# Quantitative Determination of Nicotine in a PDMS Microfluidic Channel Using Surface Enhanced Raman Spectroscopy

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Rapid and highly sensitive determination of nicotine in a PDMS microfluidic channel was investigated using surface enhanced Raman spectroscopy (SERS). A three-dimensional PDMS microfluidic channel was fabricated for this purpose. This channel shows a high mixing efficiency because the transverse and vertical dispersions of the fluid occur simultaneously through the upper and lower zig zag-type blocks. A higher efficiency of mixing could also be obtained by splitting each of the confluent streams into two sub-streams that then joined and recombined. The SERS signal was measured after nicotine molecules were effectively adsorbed onto silver nanoparticles by passing through the three-dimensional channel. A quantitative analysis of nicotine was performed based on the measured peak area at 1030 $cm^{-1}$. The detection limit was estimated to be below 0.1 ppm. In this work, the SERS detection, in combination with a PDMS microfluidic channel, has been applied to the quantitative analysis of nicotine in aqueous solution. Compared to the other conventional analytical methods, the detection sensitivity was enhanced up to several orders of magnitude.

**Key Words :** Surface enhanced Raman spectroscopy, Microfluidic chip, Nicotine, Quantitative analysis

## Introduction

Nicotine is one of the most potent pharmacological agents in tobacco and tobacco smoke, hence, it is of considerable interest to medicine and society.[1] A quantitative analysis of nicotine has been used to study the smoking behavior of smokers and the quality control of nicotine-containing products as well as the determination of nicotine amount in biological fluids.[2,3] Various analytical methods, such as HPLC,[4] UV/Vis spectroscopy,[5] GC/MS,[6] radioimmunoassay,[7] chemosensor[8] and antibody/antigen sensor,[9] have been applied to the quantitative analysis of nicotine. For example, GC/MS has been used to quantify the nicotine present in the hair of smokers and non-smokers. However, some of the problems associated with the previously reported analytical methods, such as a long sample preparation time, poor detection limit, lengthy measurement time and indirect measurement, made the detection systems less attractive.

Surface enhanced Raman spectroscopy (SERS) has also been used for the quantitative analysis of nicotine. Previous SERS studies of nicotine have used silver-coated alumina substrates,[10] silver nano particle doped cellulose acetate membranes,[11] and electrochemically roughened electrodes.[12] More recently, Bell and Sirimuthul[13] reported sensitive nicotine detection results using polymer-encapsulated silver nanoparticles. These nanoparticles were used for quantitative determination of unknown nicotine samples at the 1-5 ppm level. However, the quantitative application of SERS is known to be very challenging because it is difficult to control the experimental conditions such as the degree of aggregation, the particle sizes of the metal colloids, and the inhomogeneous distribution of molecules on the metal surface.[14,15] As a result, under ordinary sampling conditions, the expected precision from SERS is very poor. Nonetheless, a highly precise quantitative measurement can be obtained if continuous flow and homogeneous mixing conditions between the analytes and a silver nanocolloid are maintained. For this purpose, we used a polydimethylsiloxane (PDMS) microfluidic device, combined with a highly sensitive SERS detection. Recently, we have successfully used this SERS technique for the rapid and highly sensitive detection of duplex dye labeled DNA sequences,[16] cyanide ion water pollutants,[17] and methyl parathion pesticides in a PDMS microfluidic channel.[18] In the present study, we extend its applicability to the trace analysis of nicotine.

## Experimental Section

**Silver Colloid Preparation.** Silver colloids were prepared by the method recently reported by Leopold and Lendl.[19] Here, silver nitrate was reduced by hydroxylamine hydrochloride. The advantages of the hydroxylamine hydrochloride-reduced silver colloid are its fast preparation at room temperature and its immediate applicability for SERS. The detailed procedure for the preparation of silver nanoparticles has been reported elsewhere.[16] UV/Vis spectroscopy and transmission electron microscopy (TEM) were used to characterize the particle size of the produced colloids. The average particle size was determined to be 60 nm.

