



Characterization and use of a Raman liquid-core waveguide sensor using preconcentration principles

Sumalee Tanikkul^a, Jaron Jakmune^a, Mongkon Rayanakorn^a,
Kate Grudpan^a, Brian J. Marquardt^b, Gwen M. Gross^b, Bryan J. Prazen^b,
Lloyd W. Burgess^b, Gary D. Christian^b, Robert E. Synovec^{b,*}

^a Faculty of Science, Research and Development and Department of Chemistry, Institute of Science and Technology, Chiang Mai University, Chiang Mai 50200, Thailand

^b Department of Chemistry, Center for Process Analytical Chemistry, Box 351700, University of Washington, Seattle, WA 98195, USA

Received 27 August 2002; received in revised form 26 November 2002; accepted 5 December 2002

Abstract

A novel Raman sensor using a liquid-core optical waveguide is reported, implementing a Teflon-AF 2400 tube filled with water. An aqueous analyte mixture of benzene, toluene and *p*-xylene was introduced using a 1000 μ l sample loop to the liquid-core waveguide (LCW) sensor and the analytes were preconcentrated on the inside surface of the waveguide tubing. The analytes were then eluted from the waveguide using an acetonitrile–water solvent mixture injected via a 30 μ l eluting solvent loop. The preconcentration factor was experimentally determined to be 14-fold, in reasonable agreement with the theoretical preconcentration factor of 33 based upon the sample volume to elution volume ratio. Raman spectra of benzene, toluene and *p*-xylene were obtained during elution. It was found that analytically useful Raman signals for benzene, toluene and *p*-xylene were obtained at 992, 1004 and 1206 cm^{-1} , respectively. The relative standard deviation of the method was 3% for three replicate measurements. The limit of detection (LOD) was determined to be 730 ppb (parts per billion by volume) for benzene, exceptional for a system that does not resort to surface enhancement or resonance Raman approaches. The Raman spectra of these test analytes were evaluated for qualitative and quantitative analysis utility.

© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Liquid-core waveguide; Sensor; Raman; Preconcentration

1. Introduction

A liquid-core waveguide (LCW) can be made from tubing in which the wall material has a lower refractive index than the liquid-core material. Under this physical condition, it is possible for light to be launched into the core of the waveguide and be guided over a significant length [1]. A

* Corresponding author. Fax: +1-206-685-8665.

E-mail address: synovec@chem.washington.edu (R.E. Synovec).

commercially available material capable of guiding light through water is the amorphous copolymer of 2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxole. This material, trade named Teflon-AF 2400, has been investigated as an aqueous-core optical waveguide because the refractive index is $n = 1.296$ and the polymer will support internal reflection from aqueous solution (water, $n = 1.333$). Teflon-AF 2400 is quite physically and optically stable and is transparent in the spectral range from 200 to 2000 nm [2].

Teflon-AF 2400 has been used as either an LCW or liquid waveguide capillary cell (LWCC) for absorbance spectroscopy [3], fluorescence [4,5], liquid chromatography [6], gas chromatography [7], Raman spectroscopy [8–10] and used as a Raman detector for liquid chromatography [1,11]. The major advantage of using an optical waveguide for Raman spectroscopy is that the signal is significantly enhanced by the integration of Raman scattered light along the length of the waveguide. An LCW based on capillary tubing of Teflon-AF 2400 was shown by Altkorn et al. [8,9] to provide advantageous application in Raman spectroscopy. Song et al. [12] demonstrated that a 1 m LWCC made of Teflon-AF 2400 can be used to enhance the Raman signal by 20 times in aqueous solution with laser excitation at 785 nm. On the basis of the observed signal intensity and signal-to-noise enhancement, the waveguide should be well suited for use in biological, environmental or other chemical analysis applications with attributes such as small scattering cross-section, sensitivity to laser power and utility for analysis of low analyte concentration. Waterbury et al. [13] reported that an LCW extended the sensitivity of conventional absorbance spectroscopy by two or more orders of magnitude. Dasgupta et al. [7] also used a waveguide sensor based on selective chromogenic reactions and in situ long-path optical absorbance measurements. Finally, Stellman et al. [14] demonstrated the feasibility of using a long-pathlength fiber-optic Raman probe for the determination of benzene, toluene, ethyl benzene and xylene (BTEX) contaminants in aqueous environments.

