

Isotachophoresis preconcentration device for improved LOD of common pollutants in drinking water

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Real-time monitoring of common pollutants in drinking water is highly desired, given the important consequences of a contamination event. Coated SERS substrates have been successfully used to detect micromolar concentrations of different anions often present in drinking water. However, the limit of detection still needs to be improved by a factor of 10^4 in the worst case. A microfluidic preconcentration device is fabricated as a possible approach. This device can concentrate and separate the analytes of interest using isotachophoresis (ITP). Fluorescence was used to prove the phenomenon whereas coupling with Raman-related techniques is the method proposed for the final application.

Introduction

Conventional drinking water quality monitoring by the waterworks companies requires sample collection, preparation, and long analysis time in large specialized laboratories. Real-time on-chip monitoring will help minimizing the health and economic consequences associated with water contamination events. Raman spectroscopy based sensors can perform on-line detection of contaminants with high selectivity (fig. 1a). However, given the weak nature of spontaneous Raman scattering, additional techniques are often necessary to improve the sensitivity of these sensors. Surface enhanced Raman spectroscopy (SERS) sensors use metallic nano-structures to confine the electromagnetic fields from the excitation and Raman response in limited regions of the sensing area, known as “hotspots”, resulting in enhancement of the Raman signal intensity by several orders of magnitude [1][2]. In previous experiments, we successfully performed detection of perchlorate [3], nitrite and nitrate anions by using functionalized commercial SERS substrates. Nevertheless, comparison of the LOD obtained for the different analytes with their maximum legal concentration in water (fig. 1b) reveals that additional enhancement of the signal intensity by a factor of 10^1 - 10^4 is still necessary.

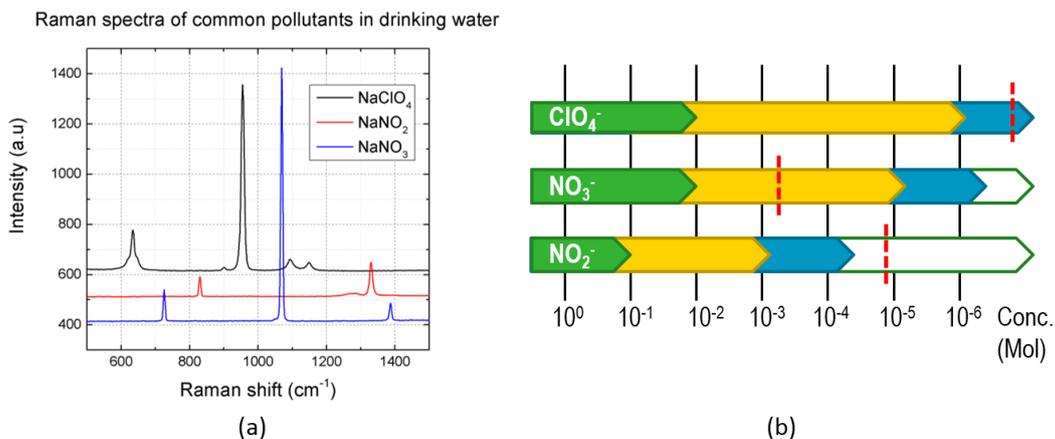


Figure 1 - (a) Raman spectra of common pollutants in drinking water; (b) limit of detection of these pollutants using conventional Raman (green) and SERS (yellow). Extra sensitivity obtained based on the pre-concentration rates achieved with our dzITP concentration device (blue). Red dashed lines indicate the maximum legal concentration.

In this work, a complementary approach is explored, namely the pre-concentration of our analytes of interest using depletion zone isotachopheresis (dzITP) [4]. In this technique, ion concentration polarization (ICP) [6] and isotachopheresis (ITP) phenomena are used to create a depletion region, near which molecules are trapped, concentrated and separated depending on their ion mobility. Focusing is achieved when the velocities of flow due to the electric field in the channel and electromigration from ICP are equal [4]. Preconcentration factors in the order of 10^4 [5] can be achieved. The simplest preconcentration device is composed of two microchannels joined by a perm-selective nanojunction. The process is driven by a voltage drop across the junction, which creates depletion (lack of ions) and enrichment (concentration) in each side of the device [6]. An applied electric field across the channel where the depletion zone is located induces the trapping of molecules at the border of this region. Two methods have been followed to fabricate the perm-selective junction: the “razor blade cut” and the “Nafion® stamp” methods. The devices were fabricated using PDMS due to its fabrication simplicity, although glass is the material proposed for the final application. The characterization was done using fluorescence as it allows real-time visualization and visual control of the events. Furthermore, the direct relation between fluorescence intensity and fluorophore concentration permits the easy quantification of the pre-concentration of the analytes.

