Electronic Supplementary Information (ESI)

Novel SERS-based process analysis for label-free segmented flow screenings

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Operational time of the SERS measurement

Table 1: Operational time of each step for the SERS measurement of 300 spots.

Step	Time
Background measurement	6 min (500 ms integration time)
Droplet spotting	3 min
Drying of the spot at RT	1 h (500 nL aqueous segments)
SERS measurement	6 min (500 ms integration time)

Preparation of the glass wafer

For the SERS array a glass wafer (Borofloat® 33) with the dimension 120 x 60 x 1.1 mm^3 was used. The glass wafer is activated by piranha solution to form hydroxy groups on the glass surface. The activated glass wafer is then inserted in a solution of 5 mM octadecyltrichlorosilane in heptane for 10 hours to make the entire glass surface hydrophobic. The hydrophilic spots on the glass wafer are created afterwards by laser ablation using a pulsed laser (10 ps) at a wavelength of 1064 nm. In this process step the surface of the glass is ablated, which ends in a cavity. The depth of the cavity can be varied from 5 µm up to 1 mm. The hydrophilic spots can then be wetted with an aqueous acrylamide monomer mixture.

Polymer composition

Table 2: Composition of the SERS polymer.

Component	Function	c [mol/kg solvent]
Silver nitrate	SERS activity	4.00×10^{-2}
Acrylamide	Matrix	4.20
N,N'-diallyltartramide (DATD)	Crosslinker	7.00×10^{-2}
2-hydroxy-2-methylpropiophenone	Photoinitiator	7.00×10^{-1}

Double distilled water and diethylene glycol were used as solvent in a 1:2 mixture. The total concentration of acrylamide in the polymer is 31.6%. The concentration of the crosslinker is 5% relating to the total concentration acrylamide.

Calibration of the segment composition

Table 3: Flowrates of the experimental setup and concentrations in the syringes

Syringe	Flow [µL/min]	Content
1	40 - 0	Demineralized water
2	0 - 40	Sodium nitrite 1.2 M (Basic Blue for calibration)
3	80	Adenine (1.5 mM) and acetic acid (1.1 M) (demineralized water for calibration)
4	200	Novec7500 + 2% Pico-Surf™

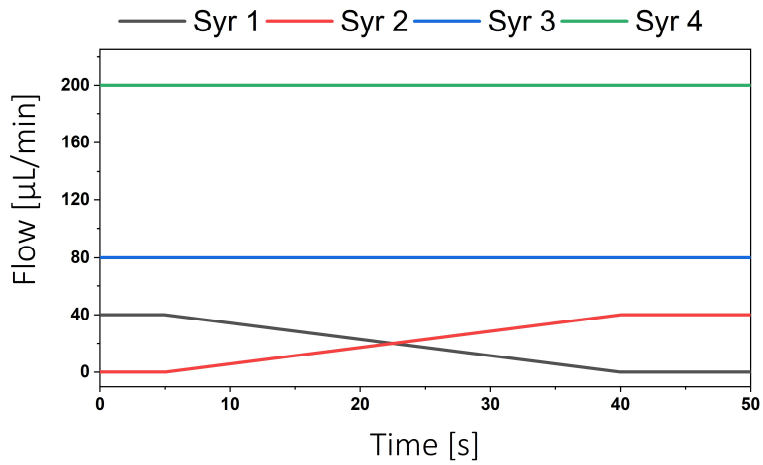

Fig. 1: Gradient scheme of the flow rates of all syringes over time for droplet generation.

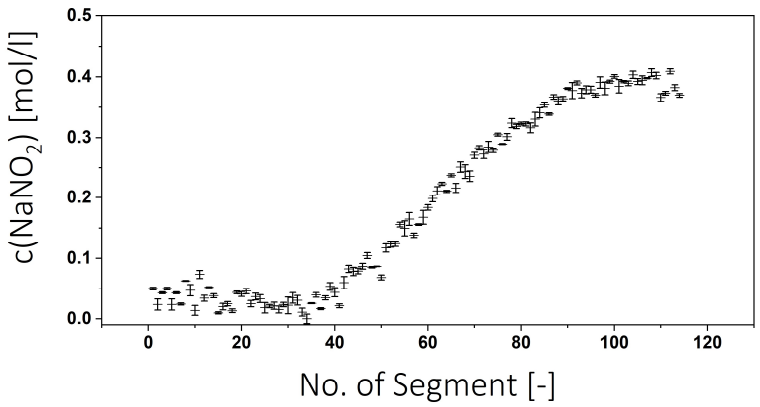

Fig. 2: Calibration curve: Mean of three measurements. The ratio of the nitrite concentration inside the segments is displayed. Error bars show the standard deviation of 3 repeated runs.

