Enhanced Sensitivity in Flow Injection Analysis Using a Long Pathlength Liquid Waveguide Capillary Flow Cell for Spectrophotometric Detection

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Incorporation of a liquid waveguide capillary flow cell into a flow injection instrument enhances the sensitivity of flow injection analysis with spectrophotometric detection by two orders of magnitude. Nitrite determination at nM levels has been used to demonstrate the feasibility of this novel technique for trace analysis. Combining the long pathlength spectrophotometry with flow injection analysis, this technique has advantages of low detection limit, good precision and high sample throughput.

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Flow injection analysis has gained a wide range of application since its inception in the 1970's. It has merits of simplicity, versatility and high sample throughput. Flow injection analysis allows the analytical method to be used in conjunction with a variety of detection techniques. Spectrophotometry, visible spectrophotometry in particular, has been predominate among the various detection techniques used in flow injection analysis.¹ Today, flow injection analysis with spectrophotometric detection has been routinely used in analyses of clinical, pharmaceutical, agricultural and environmental samples. However, the sensitivity of spectrophotometry is generally lower than that of atomic absorption and emission spectrometry and such low sensitivity hinders application of flow injection analysis with spectrophotometric detection in trace analysis.

In spectrophotometry, the utilization of absorbance signals for quantitative analysis relies upon the Lambert-Beer law, according to which the magnitude of absorbance signals is proportional to the optical pathlength, the molar absorptivity, and the concentration of the substance under investigation. The molar absorptivity is an inherent characteristic of the specific chromophoric compound utilized in the spectrophotometric measurements and therefore intrinsic to a given method. It is always a challenge to find a new chromophoric compound that is higher in both molar absorptivity and selectivity to an analyte of interest. Moreover, the molar absorptivities of most chromophoric organic compounds utilized in the modern spectrophotometry lie in a narrow range of 1×10^3 to 5×10^4 l mol⁻¹ cm⁻¹. Therefore, increasing the optical pathlength of the measurement cells becomes the most feasible approach by which a significant enhancement in the sensitivity of a spectrophotometry-based analytical system can be achieved.

A conventional cuvette longer than 10 cm requires a large sample volume and is not practical in many analytical applications. Using the lasers as a light source, long capillary cells have been developed to accommodate the requirements for both long path length and small sample volume.^{2,3} A compact design was achieved by constructing the long capillary cells in a

helical rather than a linear shape. However, the surface reflectiveness of these long capillary cells relied upon the metal coatings. Such metal-coated long cells suffered severe source light loss and non-linearity response which limit their applications to measurements for gaseous samples. To effectively utilize the long capillary cells for liquid samples, one must constrain the light propagation within a liquid medium, in other words, the cells must function as a liquid core waveguide. This requires the refractive index of the cell material to be smaller than that of the liquid contained in the cells. Due to its mechanical property being suitable for microfabrication and its high resistance to most chemicals, glass from fused silicon is often used as a material for long capillary cells. The hydrophilic property of glass also makes it a suitable material for constructing flow cells used for aqueous solution. However, the refractive index of glass (1.474) is greater than that of water (1.33), and therefore the glass cells cannot confine the light transmission in aqueous phase. Such glass cells can only be used in conjunction with higher refractive index organic solvents, such as benzene ($n = 1.50$) and carbon disulfide ($n = 1.63$).⁴ Recently Dupont (DuPont Fluoroproducts, DE, USA) manufactured amorphous fluoropolymers (Teflon AF family) that have refractive index values (1.29 – 1.31) lower than that of water. Total internal reflection of light can be achieved with a liquid waveguide capillary flow cell made by Teflon AF.⁵ Dallas and Dasgupta have provided a comprehensive review on the growing applications of liquid-core waveguides in UV-visible spectrophotometry, fluorescence and Raman spectroscopy.⁶⁻⁹ For example, such liquid waveguide capillary flow cells have been used to enhance the sensitivity of manual spectrophotometric analysis of trace constituents, such as ferrous, nitrite, molybdate and chromate ions, as well as colored dissolved organic matter in aqueous samples.¹⁰⁻¹⁹ The porous structure of amorphous fluoropolymers also provides a high gas permeability, which has been utilized to construct gas sensors.²⁰⁻²⁶ However, its porous structure is prone to adsorb surface-reactive species from aqueous samples and thence cause a decline in its waveguide performance. Furthermore, amorphous fluoropolymers have a water contact angle of 106°;

