



HAL
open science

Optimization of experimental conditions by surface enhanced Raman Scattering (SERS) spectroscopy with gold nanoparticles suspensions

Marion Berge, Antoine Dowek, Patrice Prognon, François-Xavier Legrand, Ali Tfayli, Laetitia Minh Mai Lê, Eric Caudron

► To cite this version:

Marion Berge, Antoine Dowek, Patrice Prognon, François-Xavier Legrand, Ali Tfayli, et al.. Optimization of experimental conditions by surface enhanced Raman Scattering (SERS) spectroscopy with gold nanoparticles suspensions. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* [1994-..], 2021, 268, pp.120628. 10.1016/j.saa.2021.120628 . hal-04270367

HAL Id: hal-04270367

<https://universite-paris-saclay.hal.science/hal-04270367v1>

Submitted on 22 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Optimization of experimental conditions by Surface Enhanced Raman Scattering (SERS) Spectroscopy with gold nanoparticles suspensions

Marion Berge^{1,2}, Antoine Dowek^{1,2}, Patrice Prognon^{1,2}, François-Xavier Legrand³, Ali Tfayli², Laetitia Minh Mai Lê^{1,2}, Eric Caudron^{1,2}

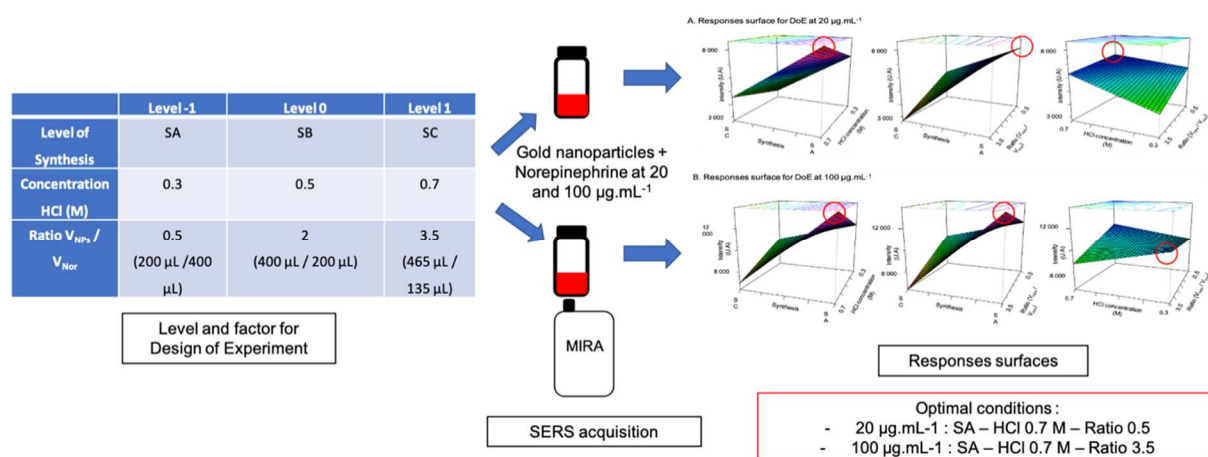
¹Service de Pharmacie, Hôpital européen Georges Pompidou, APHP.Centre Université-Paris,
20 rue Leblanc, 75015 Paris, France.

²Université Paris-Saclay, Lipides, Systèmes Analytiques et Biologiques, 92296, Châtenay-Malabry, France.

³Université Paris-Saclay, CNRS, Institut Galien Paris Sud, 92296, Châtenay-Malabry, France.

Corresponding author: Marion Berge, marion.berge@universite-paris-saclay.fr

Graphical Abstract



Abstract

Surface Enhanced Raman Scattering (SERS) spectroscopy is a rapid and innovative analysis technique involving metallic nanoparticles (NPs). The interaction between NPs and norepinephrine gives an exaltation of the Raman signal under certain experimental conditions. The control of the signal exaltation, crucial for sensitive analyses, remains one of the main limitations of this technique. The aim of this work is to optimize the exaltation conditions for an optimal SERS signal at two concentrations of norepinephrine (NOR) and spherical gold NPs in suspension. This first work will fix the optimal experimental conditions essential for the development of robust discriminant and quantitative analysis of catecholamine.

Two complete 3-factors 3-levels experiment designs were performed at 20 $\mu\text{g.mL}^{-1}$ and 100 $\mu\text{g.mL}^{-1}$ norepinephrine concentrations, each experiment being repeated 3 times. The optimization factors were the process of synthesis (variation of the quantity of gold and citrate used for the three synthesis SA, SB and SC) and HCl (0.3 M, 0.5 M, 0.7 M) as well as the volume ratio of NPs and norepinephrine (0.5, 2, 3.5) for SERS acquisition. Spectral acquisitions were performed with a handheld Raman spectrometer with an excitation source at 785 nm. For each sample, 31 acquisitions were realized during 3seconds every 8 seconds. The optimization parameter was the intensity of the characteristic band of norepinephrine at 1280 cm^{-1} .

A total of 5,042 spectra were acquired and the pre-treatment selected for all spectra was asymmetric least square combined to a smoothing of Savitsky Golay (ALS – SG). The optimal contact time between norepinephrine and NPs depends on the experimental conditions and was determined for each experiment according to the mean intensity between the three replicates. After interpretation of the experimental designs, the optimal conditions retained were the quantity of gold corresponding to SA and the HCl concentration 0.7 M for the two concentrations of norepinephrine. Indeed, the optimal volume ratio depend on the NOR concentration.

