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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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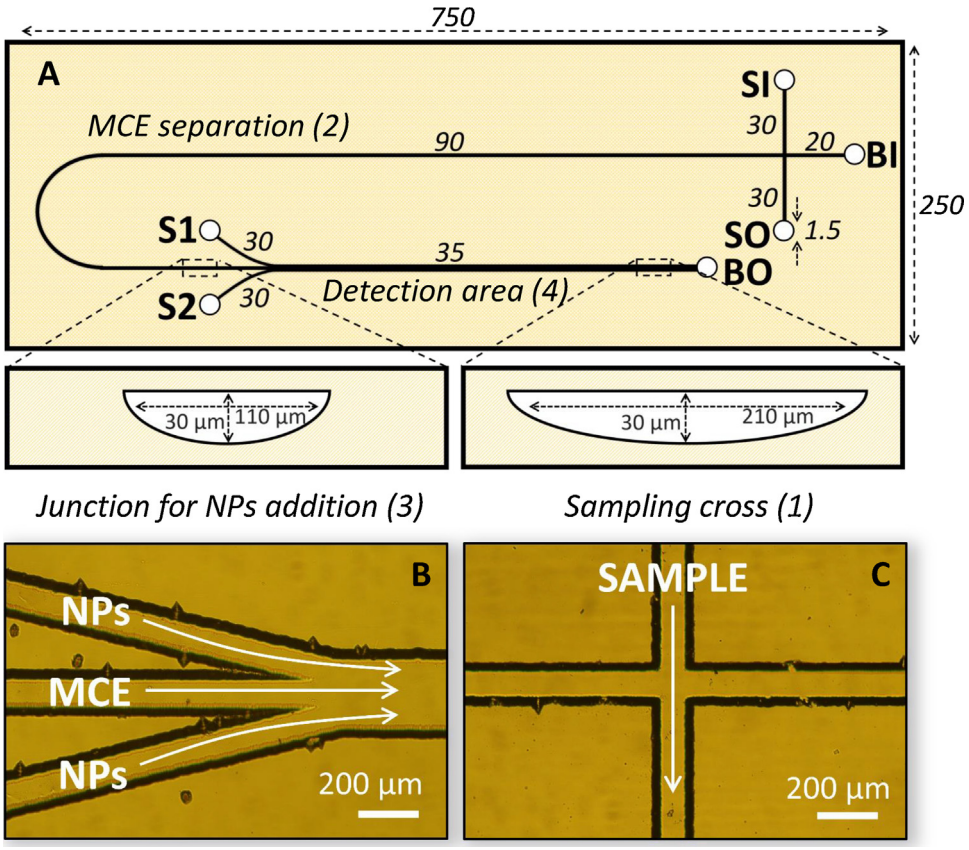

Fig. 1. A – Layout of the fabricated glass chips for MCE-SERS experiments. SI – sample inlet, SO – sample outlet, BI – background electrolyte (BGE) inlet, BO – BGE outlet, S1 and S2 – side channels. The numbers describe the length in mm. The inserted windows depict the channel profiles. B; C – Microscopic images of the dosing structure and the sampling cross, respectively.

absorption maximum at 430 nm and a silver concentration of 0.11 mg/mL.

The repeatability of NPs synthesis was evaluated via UV–vis spectra measurement of three colloid batches. This is an important issue as the shape and size distribution of the silver nanoparticles effects the SERS-signal enhancement. Comparable records with absorption maximum at 430 nm (Fig. S2A) were reached. One of the batches was investigated by scanning electron microscopy (SEM) (Fig. S2B).

In some experiments, aggregation of NPs was initiated by addition of sodium chloride (Carl Roth, Germany). To further concentrate the NPs suspension 1 mL of crude colloid was centrifuged at 13.000 rpm for 10 min (Galaxy 14D, VWR™, USA). The volume of removed supernatant was calculated with respect to the desired concentration factor. Concentrated NPs were redispersed in 100 mM MOPS (pH 6.5) and deionized water to a reach final buffer concentration of 20 mM. In case of the analysis of the rhodamine mixture, isopropanol was added to the colloid for achieving its 10% (v/v) concentration.