hence, its hydrophobic internal surface is resistant to the flowing stream and prone to adhering air bubbles. Air bubbles attached on the cell walls will scatter the incident light and cause the cell to lose its liquid waveguide characteristics. A new design of capillary cell with cladding Teflon AF on the outer surface of capillary quartz tubing has been found to minimize the potential contamination and possibility of trapping air bubbles while maintaining its liquid waveguide function.²⁷⁻²⁸

Many laboratories require an automated analytical system to promptly analyze trace constituents in a large number of samples. Recently, we have successfully incorporated a liquid waveguide capillary flow cell to a gas-segmented continuous flow auto-analyzer to significantly enhance the analytical sensitivity for nitrate,²⁹ iron,³⁰ phosphate³¹ and ammonium³² measurements in natural waters. Using this technique, detailed diurnal cycling of nitrate at nM levels in oligotrophic open ocean waters was observed for the first time.³³ In this study, we explore the feasibility of incorporation of a long pathlength liquid waveguide capillary flow cell into a flow injection instrument (Lachat Inc., USA) for determination of nM levels of nitrite in water samples.

Experimental

Liquid waveguide capillary flow cells

Three liquid waveguide capillary flow cells with pathlengths of 0.5, 2 and 4 m, respectively, have been used in this study. The liquid waveguide capillary flow cells were made of a piece of 550 μm i.d. quartz capillary tubing and the outer surface of each cell was coated with low refractive index amorphous fluoropolymers. The capillary tubing was looped in a single (0.5 m cell) turn or in multiple (2 and 4 m cells) turns and placed in a $30 \times 30 \times 2$ cm metal case. Four connection terminals, two for optical fiber (input and output) and two others for sample (in-flow and out-flow), are on the front panel of the metal case. The sample connectors are designed for 1/16-inch o.d. tubing. The optical fiber connectors are standard "ST"-type, and either of the ST mating sleeves can be used for light inlet or outlet. Two fiber optical jumper cables are used to connect a liquid waveguide capillary flow cell to the Lachat instrument, one transmitting light from the lamp to the liquid waveguide capillary flow cell and the other from the flow cell to the photodiode detector.

An Lachat flow injection analytical system

An Lachat QuikChem 8000 Automated Ion Analyzer (Milwaukee, Wisconsin, USA) was modified to adapt the liquid waveguide capillary flow cells for automated flow injection analysis. A conventional 1 cm flow cell was removed from the detector assembly. One custom-made optic mount was placed at the lamp chamber to allow the transmission of the source light from the lamp through a fiber optic cable into a liquid waveguide capillary flow cell. A detector head in QuikChem 8000 was also modified to enable one to connect an optic cable that transmits the exiting light from a liquid waveguide capillary flow cell through an interference filter (wavelength 520 nm) then to a photodiode detector. An optic cable for the reference channel was also modified to balance the light intensity change due to replacement of a 1 cm cell with a liquid waveguide capillary flow cell. Such a liquid waveguide capillary flow cell coupled flow injection system allows the source light to transmit into the liquid waveguide capillary flow cell, to be absorbed by aqueous samples within the cell and then to exit to the photodiode detector of the QuikChem 8000 flow injection analyzer.