In this paper, we describe a novel Raman waveguide sensor that preconcentrates analytes

(benzene, toluene and *p*-xylene) initially in aqueous solution and then elutes the analytes in a stronger mobile phase (with a high acetonitrile content) with simultaneous Raman detection in real-time during elution. The preconcentration and subsequent elution of analytes were investigated by passage of aqueous sample solution through an LCW, followed by solvent extraction and elution simultaneous with Raman detection. The LCW will be shown to be both an effective preconcentrating medium and flow cell for the chosen analytes and it also has the benefit of enhanced Raman sensitivity to increase the overall sensitivity and limit of detection (LOD) of the measurement. The results of the Raman signal enhancement experiments with the LCW sensor are reported.

2. Experimental

2.1. Apparatus

The experimental setup for the Raman system is shown in Fig. 1. The system consists of a Kaiser Optical Systems Hololab Series 5000 Raman instrument (Ann Arbor, MI) including a Holop-

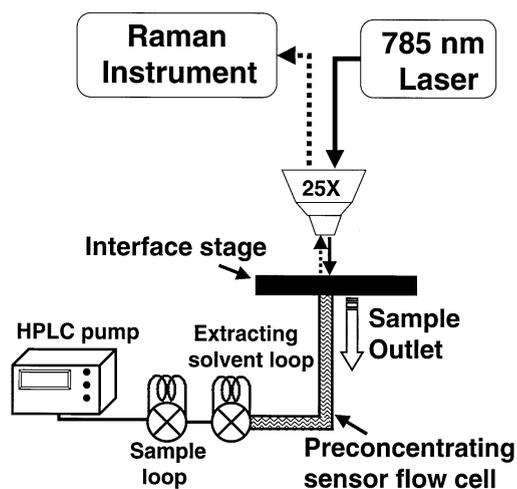


Fig. 1. A schematic diagram of the Raman LCW sensor apparatus is shown. The sample loop volume is 1000 μl , with analytes in water. The extracting solvent loop volume is 30 μl . A 70% acetonitrile/30% water mixture is used for extraction. The preconcentrating sensor flow cell is a 1 m \times 50 μm i.d. Teflon-AF 2400 capillary tube serving as a waveguide.

robe transmission holographic spectrograph interfaced to an infinity-corrected fiber-coupled microscope. A complete description of this system can be found in a previous publication [1]. The Raman system was equipped with a 785 nm stabilized external cavity diode laser operating at an average power of 100 mW. A beam splitter in the collimated space between the Raman probe head and the microscope objective provided a real-time visual image of the waveguide tubing in the Raman flow cell to facilitate laser alignment. The Raman probe was coupled to the microscope with 8 μm i.d. excitation fiber and the scattered radiation was collected using a 100 μm i.d. multimode fiber. A $25\times$ (0.65 NA) objective was used to both focus the laser and collect the scattered radiation in an epi-illumination configuration. The $25\times$ objective was chosen for its large numerical aperture, which was useful for maintaining full collection of the Raman scattered radiation from the waveguide during dynamic flowing measurements. All Raman spectra were collected using a 50 μm slit width and a detector temperature of $-40\text{ }^\circ\text{C}$. All raw Raman data collected from Raman detector was processed with MATLAB 6.0 software (Mathworks, Natick, MA).

2.2. Raman waveguide

A flow cell was designed and optimized for performing sensitive Raman measurements of flowing liquid samples, as described in previous work [1]. The cell consists of a 1 m length of 50 μm i.d. Teflon-AF 2400 waveguide tubing and a PEEK (polyetheretherketone) polymer block of the same footprint as a typical microscope slide (7.6 cm \times 3.8 cm) to facilitate use on a microscope stage. The total volume of the 1 m long LCW was 1.9 μl . The cell was equipped with three fluid ports on the underside, one port for removal of waste, another to allow purging of air bubbles and a center port, for coupling to the LCW. The three ports were connected on the surface of the cell by a channel that was laser cut into an FEP fluoropolymer gasket. The channel dimensions of the gasket were 20 mm \times 1.6 mm \times 0.25 mm ($L \times W \times D$). The gasket was placed between the PEEK top plate and the base to form a liquid-

sealed cell. A 25 mm o.d. \times 500 μm sapphire window was then secured over the center port to allow optical access to the LCW. The Raman waveguide cell was interfaced directly onto the microscope stage with the fluid connections routed under the interface stage. The excitation laser was focused with the microscope objective through the sapphire window into the core of the waveguide tubing in an epi-illumination configuration.