2.4. Chemicals

Stock solutions of rhodamine 6G (Rh 6G), rhodamine 123 (Rh 123) and rhodamine 110 (Rh 110) in concentration of 0.1 mM were prepared by dissolving in 20 mM MOPS with 10% (v/v) content of isopropanol. All three rhodamines were purchased from Sigma-Aldrich, USA. The rhodamine analyte mixture was prepared by diluting the stock solutions in BGE to a concentration of 10 µM for Rh 123 and Rh 110 and 30 µM for Rh 6G. To prepare a stock solution of riboflavin (Sigma Aldrich, USA), it was dissolved in 10 mM NaOH to obtain a riboflavin concentration of 1 mM. This stock solution was freshly prepared every week. Working solutions were obtained by dilution in BGE to a concentration of 50 µM. All stock solutions were stored in darkness at room temperature.

A sample of barbeque sauce (American Sandwich Sauce, H.J. Heinz GmbH, Germany) was prepared by dispersion of 1 g of the sauce in 1 mL of 5 mM NaOH by ultrasonification. The obtained dispersion was centrifuged in a disposable plastic vial at 13 000 rpm for 7 min resulting in three-layer system (see Fig. S3). The solution of light yellow colour from the middle layer was removed by a pipette. Prior MCE analysis the solution was filtered through disposable filters of 0.22 µm pore size (Restek GmbH, Germany) and diluted threefold using deionized water. The detailed information on the ingredients of the sauce are provided in the supporting information.

3. Results and discussion

This work aims at the development of a microfluidic approach to combine electrophoretic separation and surface enhanced Raman spectroscopy on a single device. This should combine the high separation power of chip electrophoresis and the capabilities of SERS as a vibrational spectroscopic technique for compound identification. For this purpose, we designed and fabricated glass chips with a symmetrical junction at the end of the separation channel for the post column addition of silver colloid solutions – the so-called dosing structure (see Fig. 1). This junction was implemented to decouple the electrophoretic separation and the SERS-detection area. This design keeps the separation channel free of NPs which prevents negative effects on the electrophoretic separation such as NP precipitation and related unwanted analyte interaction with the precipitates. The microfluidic element consists of two side channels

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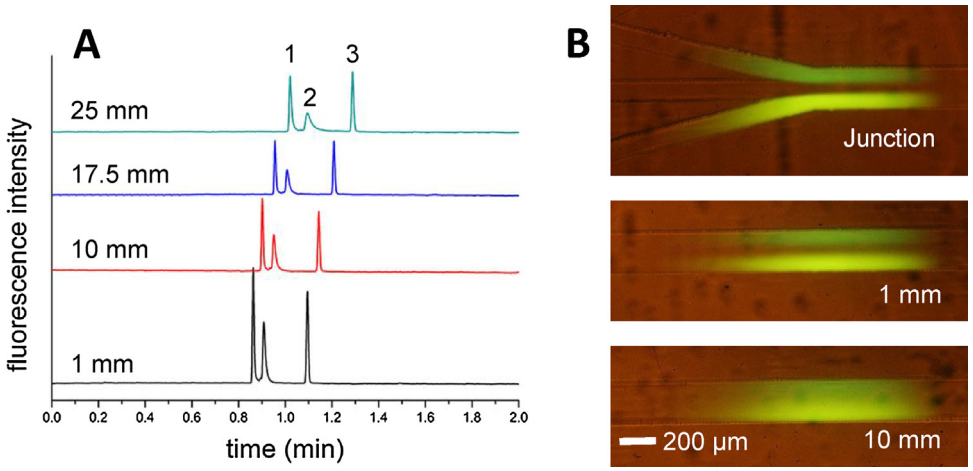

Fig. 2. A – MCE separation of (1) rhodamine 123, (2) rhodamine 6G and (3) rhodamine 110 with fluorescence detection at four different detection points (1, 10, 17.5 and 25 mm) after the junction. Addition of BGE via the dosing junction. B – Microscopic pictures of the streams inside the developed microfluidic chip using fluorescent coloured solutions.

joining the MCE channel symmetrically from both sides. The design of two side channels was developed to reach fast homogeneous distribution of NPs across the channel and to open the possibility for the addition of further reagents. After the junction (i.e. in the detection area), the channel was slightly broadened to eliminate speeding up of the MCE flow due to addition of further solutions from the sides.