Key words: Chemometric analyses, SERS spectroscopy, Experimental optimization, Experimental by design, Gold nanoparticles

Highlights:

Rational approach by experimental design to fix optimized experimental parameters in Surface enhanced Raman **scattering (SERS)** spectroscopy

Importance of controlling aggregation conditions to control SERS signal reproducibility

Different optimized conditions in function of the analyte concentration

1. Introduction

Development of innovative analysis techniques enabling the dosage of drugs is a necessity in a hospital setting. Firstly, the objective is to ensure quality control of injectable preparations to guarantee to patients the right drug (identification) at the right dose (quantification), specifically for the most toxic drugs such as chemotherapy. Secondly, analytical methods are used in precise medicine for therapeutic drug monitoring (TDM) to determine the biological concentration of drugs. TDM is particularly suitable for drugs with a narrow therapeutic range. It enables the blood concentration of the drug to be measured to determine whether the drug is effective without being toxic and thus to adjust the dosage of drug to the patient. These issues require specific analysis techniques that cover a wide range of concentrations (mg.L^{-1} for quality control; $\mu\text{g.L}^{-1}$ to ng.L^{-1} for TDM or environmental contamination). Raman spectroscopy appears as a good answer to identify and quantify drugs. Several studies have shown the feasibility of quantifying drugs by Raman spectroscopy after sampling [1,2] or directly through a plastic bag [3]. But, despite the quantitative and discriminative capacities of the technique, the lack of sensitivity of the Raman spectroscopy does not answer all quantification needs and particularly for low concentrations.

The use of Surface enhanced Raman **Scattering (SERS)** spectroscopy increases in pharmaceutical applications [4]. SERS is a potentially ultra-fast and cost-effective analytical method requiring a very small sample. SERS is an attractive technique for pharmaceutical applications compared with Raman spectroscopy because SERS measurements typically involve enhancing the Raman signal of an analyte upon adsorption on the surface of a metal nanoparticle. The excitation of local surface plasmons by the incident monochromatic light can lead to Raman enhancement factors ranging from 10^4 to 10^{11} in some specific applications [5–7]. SERS is also attractive compared with chromatographic methods since quantitative drug analysis can potentially be performed quickly and with high molecular selectivity.

An important exaltation of the Raman signal is observed when the substrate is in contact with the molecule of interest [8]. This technique has been widely developed for the detection of chemical compounds in many fields such as cosmetics [9], drugs quantification in biologic fluids [10–12] or in the food industry for the detection of chemical substances [13–15]. In

pharmaceutical sciences, SERS is used for the control of impurities or the concentration of active ingredient in finished medicines [16,17].

The main drawback to the development of this technique for quantitative applications remains the low reproducibility of the exaltation. Indeed, few of these different studies used several batches [16] of nanoparticles or SERS support or repeated SERS experiment to improve the feasibility (the repeatability, the control of interaction and the robustness) of SERS to be used in quantification.

Another work about the quantification of 5-Fluorouracil by SERS with several batches of silver nanoparticles (NPs) [18] showed the lack of reproducibility of the SERS signal to develop a quantitative model and the importance of controlling the aggregation mechanism and the interaction between NPs and the analyte to develop a robust quantitative model. Moreover, a performant and predictive quantitative model based on a complex mathematic model was developed to overcome the lack of reproducibility and minimize the synthesis variability.

Consequently, to improve the SERS technique, it is required to determine the various parameters influencing changes on the stability over time and intensity of the SERS signal of colloidal suspensions.

The aim of this study was to assess the usefulness of a DoE approach to optimize the intensity and the stability of the SERS signal to develop robust quantitative models for molecules of therapeutic interest.

The work was based on the study of the interaction between norepinephrine and gold nanoparticles (AuNPs) and the aggregation of AuNPs to obtain the most important enhancement of SERS signal. To reach this objective two conditions are necessary: first, the reproducibility of the synthesis process and second, the control of environmental parameters for the interaction between the AuNPs and the analyte. Moreover, to compare the aggregation conditions depending on analyte concentration, the study was realized for two norepinephrine concentrations at 20 and 100 $\mu\text{g.mL}^{-1}$. To show that optimal parameters of exaltation was not dependent of the analyte concentration, two full factorial designs with three factors of optimization were realized: the synthesis of gold nanoparticles to obtain three concentrations of nanoparticles, the ratio of volume $V_{\text{HCl}}/V_{\text{NP}}$, and the concentration of aggregate agent to

obtain the most important enhancement of SERS signal with a good stability of signal over time.