2.3. Preconcentration system

The preconcentrating sensor flow cell is the same optical waveguide previously described. It can be purchased in various internal diameters (Biogeneral Technologies, Inc., San Diego, CA). A tube of this material, filled with an aqueous solution (or virtually any other liquid), behaves as a liquid-core optical fiber. A waveguide of 1 m length and 50 μm core diameter was used in the system. An HPLC grade pump with an analytic micro-flow liquid head was used to pump liquid (Beckman, Model 114M, Berkeley, CA).

2.4. Chemicals

Benzene, toluene and *p*-xylene (J.T. Baker, Phillipsburg, NJ) were used in the studies and HPLC grade acetonitrile (Fisher Scientific, Fair Lawn, NJ) was also used. Deionized (DI) water, demineralized to greater than 18 M Ω with a Milli-Q system (Millipore, Bedford, MA) was used in the preparation of all solutions and as the primary mobile phase. Water was degassed prior to use with an ultrasonic bath and suction system.

2.5. Procedure

Two injection valves (EC10W, Valco Instruments Co., Inc., Houston, TX) were used as shown in Fig. 1. The first valve introduced sample solution into the preconcentrating sensor flow cell and the second valve introduced another solution to subsequently elute the preconcentrated analytes with real-time Raman detection. Thus, the preconcentrating sensor flow cell also serves as a Raman detection waveguide as shown in Fig. 1. The first valve was used to load a 1000 μl aqueous

sample solution. A second valve was then used to introduce 30 μl of the extraction solvent, 70% acetonitrile with 30% water. Deionized water was used as the carrier (mobile phase). The data collection consisted of three steps. First, in the baseline collection step, 30 μl of 70% acetonitrile/30% water was injected into the system with a flow rate of 5 $\mu\text{l min}^{-1}$ to collect the signal of the acetonitrile background. Second, a 1000 μl aqueous sample, with analytes, was loaded and injected into the waveguide at a flow rate of 60 $\mu\text{l min}^{-1}$. In this step, the aromatic analytes were preconcentrated into the waveguide tubing. Teflon-AF 2400 has a good affinity for aromatic compounds due to the hydrophobicity of Teflon-AF 2400, thus the compounds were readily extracted from aqueous solution. In the third step, the 70% acetonitrile/30% water solution was introduced to the sensor using the injection valve fitted with the 30 μl loop. This solution quantitatively extracted and eluted the aromatic analytes from the tubing while flowing through the waveguide at 5 $\mu\text{l min}^{-1}$. The Raman data from the extraction step, which includes acetonitrile and analyte signals, were baseline corrected from the signal of acetonitrile obtained in the baseline collection step. A background correction procedure was applied in order to obtain the Raman signal for the analytes of interest free from the acetonitrile interference and independent of time due to the extraction/elution process. The background correction software procedure was written in-house and will be demonstrated in Section 3.

3. Results and discussion

A three-dimensional plot of raw Raman data collected during the elution of benzene, toluene and *p*-xylene is shown in Fig. 2A. A mixture of an aqueous solution containing 70 ppm benzene (parts per million by volume), 100 ppm toluene and 100 ppm *p*-xylene in 1000 μl was initially preconcentrated into the waveguide. In the extraction and elution step, a 30 μl volume of acetonitrile in water was used to extract the analytes from the waveguide. The spectra show both the Raman response and the kinetic desorption information of

