CHAPTER 2

EXPERIMENTAL

2.1 Chemicals

All chemicals and reagents were analytical reagent grade and were used without further purification.

1 Hydrochloric acid : 37% (w/v) HCl, Merck, Germany

2 Nitric acid : 65% (w/v) HNO_3, Merck, Germany

3 Glacial acetic acid : 99.8% (w/v) CH_3COOH, Merck, Germany

4 Ammonia solution : 25% (w/v) NH_3, Merck, Germany

5 Cadmium nitrate standard solution for AAS, 1000 mg l^{-1} Cu, Merck, Germany

6 Copper nitrate standard solution for AAS, 1000 mg l^{-1} Cd, Merck, Germany

7 Lead nitrate standard solution for AAS, 1000 mg l^{-1} Pb, Merck, Germany

8 Zinc nitrate standard solution for AAS, 1000 mg l^{-1} Zn, Merck, Germany

9 Ion exchange resin, Chelex-100, sodium form, 50-100 mesh, Bio-Rad, Switzerland

10 Oxalic acid dihydrate : $C_2H_2O_4.2H_2O$, Merck, Germany

11 Tartaric acid : $C_4H_6O_6$, Merck, Germany

12 Citric acid monohydrate : $C_6H_8O_7.2H_2O$, Merck, Germany

13 Sodium hydroxide : NaOH, Merck, Germany

14 Lithium hydroxide : LiOH, Merck, Germany

15 Benzene : C_6H_6, density=0.95 g cm^{-1}, J.T. Baker, Pillipsburg, NJ, USA

16 Toluene : $C_6H_5CH_3$, density=0.87 g cm^{-1}, J.T. Baker, Pillipsburg, NJ, USA

17 *p*-Xylene : C_8H_{10}, density=0.86 g cm^{-1}, J.T. Baker, Pillipsburg, NJ, USA

18 Acetonitrile : CH_3CN, density=0.78 g cm^{-1}, Fisher Scientific, Fair Lawn, NJ, USA

2.2 Preparation of solutions

2.2.1 Standard solutions and reagents

2.2.1.1 Ammonium acetate buffer solution (1.0 M, pH 5.4)

Ammonium acetate buffer solution (1.0 M, pH 5.4) was prepared by mixing 57 ml of glacial acetic acid and 75 ml of 25% ammonia liquor and diluting to 1000 ml.

2.2.1.2 Standard cadmium solutions

A series of standard cadmium solutions was prepared from the stock cadmium standard, 1000 mg l^{-1}, by diluting the stock solutions using 0.01 M HNO_3 as a solvent.

2.2.1.3 Standard copper solutions

A series of standard copper solutions was prepared from a stock copper standard, 1000 mg l^{-1}, by diluting the 1000 mg l^{-1} of copper standard solutions using 0.01 M HNO_3.

2.2.1.4 Standard lead solutions

Similarly, a series of standard lead solutions was prepared from the stock lead standard, 1000 mg l^{-1}, by diluting the stock solutions with 0.01 M HNO_3.

2.2.1.5 Standard zinc solutions

Similarly, a series of standard zinc solutions was prepared from the stock zinc standard, 1000 mg l^{-1}, by diluting it with 0.01 M HNO_3.

2.2.1.6 Sodium citrate buffer solution pH 3

A 6.43 g portion of $C_2H_2O_4.2H_2O$ and a 2.45 g portion of NaOH were dissolved in de-ionized water and about 5.8 ml of HCl was added for adjusting the mixture to pH 3. The solution was diluted to 1000 ml with de-ionized water.

2.2.1.7 Stock benzene standard solution, 1000 mg l^{-1}

A 105 µl portion of benzene was dissolved in 100 ml of de-ionized water.

2.2.1.8 Stock toluene standard solution, 1000 mg l^{-1}

A 116 µl portion of toluene was dissolved in 100 ml of de-ionized water.

2.2.1.9 Stock *p*-xylene standard solution, 1000 mg l^{-1}

A 117 µl portion of *p*-xylene was dissolved in 100 ml of de-ionized water.

2.2.1.10 Standard benzene solutions

A series of standard benzene solutions was freshly prepared from the stock standard benzene, 1000 mg l^{-1}.

2.2.1.11 Standard toluene solutions

A series of standard toluene solutions was freshly prepared from the stock standard toluene, 1000 mg l^{-1}.