Materials and methods

Devices with single (fig. 2) and double (fig. 3) channels were designed, fabricated and characterized. The fabrication of the devices includes four processing steps: (1) mold fabrication, (2) PDMS pouring/curing, (3) creation of the perm-selective junction and (4) irreversible bonding of the PDMS to a substrate. Mold fabrication and PDMS pouring and curing were done using standard soft-lithography procedures [7].

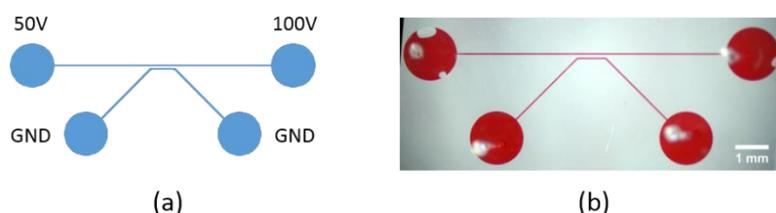


Figure 2 - (a) Single channel device with applied voltages; (b) fabricated device filled with a colorant dye

For the creation of the perm-selective junction, two different approaches were followed. In the first method, a GEM razor blade was used to make a cut, deeper than the channel height, across the different channels [8]. Then, the PDMS was bent and a 10% Nafion® solution drop was introduced in the cut, followed by a curing step at 60°C for 30 min. Finally, scotch tape was used to eliminate the excess of Nafion® in the channels and on the surface of the device. The second method investigated was the Nafion® stamp method [5]. In this method, single channels, defined in a PDMS piece reversibly bonded to the substrates were filled with Nafion®. The PDMS mold was removed leaving a Nafion® channel of the desired dimensions printed on the substrate. 10% and 5% Nafion® solutions were utilized to ease the filling of the PDMS channel. After a 30 min 60°C curing process, the fabricated devices and the substrate with the Nafion® channel printed on it were treated with an oxygen plasma for 45 seconds and put into contact for permanent bonding. Then, the devices were put again in an oven at 60°C for 30 minutes for covalent bond

strengthening. All the devices had microchannels with 50 μm width and 37 μm height. Lower heights were found to be inefficient because of the collapse of the reservoirs.

The devices were tested using an Olympus IXS1 inverted microscope equipped with two light sources: Olympus TH4-2000 (for visible light) and Olympus U-RFL-T (mercury source, for fluorescent light). A LabSmith HVS448 (3000V) high voltage sequencer was used to apply the necessary voltages to operate the devices. The voltage applied across the ITP channel is double than the voltage across the Nafion[®] membrane in order to ensure stability of the ITP phenomena [5]. External gold coated electrodes were used to create the electric connections and syringes and tubes were used to control the flow of liquid into the device inlets. Solutions of fluorescein at different concentrations (100 nM, 1 μM , 10 μM) and PBS 0.1x buffer (13.7 mM NaCl plus other ions in lower concentrations) were used during the first experiments. Captures of the fluorescence intensity near the depletion region were taken at different time intervals and later analyzed using the ImageJ software. The exposure time was adjusted for every experiment depending on the starting concentration of fluorescent (for example, 1s for a μM concentration and 200 ms for a 10 μM concentration). The shutter was closed between snapshots to avoid photobleaching.

Results and discussion

The razor blade cut method was found to be the simplest and most reproducible method, with 70% fabrication yield. A 10-fold pre-concentration rate was initially obtained after 5 minutes. Longer experimental times produced higher pre-concentration rates, obtaining a maximum of 20-fold after 40 minutes (fig. 3c). Here, we expect the pre-concentration rates to be much higher. However, good quantification of the pre-concentration rates could not be accomplished due to saturation of the CCD camera used.