3.1. Positioning of detection window

A prerequisite for achieving significant SERS signals of the electrophoretically separated compounds is a close contact of the NPs and the respective analytes. The implementation of mixer structures is an effective way of bringing NPs introduced after the separation column in close proximity to the analyte [5,25]. Unfortunately, fast mixers based on turbulent flow would lead to a loss of separation efficiency [13], eliminating one of the biggest upsides of MCE. Diffusion-based mixers are a far more promising alternative [22,26]. Their function is based on leading two streams along and driving them together just by Brownian motion. Since diffusion is a time dependent phenomenon, precise positioning of the detection window is necessary. In the proposed MCE-SERS system, the mixing of three streams is required. The middle flow is the eluate of the electrophoresis channel flanked by two lateral flows with the added silver colloid solutions.

Although the addition of NPs is vital for the surface enhancement effect, the side flows can lead to changes in peak shape and/or intensities. To investigate the influence of the side flows four detection windows were chosen in the distance of 1, 10, 17.5 and 25 mm after the junction. In a series of MCE experiments, the effect was studied applying fluorescence microscopic imaging and fluorescence detection. To fully concentrate just on the influence of the side flows and to eliminate the factor of NPs-analyte interaction, both side channels were filled with BGE in these experiments.

Due to their potential for both, fluorescence and SERS detection, a test mixture of three rhodamines (Rh 123, Rh 6G and Rh 110) was chosen for investigation of the microfluidic device properties. At the chosen pH (pH = 6.5) Rh 123 and Rh 6G were positively charged, whereas Rh 110 had a neutral net charge and was therefore also used as a marker for the electroosmotic flow.

The data from the MCE separations revealed a significant drop in intensity at the shift from the 1 mm to the 10 mm position (the observed drop was approximately 35% of the peak height). Whereas, at further points the intensity remained practically unchanged (Fig. 2A). This implies that the analytes diffused completely to the sides of the channel within the first 10 mm. This conclusion was confirmed by microscopic observations. Two organic dyes were used for fluorescent colouring of BGE in the vials at the ends of both side channels (labelled S1 and S2 in Fig. 1). The rest of the chip was filled with BGE without any additives. By running voltage conditions corresponding to previous MCE experiments, the solutions from the S1 and S2 vials were guided towards the junction and merged with the electrolyte stream originating from electrophoresis. The fluorescence microscopic images at the junction and of the channel in the distances of 1 and 10 mm after the junction clearly showed the difference in the concentration distribution (Fig. 2B). Whereas directly at the junction and at the distance of 1 mm all three streams were still apparent, at the distance of 10 mm the fluorescent compound was already spread evenly across the centre of the channel.

Besides the intensity drop observed at the shift of the detection window, a negative influence on the peak width was also observed. This is the most apparent in the case of Rh 6G, where the shift of detection window from 1 mm to 25 mm resulted in a decrease of theoretical plates from 28 000 to 10 000. For the first migrating peak (i.e. Rh 123) the separation efficiency was lowered from 60 000 to 50 000. The peak broadening can be attributed to axial diffusion with possible additional contributions of analyte-wall interactions.

3.2. MCE-SERS experiments

In previous studies we utilised the bright fluorescence of the compounds for convenient and sensitive imaging and detection. The information content of SERS measurements is however much higher as respective fingerprint vibrational spectra can be used for compound identification. Another benefit of Raman-detection compared to fluorescence is that it is, in principle, a label-free method.