2.2.1.12 Standard *p*-xylene solutions

A series of standard *p*-xylene solutions was freshly prepared from the stock standard *p*-xylene, 1000 mg l^{-1}.

2.2.1.13 70% (v/v) Acetonitrile

A 250 ml solution was prepared by mixing 175 ml of acetonitrile and 75 ml of water.

2.2.1.14 Stock tartaric acid solution, 100 mM

A 7.504 g portion of tartaric acid ($C_4H_6O_6$) was dissolved in 500 ml of de-ionized water.

2.2.1.15 Stock oxalic acid solution, 100 mM

A 6.302 g portion of oxalic acid ($C_2H_2O_4.2H_2O$) was dissolved in 500 ml of de-ionized water.

2.2.2 Sample preparation

2.2.2.1 Water samples

Water samples were collected from various areas in Bangkok, Thailand. For total metal determination, the water samples were preserved immediately after sampling by acidifying with concentrated nitric acid to pH 1.5 (1.5 ml conc. HNO_3/1000 ml sample), prior to the following acid digestion process: to 250 ml of water sample, 12.5 ml concentrated nitric acid was added, and the solution was boiled on a hot plate until it became less than 50 ml. After cooling, the solution was filtered and adjusted to pH 1.5 with sodium hydroxide solution (2.0 M), followed by dilution to 250 ml in a volumetric flask, with de-ionized water. For dissolved metal determination, the water samples were filtered with a 0.45 μm membrane filter before preserving and analyzing without digestion.

2.2.2.2 Preparation of zinc ore samples

Samples were kindly supplied by Dr. Ponlayuth Sooksamiti, the Office of the Mineral Resources (Region III), Chiang Mai. A portion (0.1 g) of zinc ore sample was accurately weighed and digested with concentrated nitric acid (25 ml) by boiling on a hot plate until it became clear (about 4 h). It was filtered and transferred into a 250 ml volumetric flask and made to volume with 1% nitric acid.

2.3 Preparation of Chelex-100 resin mini-column

Chelex-100 resin is the commercial name of a polystyrene structured resin with iminodiacetate functional groups (sodium form, 50-100 mesh; Bio-Rad Laboratories). A portion of resin was soaked in 2.0 M nitric acid, followed by de-ionized water, to convert to the H^+ form. The resins were packed in a mini-column, made of acrylic tubing (3 mm i.d. x 2 cm) similar to as previously reported [10]. Teflon frits were placed at each end of the column to prevent the loss of the resin when a solution passed through the column, as shown in Figure 2.1. The two ends of the column were connected to a 6-port injection valve by replacing a sample loop of the valve.

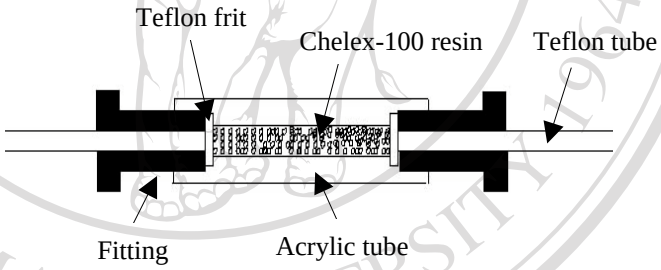

Figure 2.1 Ion exchange mini-column

The resins can be regenerated by passing 6.0 M HNO_3 solution followed by water at a flow rate of 5 ml min^{-1}.

2.4 Apparatus and instrumental setup

2.4.1 Flow injection system with in-valve ion exchanger mini-column for preconcentration of cadmium, copper, lead and zinc determination coupled to flame atomic absorption spectrometer

An in-valve cation exchanger mini-column was used to preconcentrate cadmium, copper, lead and zinc in a water sample for determination by flame atomic absorption spectrometry.

The flow manifold used is schematically shown in Figure 2.2.