Automated analysis of nitrite

The flow injection analysis with short flow cells has

Fig. 1 A schematic diagram of flow injection system coupled with a long pathlength liquid waveguide capillary flow cell (LWCFC).

previously been used for the automated determination of nitrite concentrations by spectrophotometric detection.³⁴⁻³⁸ In this study, the flow injection analytical system was coupled with a liquid waveguide capillary long flow cell; the schematic diagram of the flow injection system is shown in Fig. 1. The valve cycle period is 220 s with 90 s of a loading period and 130 s of an injecting period. The nitrite in the samples is determined by diazotizing with sulfanilamide and coupling with *N*-1-naphthylethylenediamine dihydrochloride to form a pink azo dye. The absorbance of the dye measured at 520 nm is used to quantify the concentration of nitrite in the samples.

Standards and reagents

Deionized water used for preparing standard and reagents was purified by a distilling unit, followed by a Millipore Super-Q Plus Water System that produces water with 18 M Ω resistance. To avoid contamination in trace analysis, deionized water was freshly prepared daily. Nitrite stock standard solutions (10 mM) were prepared from analytical reagent-grade pre-dried (105°C for 1 h) sodium nitrite (NaNO_2). The stock solutions were stored in a polyethylene bottle at 4°C in a refrigerator. Working standard solutions were prepared from serial dilutions of stock solutions with deionized water.

A sulfanilamide stock solution was prepared by dissolving 10 g of sulfanilamide ($\text{C}_6\text{H}_8\text{N}_2\text{O}_2\text{S}$) in 1 l of 10% (v/v) HCl solution. One gram of NED (*N*-1-naphthylethylenediamine dihydrochloride, $\text{C}_{12}\text{H}_{14}\text{N}_2 \cdot 2\text{HCl}$) was dissolved in 1 l of water as a NED solution. A 100 ml aliquot of sulfanilamide solution was mixed with the same volume of NED solution for daily analysis. One milliliter of Brij-35 surfactant (polyoxyethylene(23) lauryl ether ($\text{C}_{12}\text{H}_{25}(\text{OCH}_2\text{CH}_2)_{23}\text{OH}$, ICI Americas Inc.) was mixed with a 200 ml aliquot of the distilled water and used as a carrier solution.

Results and Discussion

Optimization of flow injection analysis

The flow rate and sample volume were optimized in line with the long flow cell. A flow rate of 0.8 ml min⁻¹ was used throughout the experiment. A 150 cm-long piece of Teflon tubing (0.042 inch i.d.) was chosen as a sample loop. A sample volume of 1.3 ml ensures minimum sample dispersion and maximum sensitivity. Timing parameters for data acquisition system were optimized using graphical events programming provided by QuikChem 8000.

Because of the extended pathlength and the small cross section of the long capillary cells that are located at the end of the flow system, the back-pressure can build up in the flow system to cause fluid leaking at various joints. It is necessary, therefore, to add a surfactant in the carrier solution to reduce the

Fig. 2 Comparison of base line noises and sample signals of nM concentrations of nitrite analysis: a) without an on-line degasser; b) with an on-line degasser.

surface tension and thereby the back pressure in the flow stream. Several surfactants have been tested for their suitability in the flow system and the Brij-35 was found to be the most effective in lowering surface tension between quartz and sample mixture. Therefore, a smooth flow can be achieved even with a 4-m long capillary flow cell in the system. This surfactant is also compatible with reagents used for nitrite analysis.²⁹

In flow injection analysis, there are no air bubbles deliberately introduced to segment the fluid stream. However, air dissolved in the reagent and carrier solutions can form micro-bubbles. These air bubbles are likely to attach on the inner surfaces of a flowcell and to reflect and scatter the incident light, generating erroneous high absorbance signals. Degassing of reagent solution by vacuum suction or sparging with a low solubility gas, *e.g.*, helium, prior to its use are a common practices in flow injection analysis. A flow injection system coupled with an online long capillary flow cell, however, requires more stringent degassing than the aforementioned conventional techniques. Microscopic air bubbles, once formed, are more likely to stick on the wall of a long capillary flow cell than that of a conventional short flow cell because of the large surface area in the former. Utilizing the high gas permeability Teflon AF as membrane material, a new on-line degassing system (Alltech, Deerfield, IL, USA) provides high degassing efficiency and fast equilibration times for flow injection analysis. It was found that incorporation of such an on-line vacuum degassing system significantly minimize the micro-bubble formation and therefore improved the stability of the baseline (Fig. 2). As a result of improved baseline, the precision of analysis was improved from 15% without a degasser to 3% with an online degasser at a level of 20 nM of nitrite.