With respect to the results from chapter 3.1, very close positioning of the detection window to the junction would be beneficial with regard to the detection sensitivity and peak shape of the separated bands. However, from the microscopic image of the streams (in Fig. 2B) it is apparent that the position at 1 mm after the junction would be not sufficient to allow a diffusion of NPs into the MCE flow. Therefore, the detection point for MCE-SERS experiments was placed at 10 mm after the junction.

Prior to MCE-SERS experiments, the interaction of rhodamines with silver colloidal solutions (Rh 6G, 123 and 110) was briefly

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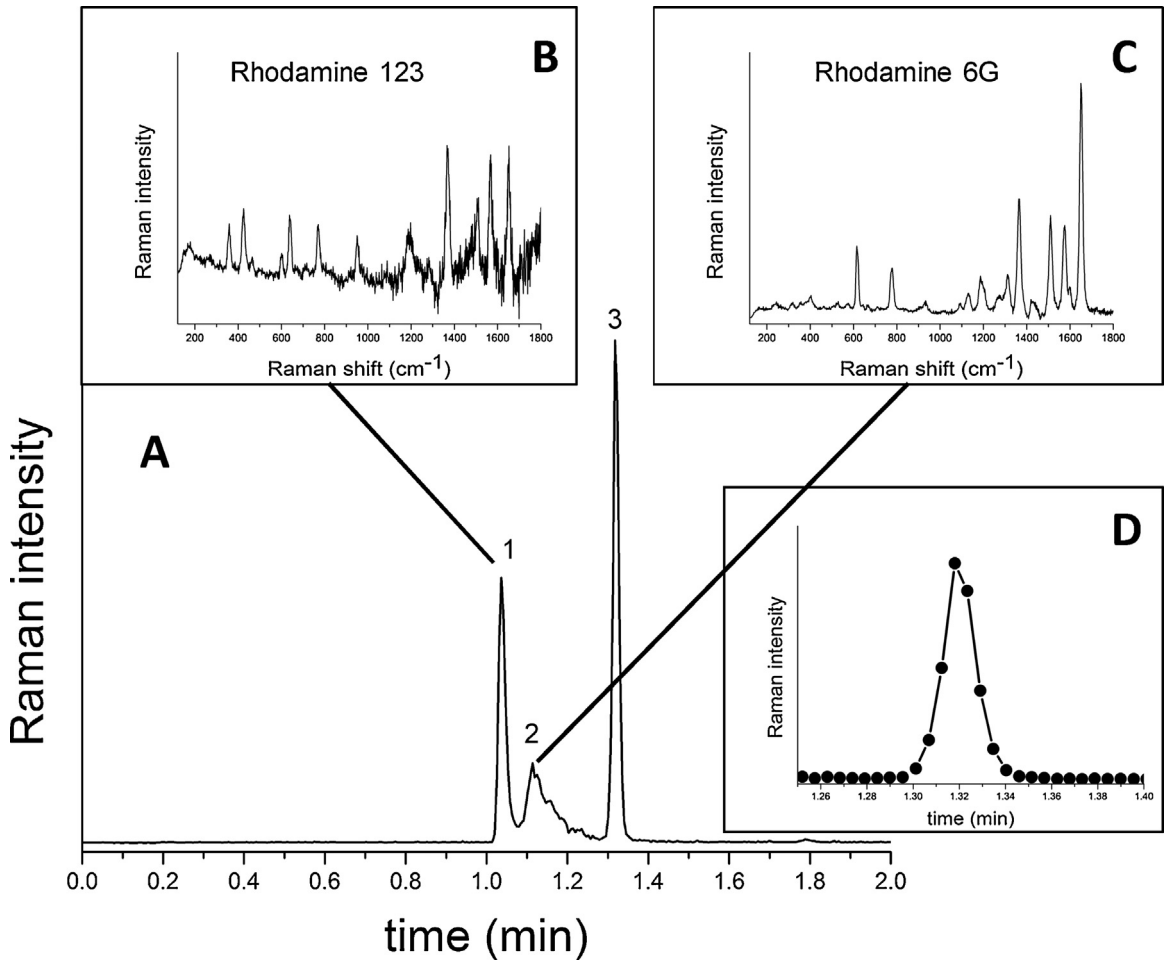