UV/Vis reflection measurement

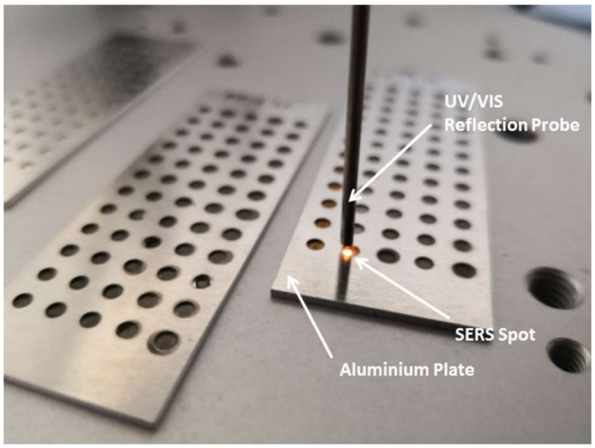

Fig. 3: Photograph of the setup for the UV/VIS measurement. A spectrometer from OceanOptics Inc. (USB 2000) with a reflection probe and a deuterium/halogen light source from Avantes BV was used.

Long-term performance

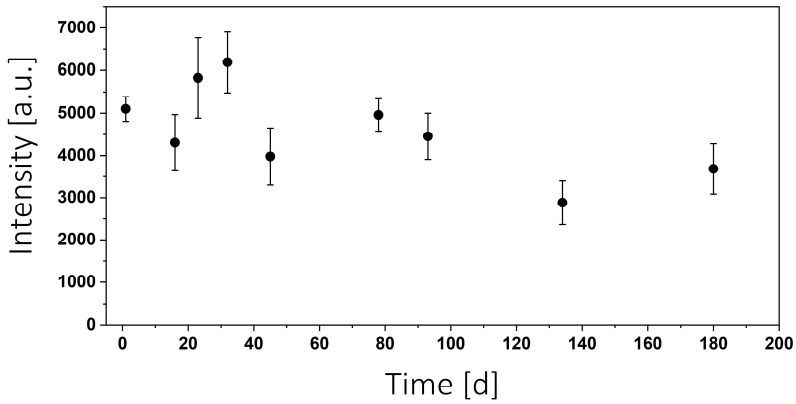

Fig. 4: Tracking Raman intensity at 735 cm $^{-1}$ of 1 µl of 100 µM adenine solution over a period of 180 days. The error bars show the standard deviation of 5 spots, each measured at 5 positions.

Raman peak shift during deamination

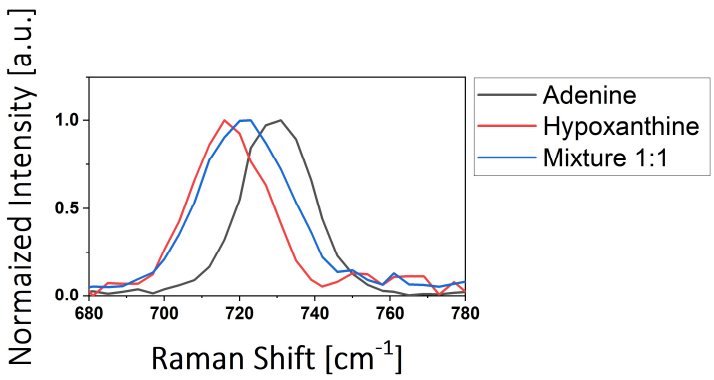

Fig. 5: Raman spectra of adenine, hypoxanthine and a mixture of both showing the shift of the ring breathing mode.

Multivariate Calibration

Unscrambler® (Camo Analytics) was used for multivariate calibration. Partial least square regression (PLS) models were set up using a calibration set (180 concentrations) of mixtures of adenine and hypoxanthine. A test set was used for validation (20 concentrations). Mixtures were prepared using the micro segmented flow setup. Different pretreatment of the spectra was examined. The transformed spectra are shown in Fig. 6. Baseline correction (BL), first derivative (dx/dy) and standard normal variate (SNV) correction were used. The results of the calibration are shown in Table 4. Using BL and SNV turned out to be the best pretreatment. Since the spectra are normalized the influence of the peak shift increases, which leads to better correlation.

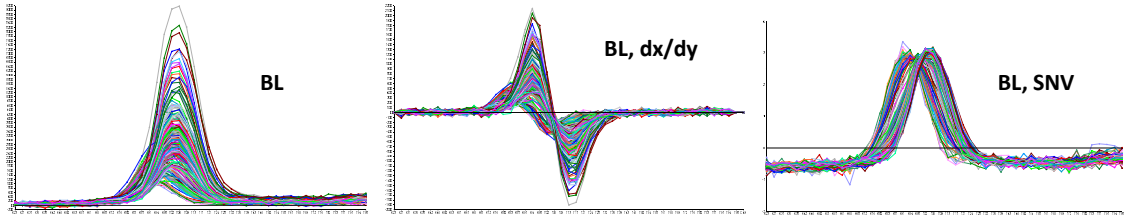

Fig. 6: SERS spectra of the segments using raw materials between 620 cm^{-1} and 800 cm^{-1}. Data transformation: Baseline correction (BL), first derivative (dx/dy) and standard normal variate (SNV).

Table 4: Results of calibration are shown. The type of transformation, the number of principal components (PC), the goodness of fit (R^2) and the root mean square error of calibration (RMSE) used for evaluation.

Transformation	PC	R^2	RMSE
BL	3	0.909	0.1075
BL, dx/dy	3	0.919	0.1016
BL, SNV	2	0.986	0.0420