2. Material and methods

2.1. Chemicals and reagents

Synthesis of gold nanoparticles (AuNPs) was performed with potassium tetrachloroaurate (KAuCl_4 , purity > 98%) obtained from Sigma-Aldrich (Saint – Louis, USA) and trisodium citrate dihydrate ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$, 98%) obtained from VWR chemicals (Leuven, Belgium). Solutions of aggregating agents were prepared from hydrochloric acid 1.0 M (HCl, European Pharmacopoeia quality) obtained from VWR chemicals. The commercial norepinephrine (NOR, Figure 1) solution at $2 \text{ mg}\cdot\text{mL}^{-1}$ was acquired from Mylan (Paris, France). Ultra-pure water was generated from Milli-Q system (Millipore, Bedford, MA, USA). Aqua regia used to clean up glassware was obtained by mixing 3 volumes of hydrochloric acid 37 % (HCl, European Pharmacopoeia quality, VWR chemicals) and 1 volume of nitric acid 65 % (HNO_3 , suprapur, Merck, Darmstadt, Germany).

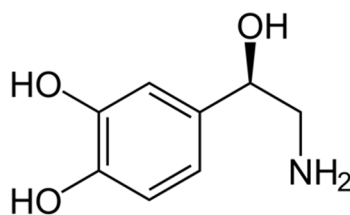


Fig. 1 : Norepinephrine molecule

2.2. Gold nanoparticles synthesis

Colloidal suspensions of AuNPs were synthesized by a reduction of gold adding citrate as a reductant and stabilizing agent following the Turkevich method [19] according to

standardized and reproducible experimental protocols. Briefly, KAuCl_4 was diluted in 100 mL of ultra-pure water and heated to 140°C with stirring at 900 rpm with a stir bare until the boiling point. Then, 4 mL of citrate solution was rapidly added, the mix solution was maintained at 140°C for 15 minutes. Prior to each synthesis, all glass materials were cleaned with aqua regia. All suspensions were kept away from the light and at $+4^\circ\text{C}$.

To evaluate the influence of KAuCl_4 introduced on the synthesized nanoparticles, increasing quantities of KAuCl_4 and citrate at 1%, 2% or 3% introduced were evaluated using a constant citrate/gold molar ratio fixed at 5.2 (table1).

Table 1 : Gold nanoparticles synthesis conditions and characterization of the three AuNPs suspension SA, SB and SC

<i>Protocols</i>	<i>SA</i>	<i>SB</i>	<i>SC</i>
<i>KAuCl_4 (mg)</i>	11.2	22.4	33.6
<i>Citrate (%)</i>	1.0	2.0	3.0
<i>Size UV-vis (nm)</i>	18.9	13.8	12.7
<i>Size TEM (nm)</i>	18.7	15.0	13.2
<i>Size DLS (nm)</i>	22.5	18.2	16.9
<i>Conc UV-vis (number of NPs /mL)</i>	3.2×10^9	8.8×10^9	14.0×10^9
<i>PDI</i>	0.097	0.174	0.223

2.3. Characterization of AuNps

In order to evaluate the nanoparticles size and the polydispersity of the suspension, nanoparticles colloidal suspensions were characterized by transmission electronic microscopy (TEM), UV visible spectroscopy (UV – vis) and Dynamic Ligth Scattering (DLS).

A 1:4 diluted suspension of AuNPs was analyzed by UV – visible spectra from 300 to 900 nm. The measurements were performed in a 1 cm plastic cell using a Varian Cary 50 UV – visible (Agilent, Les Ulis, France). The maximum wavelength (I_{\max}) and the absorbance at 450 nm (A_{450}) were acquired to characterize the sizes of AuNPs and their concentrations respectively (eq.1 and eq.2 [20]).

Equation 1 : Equation to calculate the size of AuNPs with UV-vis spectra. B is a constant equal to 3, A_{spr} corresponding to the maximal absorbance, A_{450} to the absorbance at 450 nm and C is a constant equal to 2.20.

$$Size(nm) = \exp\left(B \times \frac{A_{spr}}{A_{450}} - C\right)$$

Equation 2 : Equation to calculate the number of AuNPs with UV-vis spectra. A_{spr} corresponding to the maximal absorbance, A_{450} to the absorbance at 450 nm and d the diameter of NPs.

$$N = \frac{A_{450} \times 10^{14}}{d^2 \left[-0.295 + 1.36 \exp\left(-\left(\frac{d - 96.8}{78.2} \right)^2 \right) \right]}$$

The hydrodynamic diameter and the polydispersity index (PDI) of AuNPs was measured at 25°C by DLS using a Zetasizer Nano ZS 90 (Malvern Instrument, England). Samples were observed with a JEM 2100-Plus microscope (JEOL, Croissy, France) operating at 200 kV. The electron microscopy images were recorded with a Rio 16 (Gatan®) camera. The average AuNPs size was calculated using the ImageJ software based on the measurement of 100 AuNPs for each suspension, measured on different fields.

2.4. Design of Experiment (DoE)

In order to optimize SERS conditions to analyze norepinephrine using gold nanoparticles suspensions, two designs of experiments including a Box-Behnken and a full factorial design were tested. Experiments were performed for two concentrations of norepinephrine at 20 and 100 $\mu\text{g.mL}^{-1}$. DoE were calculated and interpreted using JMP trial 15.1.10. Each factorial design was built for 3 factors at 3 levels (table 2).

Using the Box-Behnken design, a total of 12 experiments were realized with three repetitions for central point (12 experiments + 3 central points). Using the full factorial design, a total of 81 experiments were performed and each experiment was randomly repeated 3 times (27 experiments x 3 repetitions). For both DoE, the optimization criterion was the intensity at

1280 cm^{-1} corresponding to the most intensive band of NOR [21]. The objective was to maximize the intensity at 1280 cm^{-1} .