Fig. 3. A – MCE separation of (1) rhodamine 123, (2) rhodamine 6G and (3) rhodamine 110 with SERS detection (indicator band: 1650 cm^{-1}). The detection point was localized 10 mm after the junction. Ag-NPs and NaCl solution were dosed via the junction. B – Baseline corrected SERS spectra of rhodamine 123 taken from the peak maxima. C – Baseline corrected SERS spectra of rhodamine 6G taken from the peak maxima. D – In the inserted window the sampling frequency of data collection is depicted.

tested by microscopic observations – see Fig. S4. All three rhodamines were separately added into the concentrated silver colloid. It was found, that at these conditions the positively charged analytes (Rh 6G and Rh 123) initiated NPs aggregation implying an affinity towards the Ag-NPs. On contrary, the presence of the neutrally charged Rh 110 had no effect on the overall colloid appearance. This might be explained by its neutral charge which significantly decreases the affinity towards negatively charged NPs and thus limiting aggregation.

The rhodamine mixture was used as a model sample for investigations of the characteristics of the developed MCE-SERS microfluidic device. Both side channels were filled with Ag-NPs with 0.1 mg/mL content of silver. Surprisingly, although the distance of 10 mm was proven to be sufficient for the diffusion of the analyte and NPs streams towards each other, the overall results were quite disappointing. From the three used rhodamines only Rh 6G provided a very weak Raman spectrum, whereas Rh 123 and Rh 110 showed fluorescence only. For the results see Fig. S5.

In SERS the highest signal enhancement can be achieved in places called hot-spots occurring between two or more NPs [8]. In order to promote the aggregation of the nanoparticles the addition of chlorides was therefore tested as a potential way for SERS signal improvement. In this experiment, one of the side channels was used for the introduction of NPs, whereas the second one was used for supplying a 100 mM NaCl solution. The data obtained are shown in Fig. 3A following the intensity of the Raman shift signal at 1650 cm^{-1}, which corresponds to C—C stretching mode for aromatic system occurring in all three presented rhodamines [8].

The addition of chlorides to the solution significantly enhanced the intensity of the Raman signal. Although all three rhodamines still provided a relatively strong fluorescence background (see Fig. S5), both positively charged analytes (Rh 123 and Rh 6G) showed clear Raman bands (Fig. 3B and C). From these observations we concluded, that the most promising results might be achieved by simultaneous addition of NPs and chlorides through the two side channels and by positioning the detection window 10 mm after the junction. The device was then evaluated for repeatability in a series of repetitive MCE-SERS analyses. The results were quite promising reaching RSDs of migration times in the range of 0.9–1.3% (n = 7). The RSD of Raman band intensities was determined to be 15–20% (n = 7). This rather high RSD of the Raman signals can be attributed to the fact, that the chlorides-induced formation of NPs aggregates is to certain extend a random phenomenon, resulting in variable enhancement strength.

The data acquisition time of the Raman signal affects the achievable sensitivity of the MCE-SERS measurement. In order to describe a chromatographic or electrophoretic peak, at least seven data points are required. To achieve that in our Raman detection set-up an accumulation time of 250 ms was chosen, reflecting a frequency of 4 Hz, which ensures at least seven data points per analyte zone. The sampling frequency of data is depicted in the inserted window in Fig. 3D. Much higher data acquisition rates can be achieved with