**Fabrication of the PDMS Microfluidic Channel.** The fabrication process of zig zag-type PDMS microfluidic channel has been reported elsewhere.[20,21] Briefly, the microfluidic channel were fabricated stacking two PDMS layers

having upper and lower zig zag-type block patterns. These layers were produced by pattern replication from mold masters. The cover glass was stacked onto the upper layer. Within a rectangular duct, the bar structures were located on upper and lower surfaces of the channel in a zig zag manner.

**Surface Enhanced Raman Measurements.** Raman measurements were performed with a Renishaw 2000 Raman microscope system. A Spectra Physics argon ion laser operating at $\lambda = 514.5$ nm was used as the excitation source with a laser power of approximately 20 mW. The Rayleigh line was removed from the collected Raman scattering by a holographic notch filter in the collection path. A charge coupled device (CCD) camera was coupled to a spectrograph that in combination provided a 2 $cm^{-1}$ spectral resolution. All spectral manipulations were performed using *GRAM/32* software from Galactic Industries Corporation, U.S.A.

**Confocal Fluorescence Measurements.** Confocal fluorescence image measurements were performed using a Leica TCS SP confocal fluorescence microscope with a He-Ne laser. Fluorescent Rhodamine 6G dissolved in water and non-fluorescent silver colloids were introduced through a Y-shaped inlet using microsyringe pumps to display the mixing process of the confluent streams. The confluent mixing streams were analyzed using the two-dimensional confocal fluorescence images in the $x-y$ plane located perpendicular to the optical axis using a 10 × water-immersion objective lens. The lateral resolution was estimated to be 1 mm. The laser excitation of Rhodamine 6G occurred at $\lambda = 543$ nm, and the emitted fluorescent light was detected between $\lambda =$ 590 and 620 nm. The image size was 512 × 512 pixels, and each pixel was 0.49 $\mu$m. The fluorescence intensity profiles of the flow across the channel were also measured to monitor the mixing behavior of the confluent mixing streams.

## Results and Discussion

Figure 1 shows a schematic drawing of the PDMS micromixer and the mixing process between the silver nanocolloid and the nicotine in the microfluidic channel. The silver colloids and the different concentrations of nicotine were introduced into the channel using microsyringes connected by tubes to the inlet pipettes. The flow rate was simultaneously controlled using a microsyringe pump. While the confluent streams travel along the microfluidic channel, the transverse and vertical dispersions of the fluid occur simultaneously through the upper and lower zig zag-type blocks. The confluent stream was split into two lines to improve the mixing efficiency. A high efficiency of mixing could be obtained by splitting each of the confluent streams into two sub-streams that then joined and recombined. Heule and Manz[22] previously reported that the problems of diffusion limitations in the laminar flow regime can be overcome using the multi-splitting channel. In our work, the double split design as well as the zig zag-type chaotic advection design was adopted for obtaining high mixing efficiency. After the nicotine molecules were effectively adsorbed onto