To obtain an enhancement of the signal, it was important to control the aggregation between AuNPs and the analyte. For this purpose, the two DoE implicating three factors directly implicated in aggregation process: the AuNPs quantities, the HCl concentration and the ratio between AuNPs and analyte volumes. The HCl was added with a constant volume of 10 μL for each experiment.

Table 2 : Factors and levels used for the box-Behnken and the full factorial DoE

	<i>Level -1</i>	<i>Level 0</i>	<i>Level 1</i>
<i>Gold amount for synthesis (mg/100mL)</i>	11.2 (SA)	22.4 (SB)	33.6 (SC)
<i>Concentration HCl (M)</i>	0.3	0.5	0.7
<i>Ratio V_{NPs} / V_{Nor}</i>	0.5 (200 μL / 400 μL)	2 (400 μL / 200 μL)	3.5 (465 μL / 135 μL)

To validate the DoE, several criteria must be verified such as variance analysis, the curve predicted intensity vs observed intensity and the aspect of residuals (normality, homoscedastic repartitions, low values).

2.5. Sample preparation and SERS measurement

SERS measurements were realized with a handheld Raman MIRA M-1 (Methrom® Instant Raman Analyser, France). The excitation source was a 785 nm single-mode diode laser generating a maximum of 75 mW on the sample. Analyses were conducted using the lens module at a focal distance of 8 mm with the orbital raster scan (ORS) system. **Using ORS system, a tightly focused laser beam scans circularly the sample which contributes to increase the interrogative area on the sample area. This provides high intensity of spectrum while maintaining high spectral resolution which is particularly interesting for the analysis of elements at low concentration.** The spectral region studied was 400-2,300 cm^{-1} with a spectral resolution from 12 to 14 cm^{-1} .

NOR commercial solution at 2 mg.mL^{-1} was diluted in ultra-pure water at $100 \text{ }\mu\text{g.mL}^{-1}$ and $20 \text{ }\mu\text{g.mL}^{-1}$. SERS experiments were randomly prepared and analyzed according to the experimental designs presented in section 2.4. For each experiment, AuNPs and NOR were first mixed in a glass vial for 5 seconds and then $10 \text{ }\mu\text{L}$ of HCl was added to the mixed solution and vortexed for 5 seconds. SERS spectra were immediately acquired after preparation. SERS signal were measured from 0 to 4 min every 8 seconds using 3 seconds of acquisition time. A total of 31 SERS spectra for each sample were collected.

2.6. Data analysis

Before analysis of DoE, spectral analysis and data analysis were performed with the software RStudio®: R Core Team (2020) (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria), version 1.2.5042. Several pretreatments and a combination of pretreatments as asymmetric Least Square (ALS) were used to correct the baseline, standard Normal Variation (SNV) to correct the additive and multiplicative effect and smoothing of Savitsky Golay (SG) to reduce noise.

The same pretreatment was applied to all SERS spectra acquired for the two DoE. For all experiments, one spectrum over the 31 spectra acquired was selected to interpret the DoE. The contact time was optimized for the highest intensity at 1280 cm^{-1} with a good coefficient of variation between repetitions of one experiment. The selected responses were then incorporated into DoE.

3. Results

3.1. Characterization of AuNPs

A total of three suspensions of AuNPs (SA, SB and SC) was synthesized following the protocols previously described. The characteristics of the three AuNPs suspensions determined by UV-vis, DLS and TEM are presented in the table 1. UV-visible spectra presented in figure 2 showed for each AuNPs suspension a maximum absorption between 519 nm and 521 nm (mean = 517.7 nm) with a residual standard deviation of 0.18%.