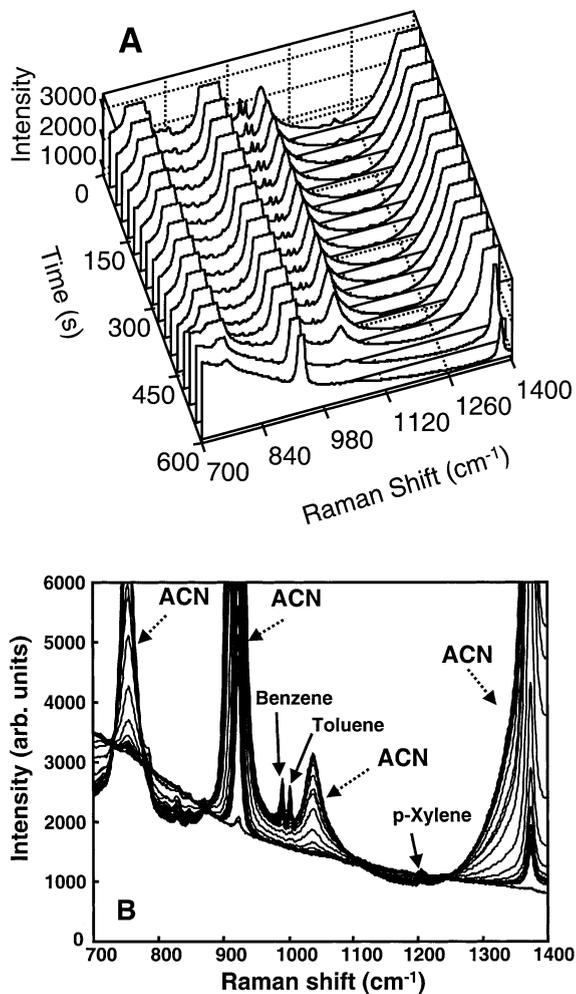


Fig. 2. (A) Three-dimensional plot of raw Raman signal during the extraction of a 1000 μl aqueous mixture containing 70 ppm benzene, 100 ppm toluene and 100 ppm *p*-xylene. Every other spectrum was omitted for clarity. (B) Two-dimensional plot of this data on the Raman shift axis. The large Raman bands for acetonitrile are also labeled (ACN). Each signal trace was obtained during the time-dependent elution of the preconcentrated sample from the waveguide.

the extracted and eluted analytes. The waveguide does not permit water to adsorb significantly onto its surface and is chemically resistant to most other solvents. Moreover, it has low solubility in selected perfluorinated solvents on the order of 3–15%. The waveguide does permit the non-polar analytes to adsorb into the inner wall of the waveguide, in the sub-micron thickness range [2] possibly by way

of the nanoporous Teflon-AF 2400 structure, and so benzene, toluene and *p*-xylene can readily be extracted and preconcentrated from aqueous solution into the waveguide inner wall. Acetonitrile was chosen as the organic component of the eluting solvent because it provided a Raman shift different from the Raman peaks of the analytes. It was shown that the elution strength of 70% acetonitrile in 30% water was optimal for extraction, since the analytes quantitatively eluted from the waveguide. Acetonitrile and the analytes, benzene, toluene and *p*-xylene, have sufficiently similar organic character and so the analytes prefer to elute from the waveguide rather than be retained on it [15]. Another reason acetonitrile was used is that it did not degas at the laser wavelength or power being used to perform the Raman analysis. Fig. 2B shows the two-dimensional plot of the same data as in Fig. 2A. The spectra were obtained during the extraction/elution phase of the experiment. A total of 40 spectra were taken during 10 min of elution. The acquisition time for each spectrum was 15 s (five exposures and three accumulations). The analyte signals are more clearly seen in Fig. 2B than in Fig. 2A. The Raman frequencies of the aromatic ring breathing modes of benzene and toluene occur at 992 and 1004 cm^{-1} , respectively. The Raman peak of *p*-xylene at 1206 cm^{-1} was indicative of the C–C ring stretch [16]. Acetonitrile bands are very intense due to the high concentration in the waveguide during the elution step; therefore, it was necessary to perform background subtraction and correction for better data analysis and clarity.

Fig. 3A shows the elution time profiles of a 75 ppm benzene sample initially preconcentrated, obtained from the analyte extraction/elution step, along with the baseline collection step (background signal). Both elution time profiles were processed at the maximum signal of benzene corresponding to a Raman shift at 992 cm^{-1} . Fig. 3B shows the elution time profile of benzene, following baseline subtraction. The figure shows that benzene can be quantitatively extracted from the waveguide tubing walls within 8 min. In this figure, the temporal information is still apparent. In the final algorithm to be applied next, the temporal information is eliminated for clarity by summing