Sensitivity enhancement

A comparison study has been conducted to evaluate the sensitivity of nitrite analysis by a conventional flow injection

Fig. 3 Comparison of sensitivity and linear dynamic range of nitrite analysis using a conventional short flow cell (1 cm) and the liquid waveguide flow cells of different pathlengths in a flow injection system.

Fig. 4 Sensitivity of flow injection analysis, shown as the slopes of calibration curves in Fig. 3, is directly proportional to the lengths of liquid waveguide flow cells used in the flow injection analytical system.

analysis with a short flow cell (1 cm) and by the system using the long pathlength liquid waveguide capillary flow cells. The sensitivity of flow injection analysis increased with increasing the length of the flow cells as predicted based on the Lambert-Beer law (Fig. 3). Using a conventional 1 cm flow cell, the detection limit of flow injection analysis is 100 nM for nitrite. By using a 0.5 m long liquid waveguide flow cell we reduced the detection limits of nitrite analysis to 6 nM. The detection limits of 2 and 4 nM have been achieved by using 4-m and 2-m liquid waveguide flow cells, respectively (Table 1). The slopes of linear calibration curves obtained from the analytical systems are directly proportional to the lengths of the capillary flow cells used in the analyses, with a correlation coefficient of 0.999999 (see Fig. 4). This indicates that the flow injection analytical system coupled with long pathlength flow cells obeys the Beer-Lambert law within the linear dynamic range of method, which in turn depends upon the length of a capillary flow cell used for the system. A typical output signal of flow injection analyses of nM nitrite using a 4 m-long liquid waveguide capillary flow cell is shown in Fig. 5. The marked peaks in Fig. 5 are samples containing 0, 2, 5, 10, 20, 50 and 100 nM of nitrite, respectively.

As commonly observed in spectrophotometry, the enhancement of sensitivity with increasing the length of the capillary flow cells is accompanied by a decrease in the linear dynamic range of analysis (Fig. 3 and Table 1). If one uses a conventional 1 cm flow cell, the linear dynamic range of nitrite determinations is 50000 nM in flow injection analysis. By

Fig. 5 A typical output signal of flow injection analysis of nitrite using a 4 m-long liquid waveguide capillary flow cell. The marked peaks are samples containing 0, 2, 5, 10, 20, 50 and 100 nM nitrite, respectively.

using the long liquid waveguide flow cells, one can reduce the linear dynamic ranges of nitrite analysis to 600, 1000 and 4000 nM for a cell length of 4, 2 and 0.5 m, respectively (Table 1). For samples containing a wide range of analyte concentrations, the sample analyses should be performed either by using a shorter flow cell or by diluting the samples with deionized water. The latter can be easily achieved in flow injection analysis by adding a dilution line to the sample flow.

Conclusion

Incorporation of a liquid waveguide capillary flow cell into a flow injection instrument enhances the sensitivity of flow injection analysis with spectrophotometric detection by about two orders of magnitude. Utilizing a compatible surfactant and an online vacuum degasser in the flow stream significantly improves the baseline stability and thereby the precision of analysis. Nitrite determination at nanomolar concentrations has demonstrated the feasibility of this novel technique. Combining the long pathlength spectrophotometry with flow injection analysis, this technique has advantages of low detection limit, good precision and high sample throughput.

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Table 1 Comparison of performance of a short flow cell (1 cm) with three long pathlength capillary flow cells of different lengths in flow injection analysis

Cell length/cm	Linear dynamic range/nM	Detection limit/nM
1	50000	100
50	4000	6
200	1000	4
400	600	2

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