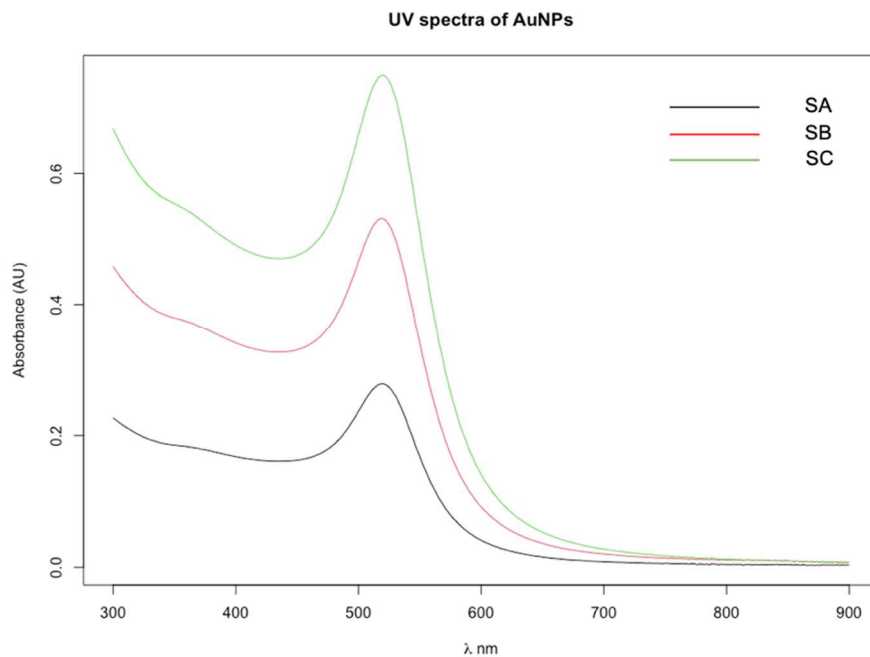


Fig. 2 : UV-visible spectra of the different AuNPs suspension

For the three suspensions of AuNPs, low polydispersity index ($PDI < 0.3$) was calculated in favor of a low polydispersity of NPs within the suspensions. Diameters estimated with DLS were hydrodynamic diameters of AuNPs, so they were higher than diameters calculated with UV-visible spectra and TEM. The TEM allowed to view the aspect of NPs and to estimate the size considering many nanoparticles. Figure 3 shows the pictures of the AuNPs for each suspension; they were spherical with low polydispersity. As reported to table 1, size of AuNPs decrease from 18,9 nm to 12,7 nm with amount of gold.

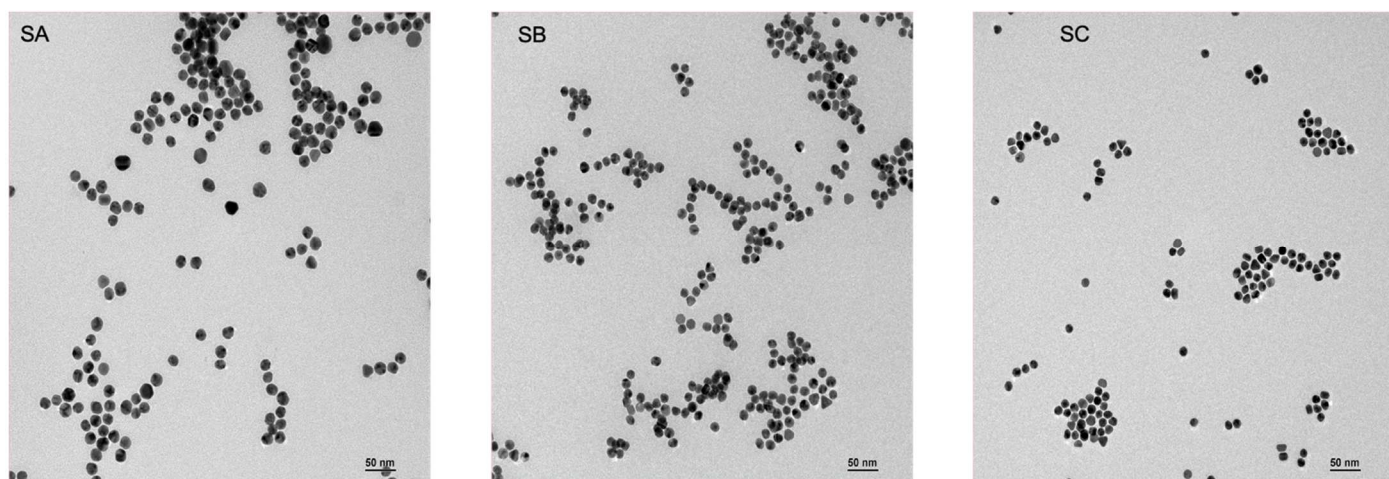


Fig. 3 : TEM images of gold nanoparticles in suspension synthesized according to the three protocols (SA, SB and SC)

3.2. SERS spectra description

A total of 5,042 spectra were acquired (2,511 spectra for each design of experiments). The 31 acquisitions over the time showed an evolution of the SERS signal on time as shown in figure 4 A. The pretreatment with the best spectra correction (effect and baseline) was ALS with a smoothing of Savitsky-Golay to reduce the noise and to correct the baseline (figure 4 B). All spectra were corrected with the same pretreatment.