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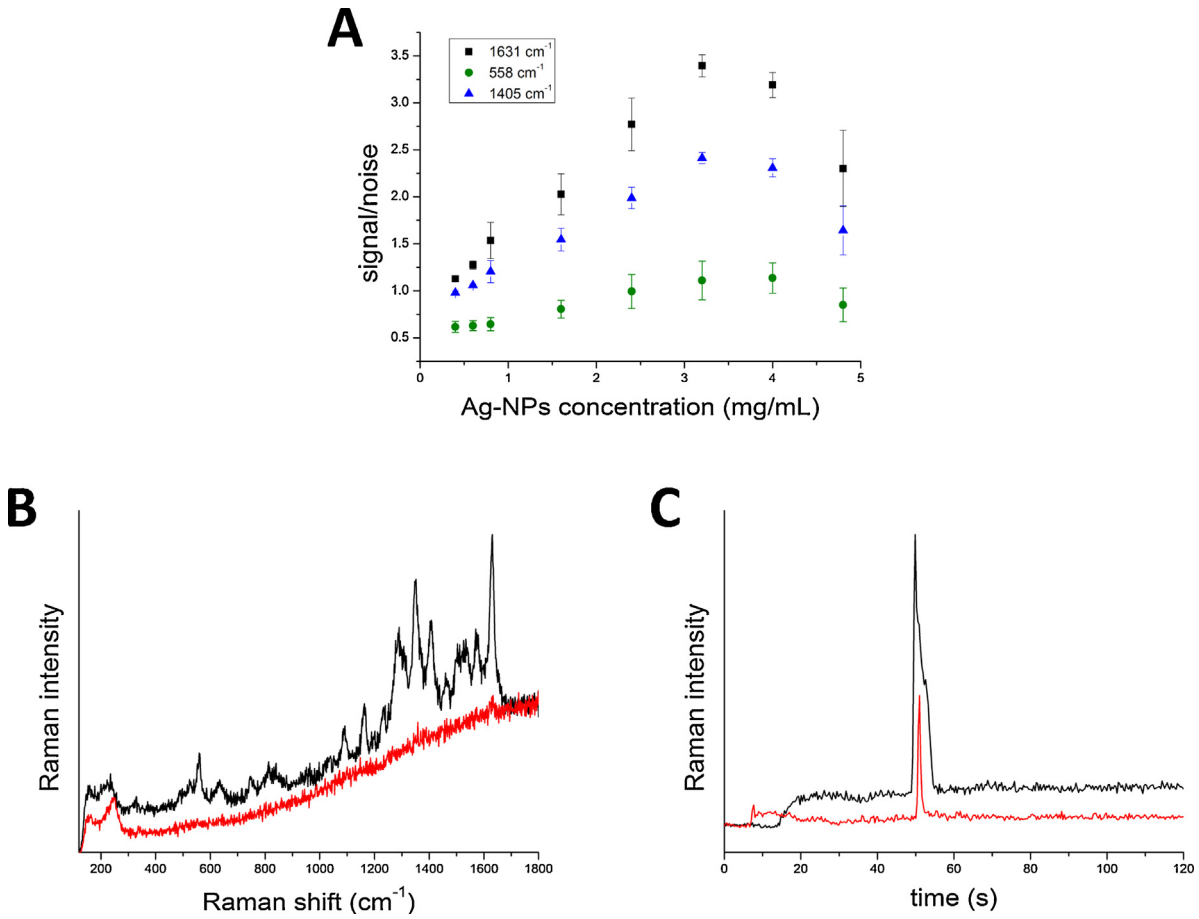

Fig. 4. A – Influence of silver colloid concentration on riboflavin (50 μM) evaluated as S/N value for three selected Raman bands. The error bars represent standard deviations (n = 3). B, C – Investigation of the influence of chlorides on the SERS signal of riboflavin. MCE analysis with SERS detection of a riboflavin standard solution (50 μM) with the addition of NaCl (red) and without addition of NaCl (black) via the side channels. B – SERS spectra taken from the peak maxima. C – MCE electropherograms with SERS detection of riboflavin standard solution (indicator band: 1631 cm^{-1}). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fluorescence detection, where we utilised 25 Hz in the respective experiments.