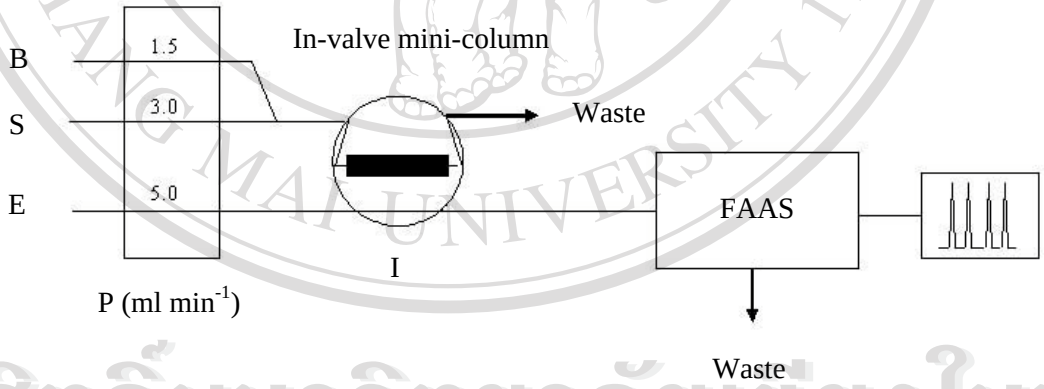

Figure 2.2 FI manifold with in-valve mini-column and FAAS; P=peristaltic pumps; B=ammonium acetate buffer; I=injection valve with in-valve mini-column; S=sample or standard solution; E=2.0 M nitric acid and FAAS=flame atomic absorption spectrometer

This on-line FI system consisted of two peristaltic pumps (MP-3, EYELA, Tokyo Rikakikai, Tokyo, Japan and Ismatec, Glattbrugg-Zurich, Switzerland), and a Rheodyne (Model 7725i) injection valve with ion exchanger mini-column (Acrylic tubing, 3 mm i.d. x 2 cm) filled with Chelex-100 resin, and FAAS (AA-Z8200, Hitachi, Japan). The FAAS with air-acetylene flame and hollow cathode lamps were used under the conditions recommended by the instrument manufacturer (Manual AA-Z8200 Hitachi, Japan). The signals were measured as the heights of the absorbance peaks.

The system operation consists of two steps. In the loading step, a standard/sample solution was first mixed on-line with ammonium acetate buffer pH 5.4 and then was loaded on the mini-column while eluent flowed to the FAAS. The cations at the specific pH were exchanged by the ions on the resin. When reaching the duration of loading, the injection valve was switched to the injection position. The elution step was started by allowing 2.0 M HNO_3 as eluent to pass through the column in the opposite flow direction of the sample loading. The reverse flow direction of the sample loading and of the elution help to prevent blockage in the column, which may be caused by accumulation of resin at one end of the column if the loading and elution were in the same direction [4]. And if the analyte is loaded at the top of the column, it is more concentrated when eluted in the reverse direction. Absorption intensity (peak height) was continuously recorded. A plot of absorbance versus time is shown in Figure 2.3.

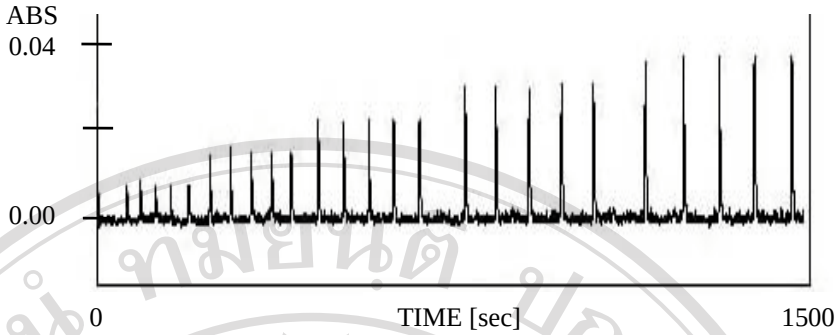

Figure 2.3 FI-FAAS peak profiles

2.4.2 Flow injection in-valve mini-column pretreatment combined with ion chromatography for cadmium, lead and zinc determination

The flow injection (FI) in-valve mini-column was used as an on-line sample pretreatment system prior to simultaneous determination by ion chromatography as shown in Figure 2.4.

