MICROFLUIDIC DEVICE FOR DETECTION OF CHEMICALS IN AQUEOUS MIXTURES USING SURFACE ENHANCED RAMAN SPECTROSCOPY

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ABSTRACT

A microfluidic flow focusing device is used to separate a mixture of two vitamins, based upon their diffusivities. The chemicals are then identified via Surface Enhanced Raman Spectroscopy. The aqueous sample to be analyzed is hydrodynamically focused by two side streams containing SERS-active nanoparticles. As different species diffuse away from the focused mixture stream at different rates, they can be detected through SERS at different locations along the channel. By interrogating cross-sections of the channel, the diffusion profiles of the chemicals are detected. A potential application is the detection of specific trace nutrients or narcotics in biological fluids.

KEYWORDS: Surface Enhanced Raman Spectroscopy, vitamin detection, colloid aggregation, microfluidic separation

INTRODUCTION

Surface Enhanced Raman Spectroscopy (SERS) has been established as a powerful tool for the detection of a variety of chemicals, including illicit substances, explosives, and molecules of biological importance [1], in solution [2] or airborne [3]. However, analysis of complex mixtures is not easily achieved, due to the complexity of the acquired spectra, fluorescence background, and other reasons.

Here, a microfluidic flow focusing device is employed, that focuses a mixture of chemicals by means of two side-streams of suspended SERS-active silver colloids. The analyte molecules diffuse away from the focused stream, and induce the colloids in the side streams to aggregate, providing a strong enhancement of the Raman signal. By interrogating different channel cross-sections along the channel, it is possible not only to trace the diffusion profiles of the different chemical species, but also to detect analytes that would otherwise be "lost" in the bulk mixture.

THEORY

In a microfluidic system with high Péclet numbers (Pé = Lv/D) diffusion of chemical species can be employed as to create well defined concentration gradients along the channel. By the Einstein-Stokes equation, the diffusivity of a chemical species in liquid, at low Reynold's number, is given by $D = k_B T / 6\pi\eta r$, where η is the viscosity of the liquid, and r the radius of the molecule. Thus, spatial separation of chemical species concentrations can be achieved, proportional to the inverse square root of the ratio of their radii.

Raman spectroscopy takes advantage of inelastic collisions of light with the molecular structure, to provide spectral signatures that uniquely identify chemicals. In SERS, plasmonic surfaces of high curvature, such as silver nanoparticles, are used to create a locally enhanced electric field, increasing the Raman scattering of molecules in their vicinity [4]. The enhancement compared to traditional Raman spectroscopy is so great that it is claimed to be able to provide single molecule detection [5]. The magnitude of enhancement is believed to be related to the degree of aggregation of the SERS-active particles, with the colloid dimer providing the highest scattering cross-section [5]. Aggregation occurs with a rate constant k, which was shown to depend on the adsorbate concentration m as follows:

$$k = k_0 e^{-V_0/(k_B T (1+\beta m)^{12/5})} \quad (1)$$

where k_0 is the maximum rate, V_0 is the colloid potential, and β is a temperature dependent constant [6].

EXPERIMENTAL

The hydrodynamic flow focusing microfluidic device was designed as described by Stiles [7] and fabricated using standard PDMS soft lithography procedures, and an SU-8 mold. The focused stream was 5 μm wide, and the microchannel was 25 μm deep throughout its length. Particle Image Velocimetry (PIV) measurements were used to determine the maximum velocity to be 10 mm/s in the center of the focused channel. Figure 1 shows the device with the focused stream indicated by fluorescent microspheres.

Raman spectra where acquired using a LabRAM Aramis by Horiba. A 633 nm, 3.8 mW laser was used for excitation, through a 50x objective for a 2 μm diameter spot size. The SERS-active nanoparticles were 20 nm diameter silver colloid (*BioPure Silver* from *nanoComposix*), diluted in water 1:100 from stock solution. The analyte mixture for these experiments was 13 μM of niacin mixed with 10 μM of thiamine (both analytical standard grade, *Supelco Inc.*) in 20 μl of phosphate buffered saline (PBS) solution. The ions from the PBS diffuse into the colloid streams inducing aggregation. As the vitamins diffuse outwards, they are enveloped by the aggregates, producing a SERS signal upon interrogation.

Two 1 s duration samples were obtained at every measurement point. Measurements were performed in 1 µm steps along cross-sections of the channel, for every 200 µm intervals along the fluid flow direction. For each cross-section a mapping was obtained as shown in Figure 1 (A & B). The peaks in the spectra signify the presence and identity of molecular species, and the signal intensity provides the degree of aggregation of the colloid at each point. COMSOL simulations of colloid aggregation, shown in Figure 1 (C), corroborate with the data. As a first approximation, the SERS signal was assumed to stem only from colloid dimers, the rate constant k was the same for single colloids as well as aggregates (Eq. 1), and the aggregate diffusivity was neglected. Spectral data processing was undertaken in MATLAB, using the PLS Toolbox from Eigenvector Research Inc.