It should be mentioned, that higher colloid concentration (more than 0.1 mg/mL of silver) were investigated for the analysis of rhodamines, too (Fig. S6). Despite the fact, that higher silver concentration led to an improved sensitivity, it resulted in extreme broadening of the Rh 6G peak, too, severely affecting separation resolution. This broadening can be attributed to the adsorption of Rh 6G to the surface and especially to the Ag-NPs [8,27] which can precipitate and accumulate at the channel walls. Whereas Rh 123 and Rh 110 adsorbed at the NPs reversibly or just flowed along the NPs, Rh 6G interacted more strongly with the NPs and induced peak broadening. Therefore, a concentration of 0.1 mg/mL of NPs was determined as the most appropriate for MCE-SERS analysis of the chosen model application.

3.3. MCE-SERS analysis of riboflavin

After we could successfully prove the performance of the approach with a rather simple model application, we evaluated the device for the analysis of a compound in a complex mixture which would usually require sample work-up procedures prior to SERS-analyses.

Riboflavin (vitamin B2) is a water-soluble compound belonging to an essential part of the human diet with a recommended daily intake of 1.0–1.4 mg [28]. Although it is naturally present in many sources (e.g. milk, egg, nuts, fish, beef, etc.), it is a common part of many dietary supplements. In food industry it also represents a popular natural yellow-green food colorant. Therefore, the reliable determination of riboflavin is an important aspect of food and drug quality control. Several methods were used for its analysis in the past – fluorescence [12,29], electroanalytical methods [30], UV–vis [19], and mass spectrometry [28] are the most frequent ones. Unfortunately, these techniques provide only unselective signals or require expensive instrumentation. In contrast, SERS, due to its fingerprint information, allows precise analyte identification without any need for complicated instrumentation. Moreover, the narrow bands enable to a certain extend the analysis of more features at the same time [6,31] and/or the possibility to identify analytes from a simple matrix [32]. Despite this fact, samples of more complex or interfering matrix require separation before SERS investigation. This is demonstrated herein by an illustrative example analysing riboflavin in a barbeque sauce. This part of the work does not aim on riboflavin quantification, but stresses the importance of a separation step prior to SERS detection.

Whereas in the case of the analysis of the rhodamine mixture, colloids containing more than 0.1 mg/mL of silver resulted in the loss of the separation resolution, for riboflavin it brought a steep increase of SERS response without any significant peak broadening (Fig. S7). Therefore, colloidal silver concentrations lying in the range of 0.4–4.8 mg/mL were systematically investigated for the MCE-SERS analysis of riboflavin. The results revealed a nearly linear signal increase up to a concentration of 3.2 mg/mL, followed by a signal drop at higher concentrations (Fig. 4A). We attributed

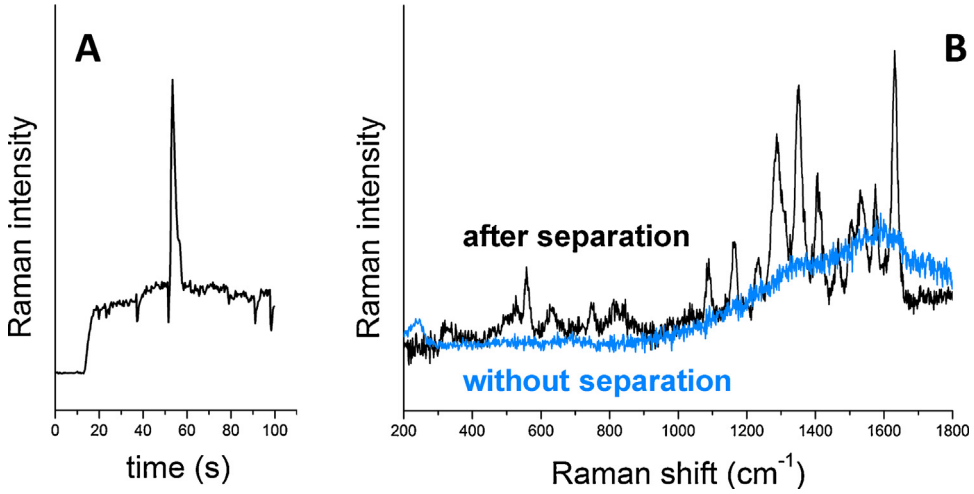

Fig. 5. A – MCE separation with SERS detection of riboflavin in BBQ sauce extract (indicator band: 1631 cm^{-1}). B – SERS spectrum of the direct analysis of the barbeque sauce extract with matrix (blue trace). SERS spectrum of the MCE separation of riboflavin in BBQ sauce extract corresponding to the data point from the maximum of the riboflavin zone (black trace). Data were baseline corrected. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