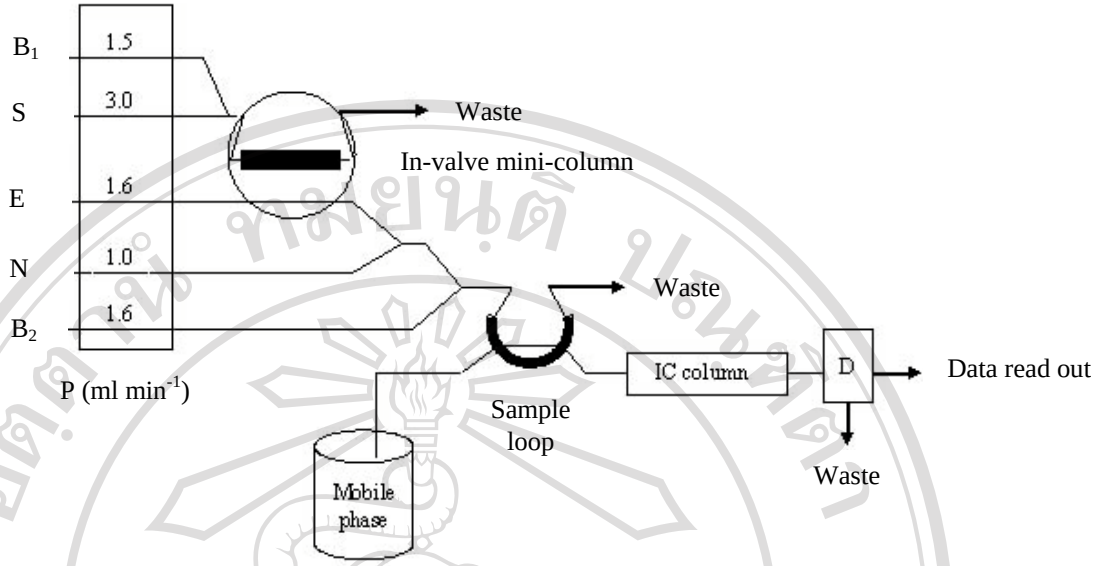

Figure 2.4 Manifold of FI-IC combination; P=peristaltic pumps; B_1=ammonium acetate buffer; B_2=sodium citrate buffer; S=sample or standard solution; E=2.0 M nitric acid; N=2.0 M sodium hydroxide; Mobile phase=mixture of tartaric acid and oxalic acid; Sample loop=20 μl loop of IC; IC column=universal cation column 100 mm x 4.6 mm; D=conductivity detector

Figure 2.4 shows the FI system with in-valve mini-column coupled to an ion chromatograph for cadmium, lead and zinc determination. The instrument setup for the FI system with in-valve mini-column was similar to that in the previous section (2.4.1), but 2.0 M NaOH and sodium citrate buffer pH 3 were used for neutralization and buffer, respectively. The ion chromatographic system without chemical suppression (Metrohm Ltd., Switzerland) was used this study. It consists of a Metrohm isocratic pump, a 6-port injection valve with 20 μl sample loop, and a

Metrohm 732 conductivity detector. The analytic column used for the cation separation was a universal cation column (100 mm x 4.6 mm, Altech, USA). The column was packed with polybutadienemaleic acid (PBDMA) coated on silica material. The chromatograms were recorded and handled with Metrohm software (Metrohm Ion Analysis, Metrohm Ltd., Switzerland) which was also used for controlling the pump and injection valve.

The FI-IC system operation was controlled by two injection valves, one for FI, another for IC, under a timer control as indicated in Figure 2.5. Standard/sample solution was flowed to mix with buffer to adjust the pH for adsorption of cations on the resin, and flowed through the column with the FI-valve in the load position, with various loading times, while the unretained cations were passed to waste. After that the valve was switched to the injection position and 2.0 M HNO_3 was flowed through the mini-column to elute cations from the column. The eluate was on-line neutralized with 2.0 M NaOH and then controlled for pH with citrate buffer. When the elapsed time was 24 seconds after switching the FI valve to the injection position, the zone of cations was moved into the sample loop and was injected into the IC via through the IC valve.