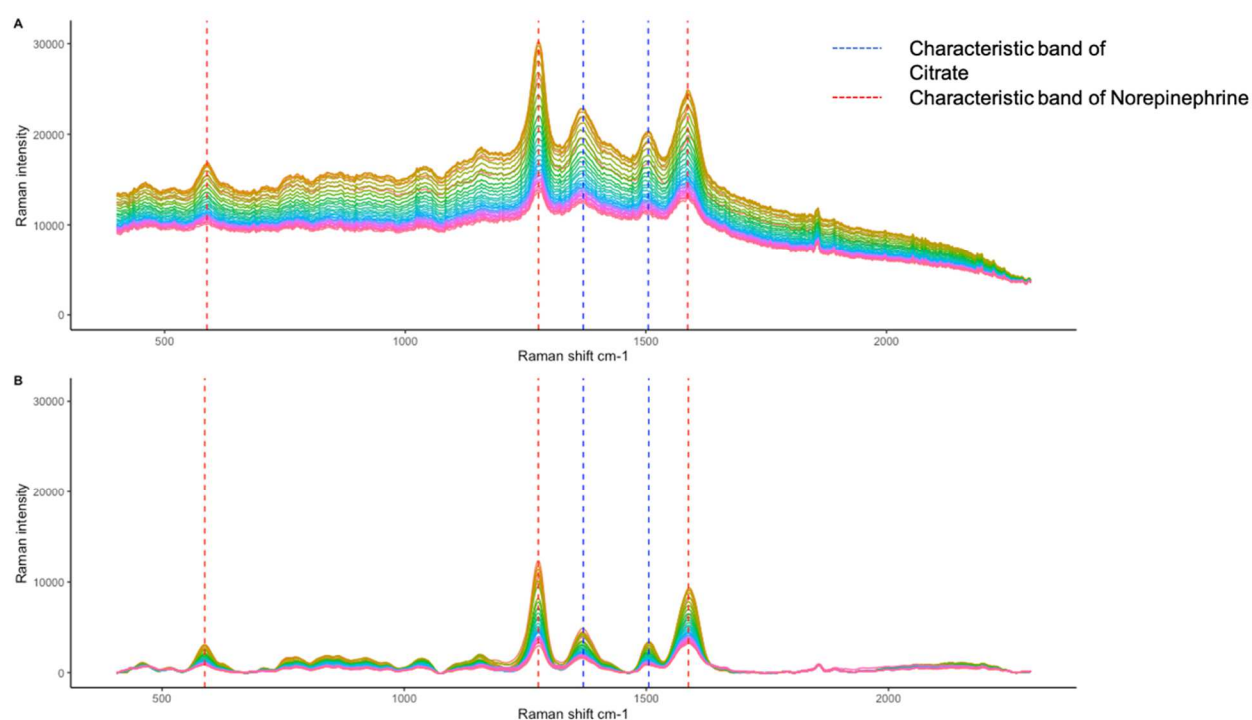


Fig. 4 : SERS spectra of Norepinephrine for the first experiment (levels SB -HCl 0.7 M – Ratio 0.5) of the DoE A) without pretreatment and B) after pretreatment by ALS - SG

As presented in figure 4, characteristic Raman shifts of citrate and norepinephrine were identified [22–24] : an intense band at 1280 cm^{-1} corresponding to the stretching C-O, a band at 1425 cm^{-1} characteristic of the bound C-N and a band at 1593 cm^{-1} corresponding to the stretching C-C of NOR. The peaks identified were known in several studies. One study was published by Munro et al. about the characterization of citrate on silver nanoparticles in SERS [25] and allowed to identify the principal band at 1417 cm^{-1} corresponding to a carboxylate symmetric stretching (ns COO^-). Another study published by Takenaka [26] allowed to identify a peak around 1530 cm^{-1} which matches with antisymmetric stretching COO^- vibrations.

3.3. SERS contact time

For each experiment, an important influence of the contact time on SERS signal was observed. Figure 5 shows the evolution of the signal for the most intensive band at 1280 cm^{-1} for three experiments of the full factorial DoE and suggests different kinetics of interaction between AuNPs and NOR. These different kinetics were classified as stable aggregation (Fig. 5A), evolutive aggregation (Fig. 5B) or sedimentation of AuNPs (Fig. 5C). Indeed, an increase of the signal intensity was assimilated to an aggregation of AuNPs and an improvement of the interaction between AuNPs and NOR. A decrease of intensity over time was related to a sedimentation phenomenon of AuNPs. Figure 5 A was assimilated to an aggregation of AuNPs whereas Figure 5 C was associated to a sedimentation of AuNPs. Figure 5 B was related to low aggregate phenomena with an increase of the intensity and then a decrease until sedimentation of the AuNPs-NOR aggregate.

This result shows that the control of AuNPs aggregation and AuNPs/NOR interaction is complex to reproduce whereas it is an essential element to control in the perspective of a quantitative analysis.