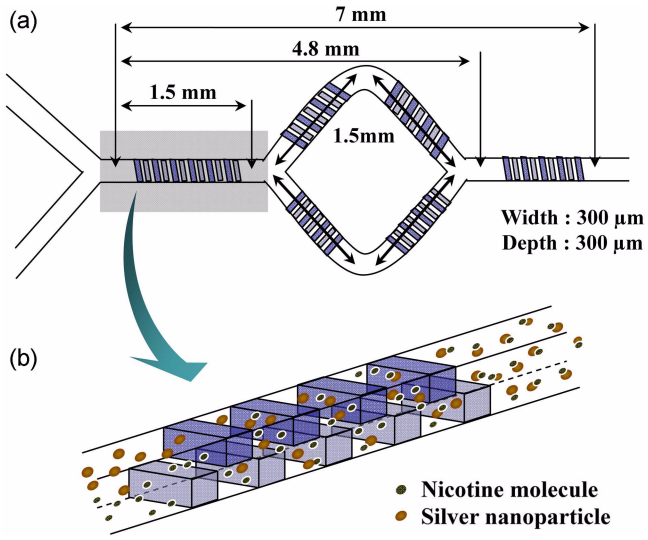


**Figure 1**. (a) A schematic view of the zig zag-type PDMS microfluidic channel. (b) A mixing process of confluent streams, colloidal silver nanoparticles, and nicotine solution. While laminar flow streams traverse the microfluidic channel, the transverse and vertical dispersion of the fluid occurs simultaneously through the upper and lower blocks.

the silver nanoparticles on traveling along the blocks and split channel lines, the SERS signal was measured.

In order to investigate the mixing efficiency of the channel used, the fluorescence image was measured using a confocal laser scanning microscope. Laser-grade Rhodamine 6G was dissolved in distilled water to produce a 0.05 M solution that formed one of the confluent streams in the channel. The other stream contained silver colloids. These two streams were introduced into the PDMS channel from microsyringes connected by the tubes to the inlet pipettes. The flow rates were controlled simultaneously using a KD Science microsyringe pump. Figure 2 shows the captured fluorescence images and corresponding intensity profiles at the location

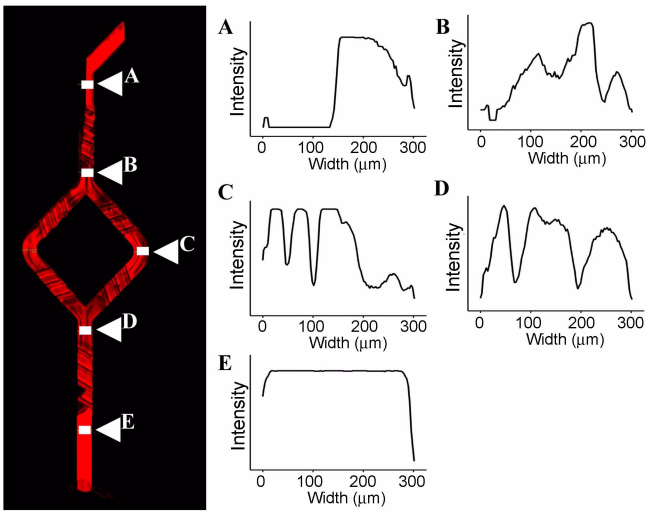


**Figure 2**. Confocal fluorescence images of lateral confluent streams along the channel and corresponding intensity profiles at the locations indicated by the solid lines.

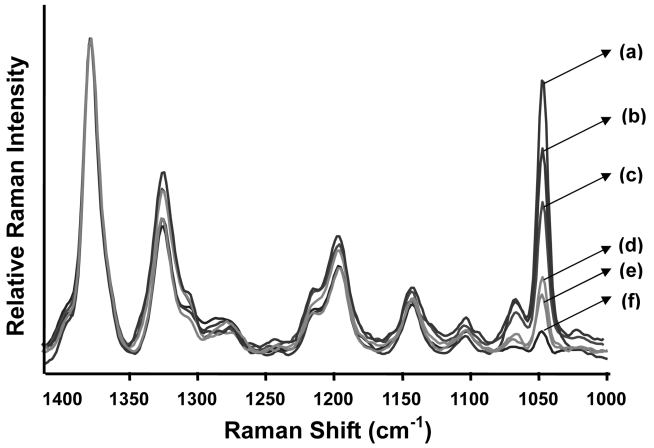


**Figure 3**. Confocal SER spectra for varing concentrations of nicotine in the microfluidic channel: (a) 10 ppm, (b) 7.5 ppm, (c) 5.0 ppm, (d) 2.5 ppm, (e) 1.0 ppm, and (f) 0.1 ppm.

indicated by dashed lines at five different channel positions. The confluent streams display a heterogeneous mixing behavior with increasing channel distance by the chaotic advection. To determine the optimum conditions for the SERS measurements, the flow rate of two confluent streams was varied in the range 1-170 $\mu$L/min. According to our experimental data, the optimum rate was determined to be 100 $\mu$L/min. As shown in Figure 2, the mixing of the red fluorescence dye occurred from half of the channel (A), to over the full channel (E) along the channel distance. This means that the laminar flow mixing was efficiently driven by the chaotic advection as well as the splitting into two sub-streams. As a result, the confluent streams were completely mixed at the end of the channel (E).