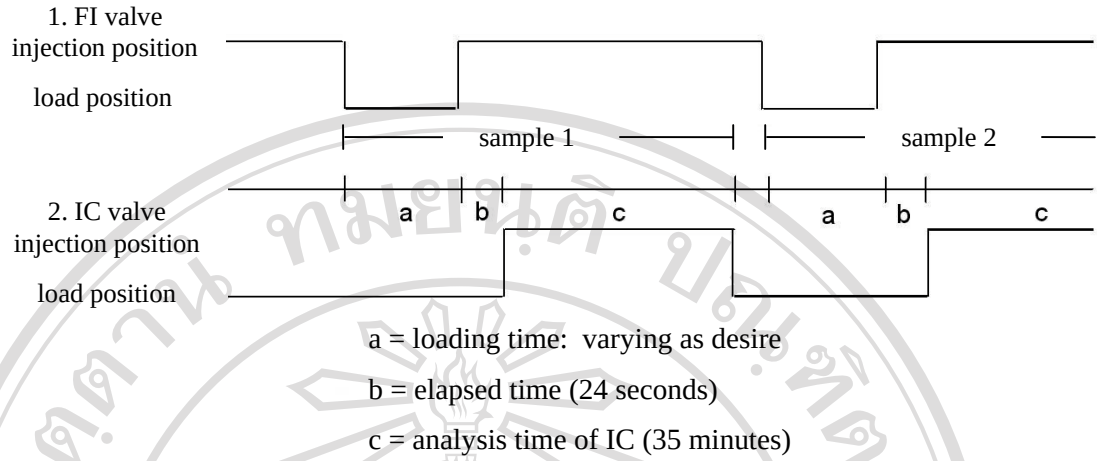

Figure 2.5 Schematic diagram of timer control for operation of valve in the FI-IC system

2.4.3 On-line preconcentration and quantitation of benzene, toluene and *p*-xylene by using Raman liquid-core waveguide sensor

For determination of benzene, toluene and *p*-xylene, a Raman liquid-core waveguide (LCW) with used as a preconcentrating flow cell (Figure 2.6), connected to a liquid flow cell (Figure 2.7).

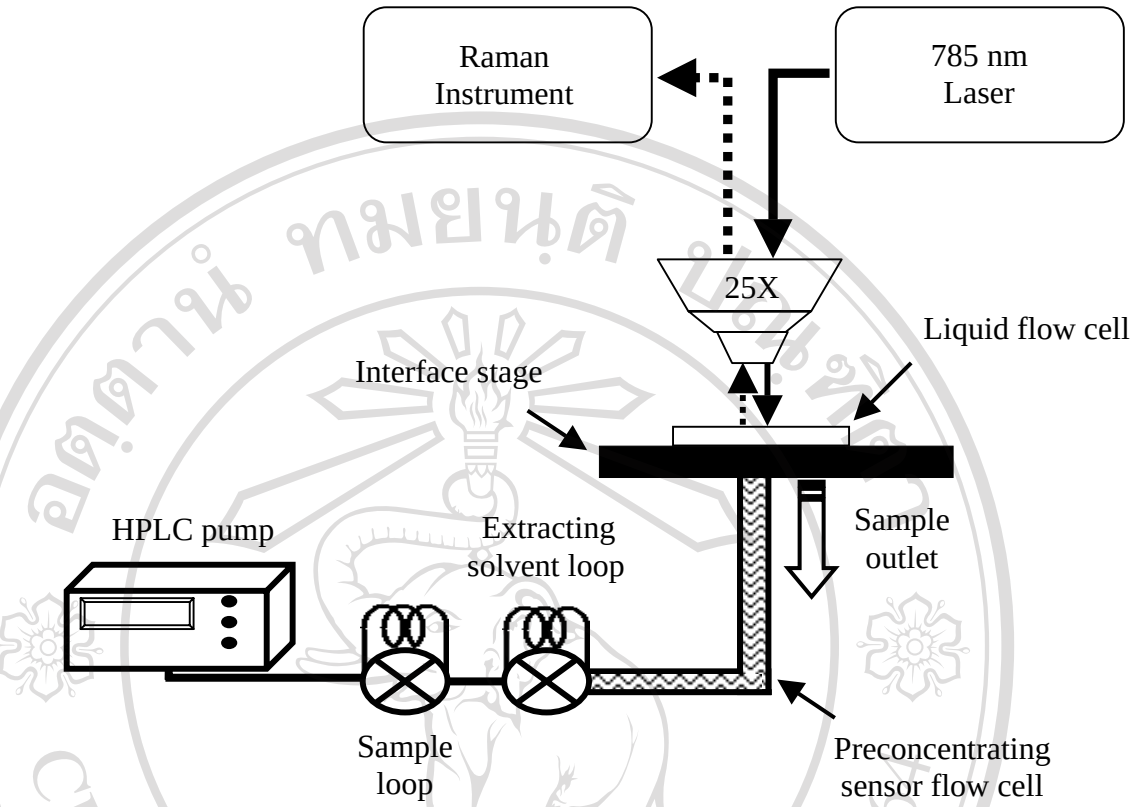

Figure 2.6 A schematic diagram of the Raman LCW sensor apparatus. The sample loop volume is 1000 μl for analytes in water. The extracting solvent loop of 30 μl volume was for extraction. The preconcentrating sensor flow cell of 1 m x 50 μm i.d. Teflon-AF 2400 capillary tube served as a waveguide.