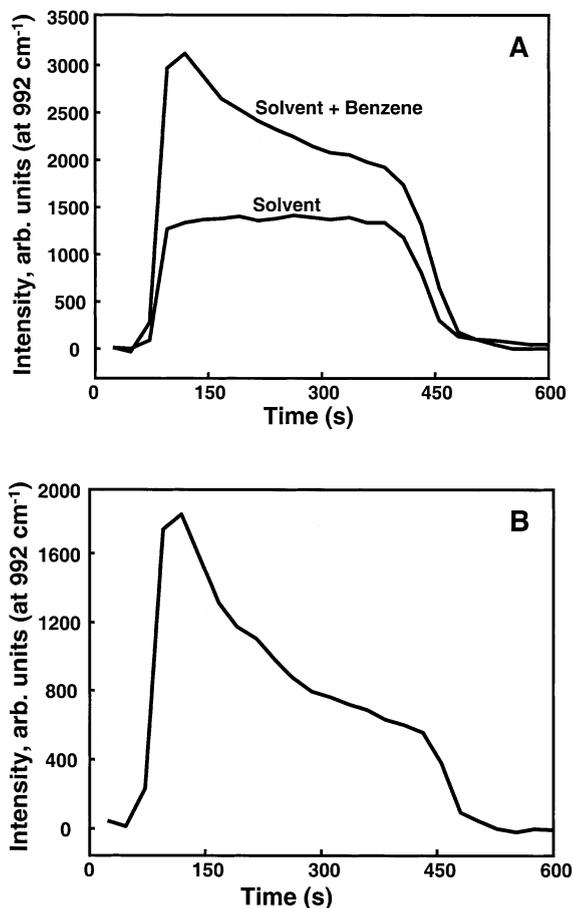


Fig. 3. (A) Elution time profile of extraction solvent only (70% acetonitrile/30% water) and the extraction of 75 ppm benzene (as single analyte in sample). These profiles contain the summation of signals at 992 cm^{-1} , which is the maximum sensitivity for benzene. (B) The elution time profile of benzene after background correction.

the baseline-corrected response at each wavenumber and then plotting the resulting Raman spectra as a function of wavenumber only.

The results shown in Fig. 4 illustrate the Raman spectrum of the analyte mixture shown previously in Fig. 2 after application of the background correction procedure. The procedure removed the acetonitrile signal contribution as well as reduced the data density by eliminating the time dependence. In the first step of background correction, the two-dimensional matrices of sample data and background data are reduced to vectors of back-

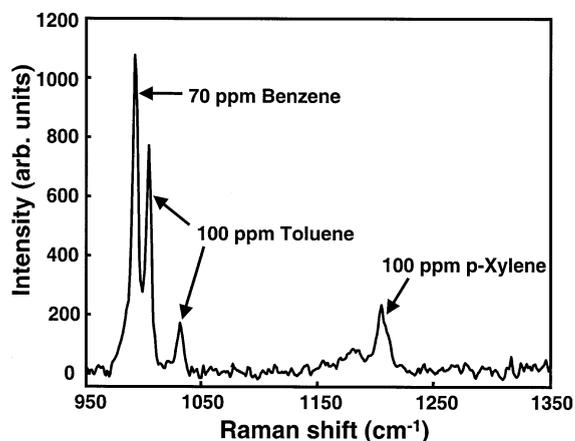


Fig. 4. Background-subtracted Raman spectrum of mixture containing 70 ppm benzene, 100 ppm toluene and 100 ppm *p*-xylene using the data presented in Fig. 2. The data have also been summed at each time interval, thus eliminating the time axis.

ground data and sample data along the spectra axis. This is completed by averaging the signal during the elution of the sample plug. A first-order polynomial fit of the background vector to the sample vector was then subtracted from the sample vector. The polynomial fit accounts for small fluctuations in the baseline and laser intensity. The benefits of the acetonitrile background subtraction and correction routine are evident in the appearance of the 1031 cm^{-1} Raman peak of toluene peak that can easily be resolved from the background. The background-corrected spectrum shows very good selectivity and overall sensitivity for benzene, toluene and *p*-xylene.