Figure 3 illustrates the SER spectra for different concentrations of nicotine solution in a PDMS microfluidic channel. The characteristic Raman peak of nicotine at 1030 $cm^{-1}$ was monitored at different concentrations. The areas of the Raman peaks increased concomitantly with the increase in the concentration of nicotine. This peak was used for the

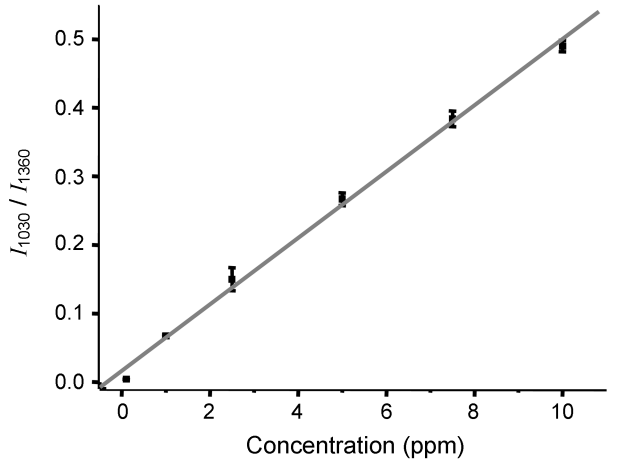


**Figure 4**. Variation of the peak area ratio ($I_{1030}/I_{1360}$) as a function of nicotine concentration. (Correlation coefficient, R = 0.998)

quantitative determination of nicotine. In order to correct the sample-to-sample fluctuations in the Raman intensities,[23,24] the Raman peak at 1030 $cm^{-1}$ was normalized to the peak at 1360 $cm^{-1}$. This is the peak for the reducing agent, hydroxylamine, and is used as an internal standard.

The calibration curve is shown in Figure 4. A very good linear response of peak area ratio ($I_{1030}/I_{1360}$) was found in the concentration range 0.1-10 ppm. The limit of detection (LOD) was determined to be below 0.1 ppm assessed from six standard deviations above the background. This means that the highly sensitive quantitative determination of nicotine in a PDMS microfluidic channel is possible using the surface enhanced Raman technique.

## Conclusions

Lab-on-a-chip technology is attracting great interest as the miniaturization of reaction systems offers practical advantages. Our proposed analytical method, using a PDMS microfluidic channel and a SERS detection technique, offers rapid and highly sensitive detection applicable to the quantitative determination of nicotine. Rapid mixing of the fluids flowing through a microchannel is very important for various applications of microfluidic systems. In addition, highly sensitive on-chip detection techniques are essential for the *in situ* monitoring of chemical reactions because the detection volume in a channel is extremely small. For this purpose, a zig zag-shaped split PDMS channel was fabricated. In this channel, the problems of diffusion limitations in the laminar flow could be overcome using the double split and zig zag- type chaotic advection design. According to our fluorescence images, measured using a confocal laser scanning microscope, the confluent streams display a complete mixing behavior after they pass through the zig zag-type PDMS blocks. The SERS detection technique has also been used for the highly sensitive analyte detection. In this work, the SERS detection, in combination with a PDMS microfluidic channel, has been applied to the quantitative analysis of nicotine in its aqueous solution. Compared to the other conventional analytical methods, the detection sensitivity was enhanced up to several orders of magnitude.

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