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Surface enhanced Raman spectroscopy in microchip electrophoresis☆

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ABSTRACT

Coupling microchip capillary electrophoresis to surface enhanced Raman spectroscopy (MCE-SERS) combines the high separation power of capillary electrophoresis with the capability to obtain vibrational fingerprint spectra for compound identification. Raman spectroscopy is a structurally descriptive and label-free detection method which is particularly suited for chemical analysis because it is non-destructive and allows the identification of analytes. However, it suffers from poor sensitivity and sometimes even requires acquisition times far longer than the typical peak width of electrophoretic separations. The Raman intensity can be drastically improved if the analyte is brought into close proximity to nanostructured metal surfaces or colloids due to the surface enhancement effect. This paper presents a novel approach in the field of MCE-SERS on-line coupling. The key element of the developed glass microfluidic device is a dosing structure which consists of two side channels joining the MCE channel symmetrically after the electrophoretic separation of the analytes. The dosing channel supplies silver nanoparticles (Ag-NPs), to the separated electrophoretic zones which facilitates an on-the-fly recording of SERS-spectra of the separated compounds. The functionality of the MCE-SERS chip was evaluated by the analysis of a rhodamine model mixture within 90 s achieving RSD of migration times below 1.5%. The approach was successfully applied for the analysis of the food additive riboflavin in a barbecue sauce.

1. Introduction

Raman spectroscopy is a valuable analytical tool for the chemical identification of compounds, based on monitoring vibrational changes in a molecule after inelastic scattering of interacting photons. However, since inelastic scattering is a rare phenomenon (it is estimated that only 1 photon from 10^6 provides Raman signals), Raman spectroscopy suffers from low signal intensity. In the late 1970, it was discovered, that the adsorption of analytes to noble metal nanostructures leads to a significant improvement in sensitivity with enhancement factors of up to 10^6–10^9. Even sensitivities down to the single molecule level have been reported [1,2]. This technique, called surface enhanced Raman spectroscopy (SERS), has gained increased importance in modern analytical chemistry and has also been applied in microfluidics [3–10].

The SERS effect is to a large extend the result of the electromagnetic field occurring at the surface of metal nanostructure after the interaction with photons of suitable energy. The term hot-spot is used for a space in between two (or more) nanostructures, where this electromagnetic field becomes particularly intense leading to higher enhancement factors [8]. In the case of metal nanostructures, firmly embedded in the detection area, hot-spots are achieved by creation of precise (often hierarchical) structures [9]. Approaches based on in-solution nanoparticles (NPs) rely on controlled aggregation, usually initiated by an increase of ionic strength – typically via addition of chlorides (e.g. NaCl or KCl) [8,10].

Despite the fact that an on-line coupled separation step would dramatically increase the sphere of potential applications, the combination of separations technologies with SERS detection has only rarely been applied [4,11]. Challenges preventing SERS as an online analysis tool in separation science is the integration of suitable SERS-substrates and the necessity for rapid data acquisition, especially for real-time monitoring of fast separations like HPLC or capillary electrophoresis (CE). The limited spectra recording time severely restricts the achievable sensitivity.

In one of the first works aiming for CE-SERS coupling the accumulation time was extended up to 1 s by employing a rather low electrical field strength (16 V/cm) [12]. However, this resulted

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in relative long separation times and broad peaks. Online SERS-detection was facilitated straightforwardly by the addition of a silver colloidal solution to the background electrolyte (BGE). This however affected the peak shape and the deposition of silver nanoparticles (NPs) in the detection window posed difficulties for subsequent analyses. For the combination of HPLC with SERS-analysis Zaffino et al. used a post column addition strategy to dose a NPs solution to the effluent [13]. A knitted coil located before the SERS detection window acted as a mixer for NPs and analytes. While mixing was beneficial for the intensity of the SERS signal, it impaired the separation resolution.

The creation of silver spots inside the separation channel from NPs [14] or NPs precursors [15] by laser-induced photo deposition was tested as an alternative approach for CE-SERS and revealed a weakness of firmly embedded nanostructures. Since analytes closely interacted with the created silver spot, severe peak tailing was observed. Significant improvements were achieved by the Schultz group, who proposed a design based on the placement of the silver substrate directly after the separation capillary [16]. Additional liquid was pumped along the capillary outlet focusing separated analytes onto the substrate and providing its refreshment at the same time. In this context, they reported the analysis of biological samples by CE-SERS [17,18] as well as a pioneering work in the field of capillary LC-SERS [19].

Chip based microfluidics facilitates system integration, and shows high potential to combine liquid phase separation and Raman or SERS detection on a single device. Connaster et al. introduced a concept for at-line coupling of microfluidic capillary electrophoresis (MCE) to SERS [20]. The separation was performed in a microfluidic PDMS platform involving a region of three-dimensional clusters of silver. After electrophoretic separation, the voltage driving the separation was stopped and a laser scanned over the silver section. Although this approach is very innovative and allows practically unlimited accumulation time, the scanning is time-consuming and requires sophisticated instrumentation. A challenge with stationary SERS targets is the common irreversible analyte adsorption which prevents consecutive SERS measurements with one target in continuous flow. A solution to that problem could be a recently published approach for electrically assisted regeneration of on-chip SERS substrates [21].