this intensity drop to a removal of stabilizing citrate from the colloid (due to the concentration step) leading to too high levels of NPs aggregation. Based on these observations, a colloid mixture with 3.2 mg/mL of silver was used for further experiments with riboflavin. In a series of experiments the RSD of migration time was determined to be below 0.5% (n = 5) and the limit of detection (LOD) was at the level of 1 μM.

The standard solution of riboflavin was analysed by MCE-SERS using the developed microfluidic chip with the two side channels design for supplying NPs and chlorides as optimized in the previous section. To our surprise, the addition of chlorides dramatically decreased the SERS signals of riboflavin. This unexpected phenomenon is documented in Fig. 4B and C, where a zone of riboflavin was analysed by MCE-SERS without (black line) and with the addition of chlorides (red line) supplied via one of the side channels. Whereas the addition of Ag-NPs alone provided clear SERS spectra (Fig. 4B), the addition of chlorides completely diminished Raman bands and the recorded electropherogram (Fig. 4C) was to a large extend the result of fluorescence. The influence of iodides, chlorides, and bromides on the riboflavin SERS spectra were studied in detail by Liu et al., who observed significant intensity drops and slight changes in Raman shifts for all three tested halides [33]. This behaviour was attributed to competitive adsorption between halides and NPs. Due to this fact in this work, both side channels were used for supplying silver nanoparticles only and chlorides were omitted from experiments with riboflavin.

After this method development Riboflavin was analysed from a sample of barbeque sauce. In the sauce, riboflavin is used as a yellow-green food colorant. It is important to highlight, that besides other ingredients (see support information) sodium chloride was present in the sauce, too.

A direct SERS analysis of the barbeque sauce without prior separation showed that the matrix severely hampers the spectral analysis as shown in Fig. 5B, blue trace. The respective analysis in our MCE-SERS chip revealed the high potential of such a combined separation and SERS device. During the electrophoretic separation, the analyte was effectively separated from the interfering matrix. This resulted in a clear SERS spectrum of the electrophoretic peak (Fig. 5A) as shown in (Fig. 5B, black spectrum).

This very simple example demonstrates the usefulness of the MCE separation step prior to SERS analysis for samples with interfering matrix. This approach should also be advantageous for other complex matrixes containing halides such as urinary samples [34].

4. Concluding remarks

The introduced glass device seamlessly integrates an electrophoresis channel and a downstream SERS-detection channel for the online acquisition of Raman spectra in real time. The design of the device is based on two side channels supplying a nanoparticle solution and an optional aggregation agent to the effluent from the electrophoresis channel flow. As the mixing of all three streams is driven by diffusion only, this reduces peak broadening and loss of separation resolution, compared to turbulent mixers. The integration of all fluidic channels on a single chip made the technical realisation relatively straightforward compared to more traditional approaches using connection tubing, and respective fittings and interconnections.

The integrated electrophoresis-SERS chip was characterized via analysis of a mixture of three rhodamines. The correct positioning of the detection window as well as an optimised colloid concentration proved essential for the proper functionality of the device. The potential of such a device for the SERS analysis of compounds in complex mixtures was evidenced with the analysis of the food colorant riboflavin in barbeque sauce.

Despite the fact, that the device provided very reliable RSDs of migration times (below 1.5%), there is still potential for sensitivity improvement. To boost sensitivity, in future work we would like to focus on the implementation of a concentration step (e.g. field amplified stacking or transient isotachophoresis) and/or designing the device with on-chip synthesis of NPs [35].

Conflict of interest

The authors have declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.chroma.2018.02.014.

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