manufacturing. The Nafion membrane showed affinity for some analytes which can be lost during pervaporation. Other membrane materials need to be used for these molecules.

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