In chip-HPLC, Raman detection was only recently reported by Geissler et al. utilising coherent anti-Stokes Raman scattering [23]. The post column addition of a colloidal silver solution as SERS-substrate has also been realised in chip-HPLC as shown by Taylor et al. They developed a device with a pillar-based column and a post-column diffusion controlled addition of NPs via a side channel for on-line SERS detection [22]. A challenge, in this context, was to deal with the different backpressure of the separation column and the side channel. In electroosmotically driven separations, such as in microchip electrophoresis, such backpressure issues as reported above are of no concern.

Here, we present an approach for a seamless combination of microchip electrophoresis (MCE) and SERS on a single substrate. The proposed design of in-house built MCE glass chip involves two symmetrical side channels for the introduction of silver NPs after the electrophoretic separation. The NPs are mixed with the stream of analyte by radial diffusion thereby limiting a potential risk of zone broadening occurring in turbulence-based mixers. Two (instead of one) side channels ensure faster, homogeneous distribution of NPs across the channel and allow the addition of a second additive (e.g. an aggregation agent) to promote SERS detection. The characteristics and potential of the developed device are investigated using a model mixture of three rhodamines. Finally the system was applied to identify a compound in a complex interfering mixture with a food sample as a real-world application.

Table 1
Potentials in kV applied for injection and MCE separation, respectively.

	SI	SO	BI	BO	S1	S2
Injection	3.0	0	2.0	6.5	Floating	Floating
Separation	3.3	3.3	5.3	0	1.6	1.6

2. Materials and methods

2.1. Microfluidic device fabrication

The microfluidic chips for MCE-SERS analysis were prepared in-house and consisted of two 1 mm thick soda-lime glass slides (76 × 26 mm). Common photolithography followed by a wet-etching procedure and high temperature bonding was performed. For detailed description of the fabrication process and device parameters see the support information. The structure of fabricated device can be divided into four parts according to their functionality: (1) sampling cross for pinched injection, (2) channel for MCE separation, (3) symmetrical junction for introduction of SERS supporting additives, and (4) detection area. The overall design is depicted in Fig. 1.

2.2. MCE with fluorescence and SERS detection

The MCE separations were run in 20 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (Sigma Aldrich, USA) adjusted to pH = 6.5 as the background electrolyte (BGE). For experiments with the rhodamine mixture sample, 10% of isopropanol (Merck, Germany) was added into the BGE. This BGE was freshly prepared before every analysis to avoid changes in isopropanol content due to its evaporation. Between the analyses the chip was flushed with 1 M HNO_3 (Merck, Germany) and 0.1 M NaOH (Sigma Aldrich, USA) to refresh the bare glass surface and to prevent memory effects. During the MCE analysis the microfluidic device was held at potentials given in Table 1 achieving pinched injection of the sample as well as electrophoretic separation. The voltage was provided by two four-channel power supplies (FuG Electronik GmbH, Germany) via six platinum electrodes attached by Teflon screws to a plastic electrode plate.

For MCE analyses with fluorescence detection the microfluidic chip was placed on an IX-70 microscope (Olympus, Japan) equipped with a 40-fold objective (Olympus, Japan) focussed into the channel. A Hg-lamp (HBO 103 W/2, Osram, Germany) was used for sample excitation in combination with an U-MSWB2 filter cube (Olympus, Japan) with λ_{ex}: 420–480 nm, λ_{em} > 520 nm, dichroic mirror: 500 nm. Fluorescence signals were collected by a photomultiplier tube (H9307-03, Hamamatsu, Germany) and recorded via the Clarity software (DataApex, Czech Republic) with a 25 Hz data acquisition rate.

In MCE-SERS experiments, the microfluidic chip was placed on an IX-71 epifluorescence microscope (Olympus, Japan), which was part of a modular confocal Raman system. The Raman instrumentation used a 473 nm laser (50 mW, Cobolt, Sweden) focused in the middle of the chip channel by 40-fold objective (Olympus, Japan). Raman spectra were recorded with an Acton SP2750 monochromator (Princeton Instruments, USA) using a 600 lines/mm grating. The accumulation time was set to 250 ms which enabled more than 7 data points for every analyte zone in the data presented herein.

2.3. Silver nanoparticles

The silver nanoparticles (Ag-NPs) were synthetized according to the Lee-Meisel protocol [24] based on the reduction of silver nitrate (Sigma-Aldrich, USA) by sodium citrate dihydrate (Icommerz, Germany). The obtained colloid suspensions had an

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