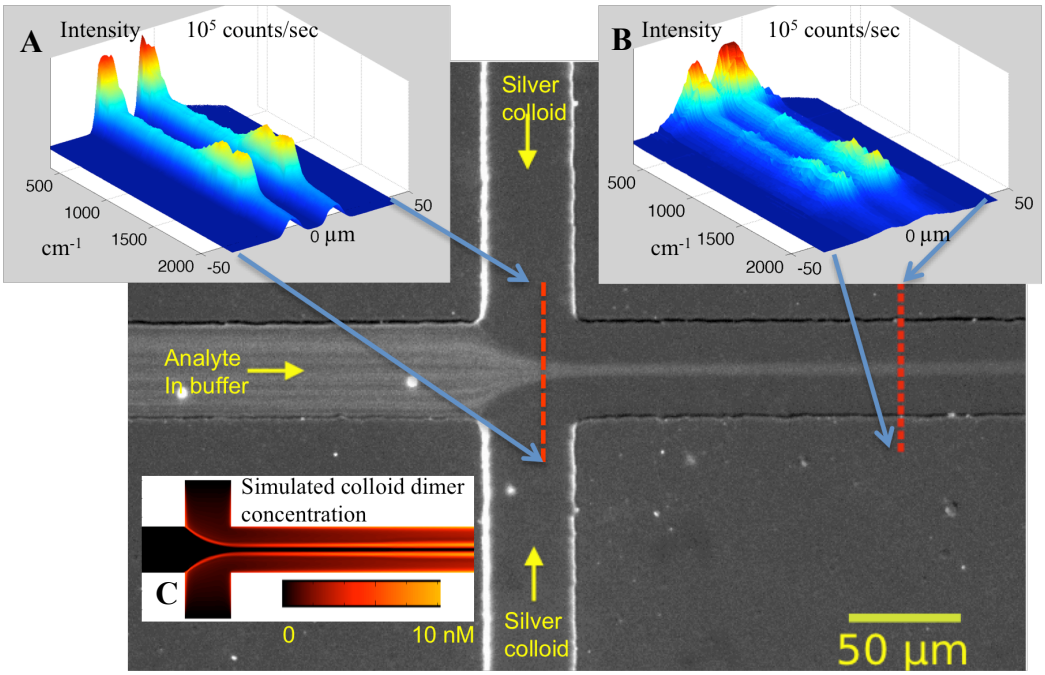

Figure 1: Fluorescent particles demonstrate the flow focusing. Spectral maps at cross-sections at 0 µm (A) and 200 µm (B) from the junction show the chemicals diffusing from the focused stream and the induced aggregation of the colloids. The intensity of the spectra can be correlated against simulations of colloid aggregation kinetics (C).

RESULTS AND DISCUSSION

The flow focusing device enabled the control of the concentration profile of the analytes in the channel. Mappings of spectral data were collected for cross-sections up to 800 µm away from the junction in 200 µm steps. In order to be able to identify the detected chemicals, sample spectra were collected in a separate control experiment by interrogating droplets of solution on a silicon wafer. The SERS signatures of the two vitamins acquired are shown in Figure 2.

This device also enables the study of aggregation kinetics of the silver colloids. By correlating concentration of each species with the intensity of the Raman spectrum a quantitative relation can be obtained between the concentration of the chemical and the degree of aggregation, thus deriving the unknown parameters from Eq. 1. The deduced concentration of colloid dimers is shown in Figure 3.

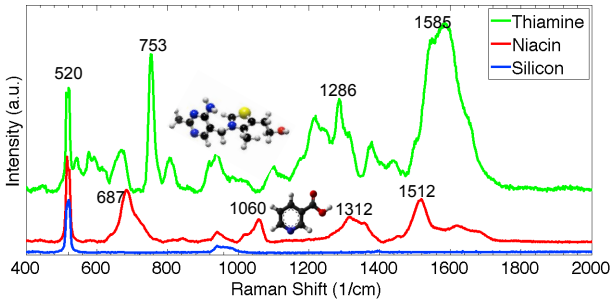

Figure 2: Surface enhanced Raman spectra for thiamine and niacin solutions on silicon. The specified peaks can be used to identify each of the chemicals. The silicon spectrum is also provided for comparison.

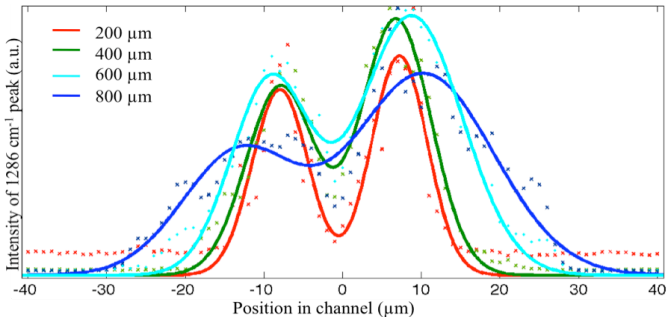

Figure 3: By plotting the intensity of a thiamine peak (1286 cm^{-1}) as a function of position across the channel, the diffusion profile of the chemical at different channel cross-sections can be visualized, here fitted using Gaussians.