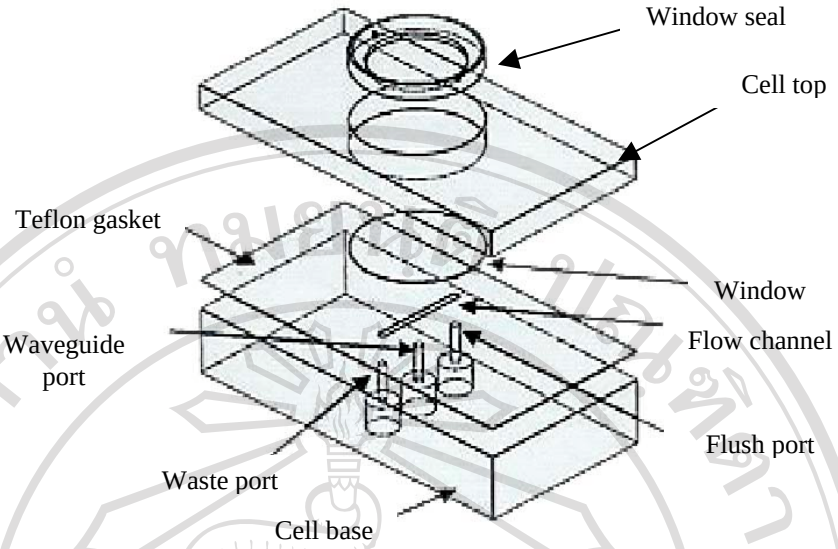

Figure 2.7 The liquid flow cell

An HPLC pump with an analytic micro-flow liquid head (Beckman, Model 114 M, Berkeley, CA) was used to pump de-ionized water as a carrier stream. Two injection valves (EC10W, Valco Instruments Co., Inc., Houston, TX) were used. The first valve was used to load a 1000 µl aqueous sample solution. A second valve was used to introduce 30 µl of the extraction solvent (70% acetonitrile/30% water). The preconcentrating flow cell was 1 m long, and 50 µm in i.d. Teflon-AF 2400 tubing (Biogeneral Technologies, Inc., San Diego, CA) connected to a liquid flow cell. The liquid flow cell consisted of a PEEK (polyetheretherketone) polymer block of the same footprint as a typical microscope slide (7.6 cm x 3.8 cm) to facilitate use on a microscope stage. It 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 a FEP fluoropolymer gasket. The channel dimension

of the gasket was 20 mm x 1.6 mm x 0.25 mm (L x W x D). The gasket was placed between the PEEK top plate and the base to form a liquid sealed cell. A 25 mm o.d. x 500 μm sapphire window was then secured over the center port to allow optical access to the LCW as shown in Figure 2.7. 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. The Raman instrument system consists of a Kaiser Optical Systems Hololab Series 5000 Raman instrument (Ann Arbor, MI), including a Holoprobe transmission holographic spectrograph interfaced to an infinity-corrected fiber-coupled microscope. 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 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 an 8 μm i.d. excitation fiber, and the scattered radiation was collected using a 100 μm i.d. multimode fiber. A 25X (0.65 NA) objective was used both to focus the laser and to collect the scattered radiation. The objective was chosen for its large numerical collection of the Raman scattered radiation from the waveguide during dynamic flowing measurements. The data collection consisted of three steps. First, in the baseline collection step, 70% acetonitrile was injected into the system with a flow rate of 5 μl 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 μl min^{-1}. In the third step, the 70% acetonitrile solution was introduced to the sensor at a flow rate of 5 μl min^{-1} using the injection valve fitted

with the 30 μl loop. All Raman spectra were collected using a 50 μm slit width and a detector temperature of -40°C. All raw Raman data collected from the Raman detector were processed with MATLAB 6.0 software (Mathworks, Natick, MA).