Calibration data were obtained from Raman signals of individually run analytes, which after baseline correction are between 975 and 1005 cm^{-1} Raman shift for benzene and between 985 and 1015 cm^{-1} Raman shift for toluene. The peak signals at 992 cm^{-1} for benzene and at 1004 cm^{-1} for toluene were used for calibration, as shown in Fig. 5A and B, respectively. The calibration plots for benzene and toluene were constructed using the peak areas from the signal calibration data. Calibration graphs for a concentration range from 10 to 75 ppm are shown in Fig. 6. The correlation coefficients of linear regression analysis were 0.9814 for benzene and 0.9977 for toluene. The

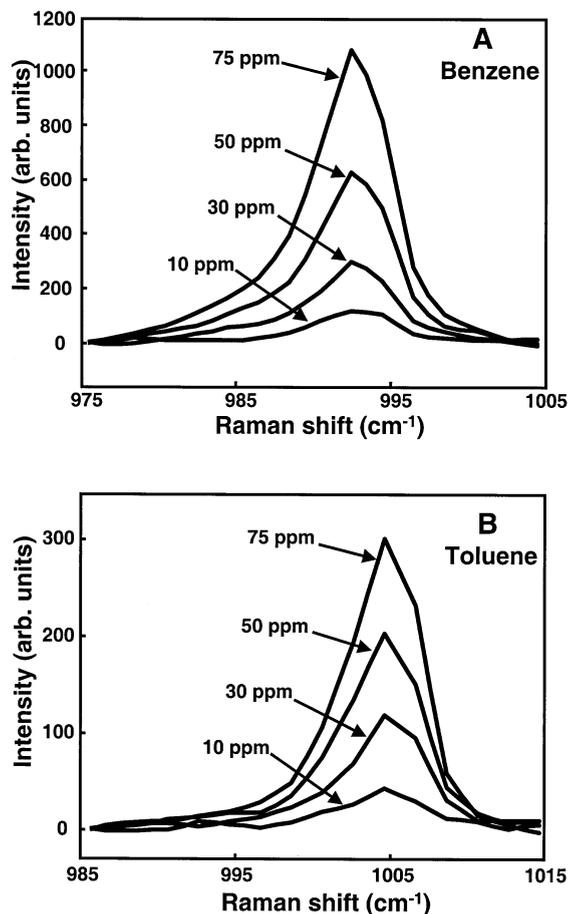


Fig. 5. Raman spectra of samples over a concentration range 10–75 ppm of (A) benzene and (B) toluene, following the background correction procedure. Samples were initially pre-concentrated in water and then eluted in 70% acetonitrile/30% water.

relative standard deviation obtained in this analysis was 3.0% for three replicate measurements at 50 ppm benzene. The waveguide material was not extensively studied above 75 ppm as demonstrated in Fig. 6, since above 75 ppm the same apparatus could be applied for the analysis without requiring pre-concentration. For this reason, we did not explore the upper concentration limit up to the point of saturation during pre-concentration. The LOD was calculated from the peak height of the 10 ppm benzene signal and representative baseline noise. Please see Fig. 7 for typical data used for LOD calculation. The LOD was determined to be

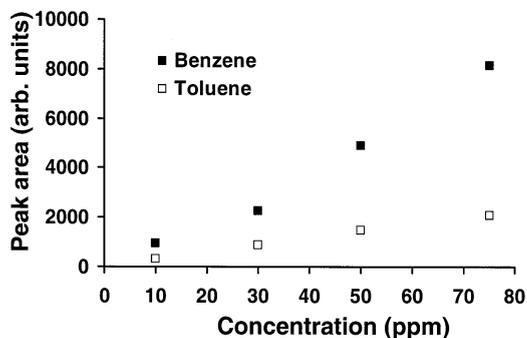


Fig. 6. Calibration graphs are shown with the intensity of the Raman signal plotted versus the concentration of benzene and toluene. Samples were initially preconcentrated in water and then eluted in 70% acetonitrile/30% water.

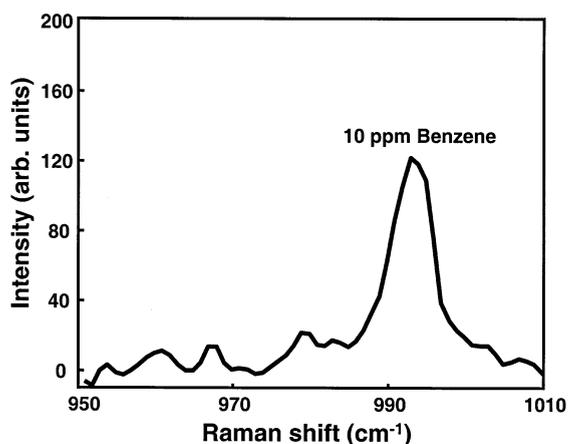


Fig. 7. Raman spectra of a 10 ppm benzene sample and representative baseline noise. The LOD of the method was 730 ppb for benzene.