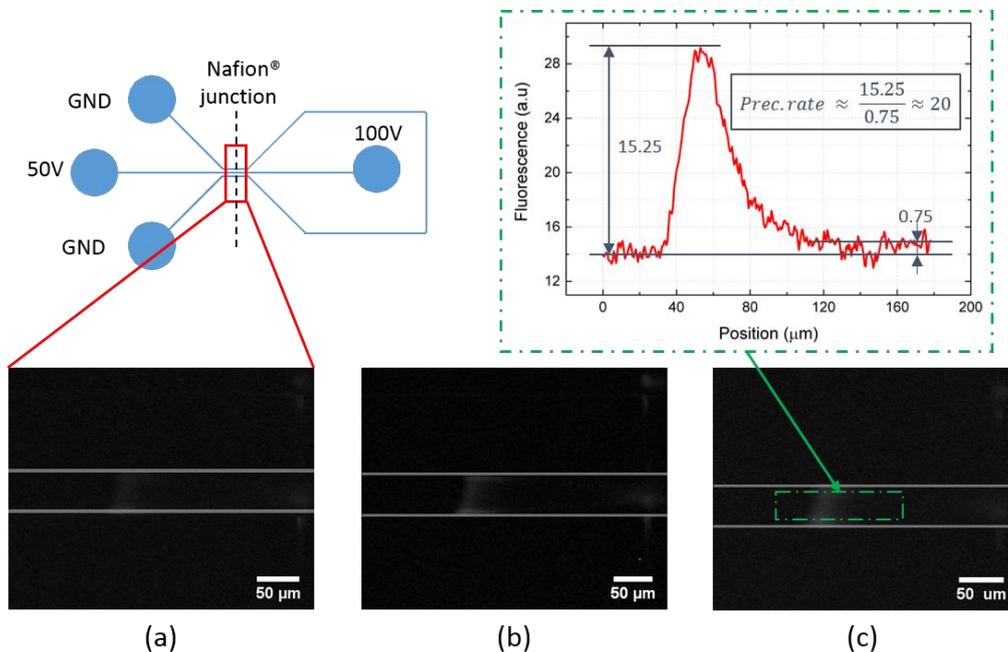


Figure 3 – Double channel device with applied voltages (top) and pre-concentration plug after: (a) 12 minutes, (b) 25 minutes, and (c) 40 minutes.

The Nafion[®] stamp method was expected to produce much better results, according to the literature [5]. This is because of the wider Nafion[®] junction cross-section obtained after the stamping process, what results in higher ion transport through the junction. However, the fabrication of the devices by this method turned out to have much lower yield in comparison to the razor blade method. Moreover, the measured pre-concentration rates were similar to the ones of the devices fabricated by the razor blade cut method (between 10 and 20-folds).

Conclusion and outlook

Successful fabrication and characterization of different dzITP pre-concentration devices was achieved. Preliminary pre-concentration rates of about 20-fold were obtained in most of the experiments. These rates are much lower compared to the ones found in the literature. However, the fabricated devices (combined with SERS substrates) will allow the detection of perchlorate anions with a LOD below the legal limit (fig. 1b). Optimization of the devices is still necessary to increase their pre-concentration rate by a factor of 10^2 - 10^3 and improve the detection of other pollutants with lower Raman cross-section such as nitrite anions.

Several options are being studied in order to obtain devices with a better performance. A higher flow (higher analyte supply to the concentration-plug) together with the application of higher voltages (to keep the depletion zone stable) is expected to increase the pre-concentration factor. Fabrication of higher quality perm-selective junctions, much more repeatable and with enough transversal area of perm-selective material, would also increase the performance. Nafion[®] assisting microchannels [9] and Nafion[®] reservoirs will be explored as possible solutions. The new devices include additional channels, reservoirs and valves [10] to fabricate the Nafion[®] junction in a controlled way.

A proof of concept in PDMS has been obtained. Future experiments will focus in exploring the combination of Raman-related techniques and dzITP for the detection of our analytes of interest. On-chip integration of a glass-made pre-concentration device is our final objective.

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References

- [1] Nuntawong, N.; Eiamchai, P.; Limwichean, S.; Wong-Ek, B. *Forensic science international* 2013, 233(1), 174–178.
- [2] Stiles, P. L.; Dieringer, J. A.; Shah, N. C.; Van Duyne, R. P. *Anal. Chem.* 2008, 1, 601-626.
- [3] Noordam, C.T.N.; Muñoz, P.; Egberink, R.J.M.; Huskens, J.; García Blanco, S.M.; *Proceedings of the 20th Annual Symposium of the IEEE Photonics Society Benelux Chapter*, 2015, 215-218.
- [4] Quist, J.; Janssen, K. G. H.; Vulto, P.; Hankemeier, T.; van der Linden, H. J. *Anal. Chem.* 2011, 83, 7910-7915.
- [5] Lee, J. H.; Song, Y.; Han, J. *LabChip*, 2008, 8, 596-601.
- [6] Kim, S. J.; Song, Y.; Han, J. *Chem. Soc. Rev.* 2010, 39, 912-922.
- [7] Fürjes, P.; Holczer, E.G.; Tóth, E. et al. *Microsyst Technol* (2015) 21: 581.
- [8] Kim, S. J.; Han, J. *Anal. Chem.* 2008, 80, 3507-3511.
- [9] Liu, V.; Song, Y.; Han, J. *Lab Chip*. 2010, 10, 1485-1490.
- [10] Ko, S. H.; Song, Y.; Kim, S. J.; Kim, M.; Han, J.; Kang, K. H. *Lab Chip*, 2012, 12, 4472-4482.