As niacin is a smaller molecule than thiamine, it diffuses away from the central stream at a faster rate. This effectively separates some niacin molecules from the mixture, allowing the detection of its SERS signature, as shown in Figure 4. This demonstrates the ability of microfluidic devices operating at high Péclet numbers, to separate mixtures in order to detect traces of fast diffusing molecules.

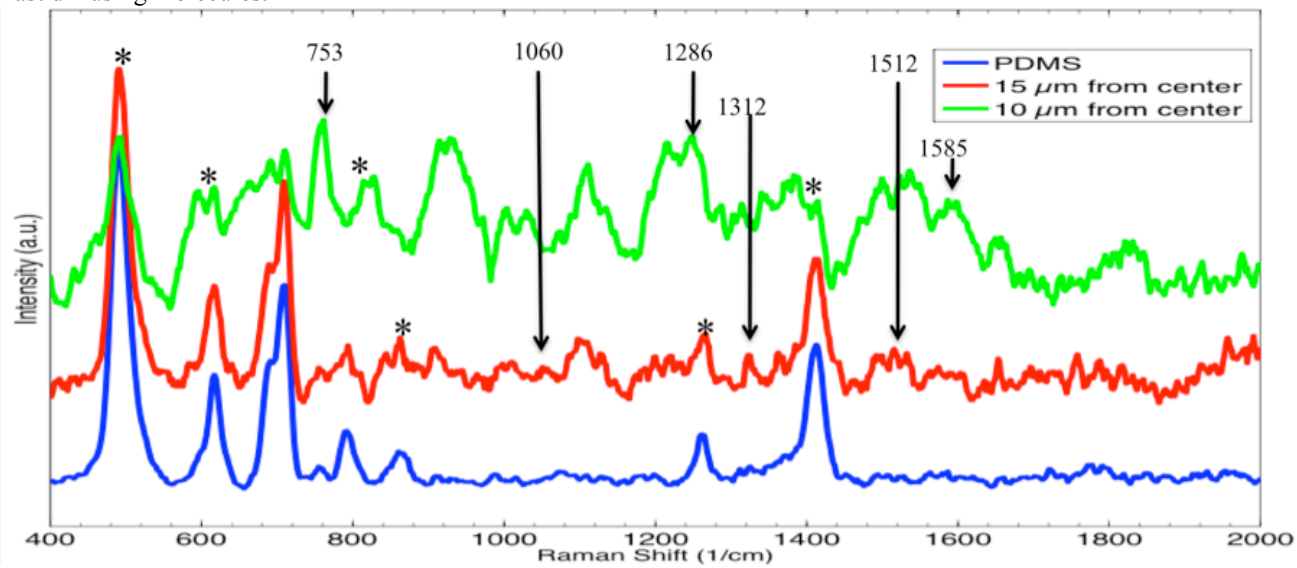

*Figure 4: Spectra obtained at three positions across the channel, 400 μm from the junction. Peaks corresponding to niacin were detected first (red line), further from the center of the focused stream, whereas the ones corresponding to thiamine were detected more towards the focused stream (green line). The spectrum of PDMS is pervasive, and its corresponding peaks are denoted with *.*

CONCLUSION

A hydrodynamic flow focusing microfluidic device was employed to confine a mixture of two vitamins in a focused stream. SERS-active silver nanoparticles in the side streams aggregated in the presence of the chemicals to induce SERS effect. By interrogating cross-sections of the channel, SERS mappings were acquired, allowing for the detection of vitamins and tracking the diffusion profiles of individual species. The correlation between concentration of different chemicals and colloid aggregation can be inferred based on the intensity of SERS signal, enabling the investigation of colloid aggregation kinetics.

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REFERENCES

[1] K. Hering, *et al.*, "SERS: a versatile tool in chemical and biochemical diagnostics" Anal Bioanal Chem, vol. 3902008
[2] X. Qian, and S. Nie, Single-molecule and single-nanoparticle sers: from fundamental mechanisms to biomedical appli cations, Chemical Society Reviews, 2008.
[3] B. D. Piorek, S. J. Lee, J. G. Santiago, M. Moskovits, S. Banerjee, and C. D. Meinhart, Free-surface microfluidic control of surface-enhanced Raman spectroscopy for the optimized detection of airborne molecules, PNAS, 2007
[4] M. Moskovits, Surface roughness and the enhanced intensity of Raman scattering by molecules adsorbed on metals, Journal of Chemical Physics, 1978
[5] M. Moskovits, L. L. Tay, J. Yang, and T. Haslett, SERS and the Single Molecule, Topics in Applied Physics, 2002
[6] M. Moskovits, and B. Vlckova, Adsorbate-induced silver nanoparticle aggregration kinetics, Physical Chemistry B, 2005.
[7] T. Stiles, R. Fallon, T. Vestad, J. Oakley, D. W. M. Marr, J. Squier, and R. Jimenez, Hydrodynamic focusing for vacuum-pumped microfluidics, Microfluid Nanofluid, 2005

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