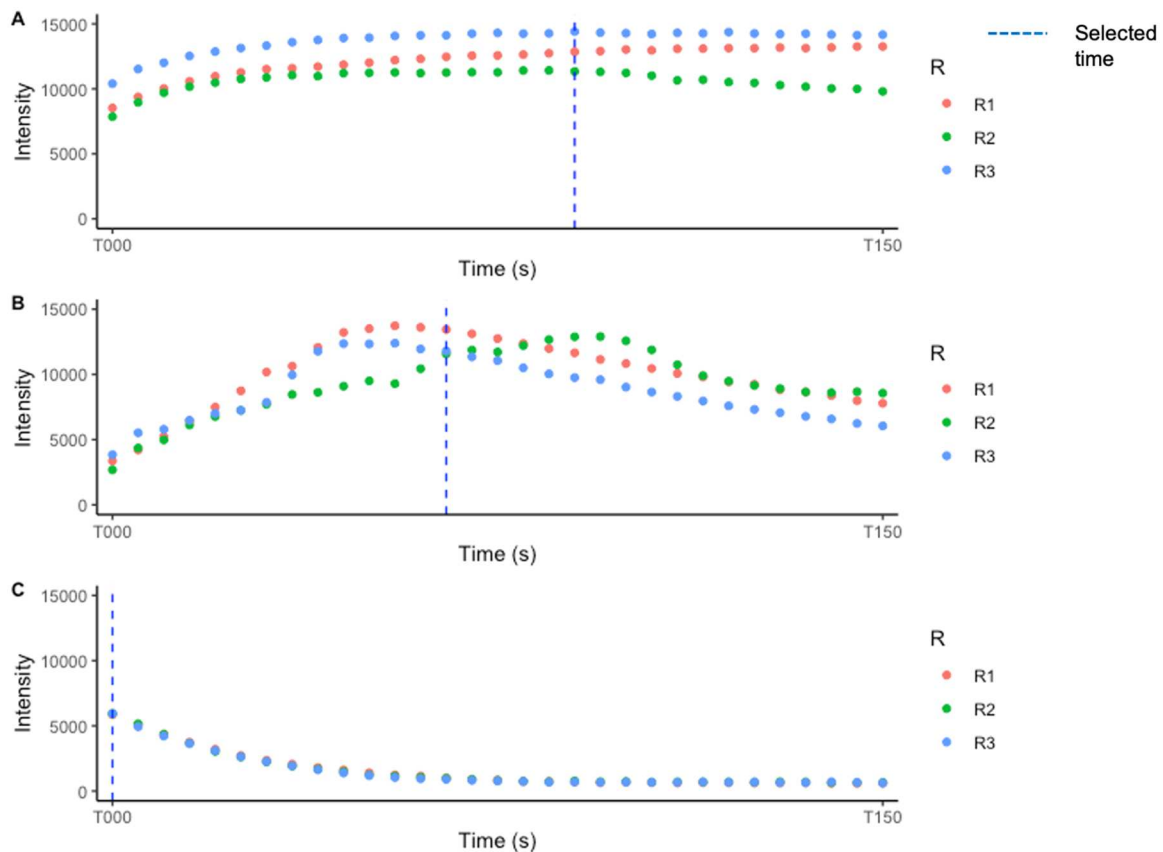


Fig. 5 : Evolution of the SERS signal over the time for three the repetition (R1, R2, R3) of three different experiments. A- Experiment levels SB – HCl concentration 0.3 M – Ratio 2. B – Experiment levels SA – HCl concentration 0.3 M – Ratio 2. C – Experiment levels SC – HCl 0.7 M – Ratio 3.5. The blue dotted lines represented the time selected for the analysis of DoE.

3.4. DoE analysis

For the Box-Behnken analysis, outliers were not removed because there are no repetitions for the experiment. For the full factorial design, evolution of the SERS signal over the time for the three repetitions was not reproducible: if one repetition of one experiment had a different evolution from the other repetition of the same experiment, the repetition was considered as an outlier and was removed for the full factorial analysis.

As previously observed, various evolution of the SERS signal over the time were observed among the conditions of the DoE. To determine the optimal condition of aggregation for each condition, we selected the time with the maximal mean intensity at 1280 cm^{-1} as represented fig. 5.

3.4.1. Box-behnken design analysis

First, a Box-Behnken design analysis was evaluated. For the first concentration at $100\text{ }\mu\text{g.mL}^{-1}$, the DoE was validated with a correlation coefficient at 0.96 and a variance analysis significant (p-value = 0.00069). On contrast, the DoE for the concentration at $20\text{ }\mu\text{g.mL}^{-1}$ was not significant (p-value = 0.39). As the objective of the study was to determine the optimal conditions for analysis over a concentration range of 20 to $100\text{ }\mu\text{g.mL}^{-1}$, the Box-Behnken design was not retained and a full factorial design was preferred.

3.4.2. Full factorial design analysis

Using a full factorial design, the DoE was validated for the two concentrations (Table 6). The correlation coefficient (R^2) between predicted and observed values was 0,82 and 0,76 for the DoE at $20\text{ }\mu\text{g.mL}^{-1}$ and $100\text{ }\mu\text{g.mL}^{-1}$ respectively, which reveals a good prediction of the developed model, specifically for SERS measurements. Indeed, there are many sources of uncontrolled or unknown signal variability, which may lead to a lack of linearity and fit

between observed and predicted responses by the model. The residuals were also homoscedastic, low and had a normal distribution. The variance analysis (ANOVA) for these DoE was significant (p-value < 0,05): the model error was lowest than the residual error as shown in the table 3.

Table 3 : Table of ANOVA for the two DoE

Intensity 1280 cm ⁻¹ at 100 µg.mL ⁻¹				
	Degrees of freedom	Medium square	F exp	p-value
Model	7	4.89E+07	26.49	< 0.0001
Error	57	1.85E+06		
Total	64			
Intensity 1280 cm ⁻¹ at 20 µg.mL ⁻¹				
Model	7	3.09E+07	15.0681	< 0.0001
Error	54	2.05E+06		
Total	61			

The most significant effects in these two DoE were the quantity of AuNPs and the interaction between the quantity of AuNPs and the volume ratio and between the quantity of AuNPs and the concentration of HCl. The two recapitulative tables for all effects are presented in table 4 and 5.