730 ppb (parts per billion by volume) for benzene. Note that LOD is a figure-of-merit and at LOD the signal would just begin to be discernable from the baseline noise. LOD for benzene with the same waveguide system but without using the preconcentration procedure was determined to be 10 ppm (data not shown for brevity). Thus, a factor of 14 enhancement in LOD is provided by the preconcentration procedure with the enhancement factor for benzene calculated as LOD without precon-

centration divided by LOD using preconcentration. This experimental enhancement is in reasonable agreement with the theoretical enhancement of 33 calculated by taking the ratio of the sample volume (1000 μ l) divided by the elution volume (30 μ l). The agreement is reasonable given the noise in the baseline does not proportionately relate to the analyte signal during the data reduction procedure applied with the preconcentration procedure. The experimental LOD obtained using the preconcentration procedure is quite satisfactory for many applications.

4. Conclusions

This work demonstrates that a Teflon-AF 2400 optical LCW can be used to both preconcentrate analytes (benzene, toluene and *p*-xylene) and detect them to greatly improve the sensitivity of a Raman measurement of selected aromatic compounds. The optical properties (lower refractive index than water) of Teflon-AF 2400 allow it to be used with aqueous solutions. A small volume of eluting solvent, 70% acetonitrile in water, was useful for extracting the analytes from the waveguide during Raman detection mode. The excitation was carried out at 785 nm from the laser source. The Raman waveguide sensor can be used for preconcentration, characterization and analysis of mixtures of benzene, toluene and *p*-xylene. The sensor is likely to be broadly applicable for simple to moderately complex aqueous samples. The combination of separation and spectroscopy technologies is a novel approach for leveraging the strengths of both technologies.

Acknowledgements

The authors thank the Center for Process Analytical Chemistry (CPAC) and the Thailand Government Ministry of University Affairs (MUA) for financial support of the work.

References

- [1] B.J. Marquardt, P.G. Vahey, R.E. Synovec, L.W. Burgess, *Anal. Chem.* 71 (1999) 4808.
- [2] <http://www.dupont.com/teflon/af/performance.html>.
- [3] W. Yao, R.H. Byrne, *Talanta* 48 (1999) 277.
- [4] P.K. Dasgupta, Z. Genfa, J. Li, C.B. Boring, S. Jambunathan, R. Al-Horr, *Anal. Chem.* 71 (1999) 1400.
- [5] R.H. Byrne, W. Yao, E. Kaltenbacher, R.D. Waterbury, *Talanta* 50 (2000) 1307.
- [6] C. Gooijer, G.P. Hoornweg, T. de Beer, A. Bader, D.J. van Iperen, U.A.T. Brinkman, *J. Chromatogr. A* 824 (1998) 1.
- [7] P.K. Dasgupta, Z. Genfa, S.K. Poruthoor, S. Caldwell, S. Dong, *Anal. Chem.* 70 (1998) 4661.
- [8] R. Altkorn, I. Koev, A. Gottlieb, *Appl. Spectrosc.* 51 (1997) 1554.
- [9] R. Altkorn, I. Koev, M.J. Pelletier, *Appl. Spectrosc.* 53 (1999) 1169.
- [10] M. Holtz, P.K. Dasgupta, G. Zhang, *Anal. Chem.* 71 (1999) 2934.
- [11] R.J. Dijkstra, C.J. Slooten, A. Stortelder, J.B. Buijs, F. Ariese, U.A.T. Brinkman, C. Gooijer, *J. Chromatogr. A* 918 (2001) 25.
- [12] L. Song, S. Liu, V. Zhelyaskov, M.A. El-Say-Ed, *Appl. Spectrosc.* 52 (1998) 1364.
- [13] R.D. Waterbury, W. Yao, R.H. Byrne, *Anal. Chim. Acta* 357 (1997) 99.
- [14] C.M. Stellman, K.J. Ewing, F. Bucholtz, I.D. Aggarwal, *Sens. Actuators B* 53 (1998) 173.
- [15] http://home.planet.nl/~skok/techiqes/hplc/eluotropic_series_extended.html.
- [16] <http://www.ijvs.com/volume2/edition2/section1.htm>.