Table 4: Summary table of effect for DoE at 20 µg.mL⁻¹ (* = significant effect)

<i>Terme</i>	<i>Estimation</i>	<i>Erreur standard</i>	<i>t ratio</i>	<i>Prob. > t </i>
<i>Constant</i>	5894.33	184.14	32.01	<0.0001*
<i>Quantity of gold NPs</i>	-1927.82	224.65	-8.58	<0.0001*
<i>HCl concentration</i>	669.38	220.88	3.03	0.0037*
<i>Ratio</i>	-590.83	223.53	-2.64	0.0107*
<i>Quantity of gold NPs*HCl</i>	-79.19	280.13	-0.28	0.78
<i>Quantity of gold NPs*Ratio</i>	-615.20	264.26	-2.33	0.024*

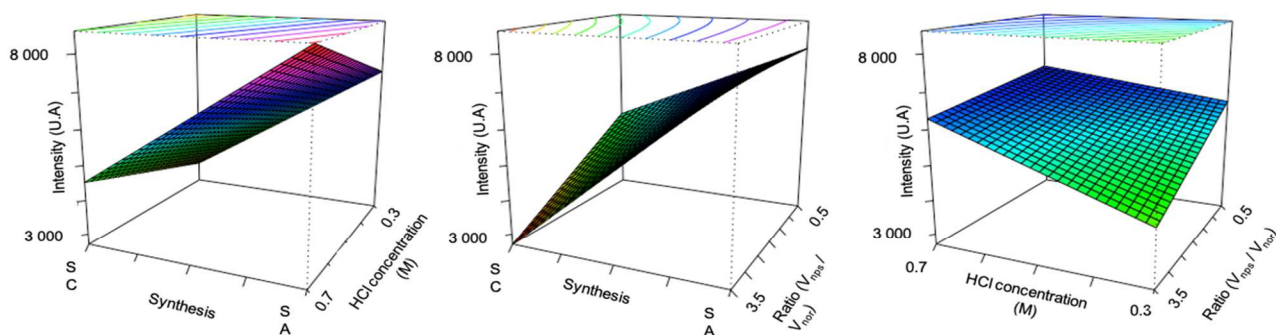
<i>HCl*Ratio</i>	320.04	264.02	1.21	0.23
<i>Quantity of gold NPs *HCl*Ratio</i>	-21.06	323.44	-0.07	0.95

Table 5 : Summary table of effect for DoE at 100 $\mu\text{g.mL}^{-1}$ (* = significant effect)

<i>Terme</i>	<i>Estimation</i>	<i>Standard error</i>	<i>t ratio</i>	<i>Prob. > t </i>
<i>Constant</i>	10378.21	175.282	59.21	<0.0001*
<i>Quantity of gold NPs</i>	-2268.415	211.57	-10.72	<0.0001*
<i>HCl concentration</i>	-59.86645	213.0975	-0.28	0.78
<i>Ratio</i>	-292.7717	208.822	-1.40	0.17
<i>Quantity of gold NPs *HCl</i>	-1170.13	263.2111	-4.45	<0.0001*
<i>Quantity of gold NPs *Ratio</i>	-1370.023	265.6268	-5.16	<0.0001*
<i>HCl*Ratio</i>	-801.7732	260.0053	-3.08	0.0031*
<i>Quantity of gold NPs *HCl*Ratio</i>	323.5982	337.0268	0.96	0.34

The best response for the DoE at 100 $\mu\text{g.mL}^{-1}$ was obtained for SA (level -1), HCl 0.7 M (level +1), and a ratio of 3.5 (level +1) as shown by the surface responses shown in figure 6 a. In contrast, the maximization of the SERS signal enhancement obtained at 20 $\mu\text{g.mL}^{-1}$ was shown on response surfaces (figure 6 b) and corresponded to SA (level -1), HCl 0.7 M (level +1), and a ratio of 0,5 (level -1).

A. Responses surface for DoE at 20 $\mu\text{g.mL}^{-1}$



B. Responses surface for DoE at 100 $\mu\text{g.mL}^{-1}$

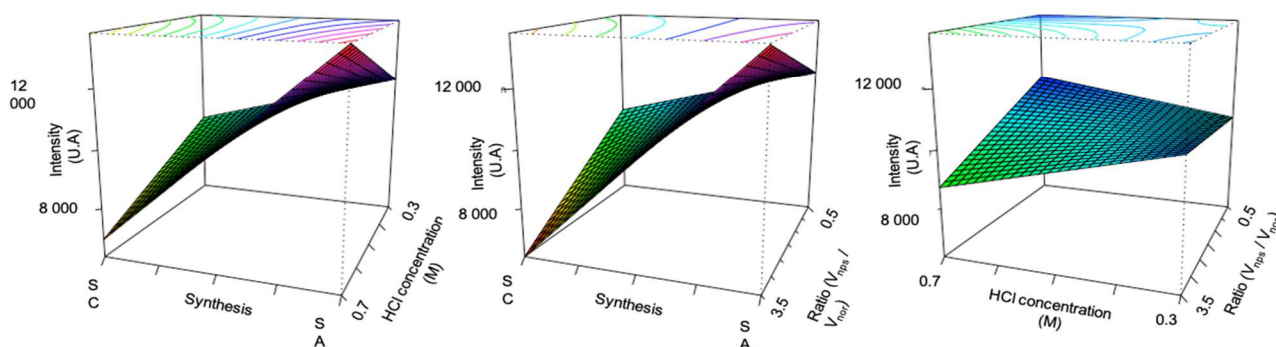


Fig. 6 : Responses surfaces for the two DoE – Conditions: Synthesis SB – HCl concentration 0.5 M – Ratio 2.0

Finally, optimal conditions depended on the analyte concentration. For the range 20 – 100 $\mu\text{g.mL}^{-1}$, the optimal conditions were the synthesis SA and the concentration of HCl at 0.7 M. The ratio could be added function of the concentration.

4. Discussion

This work showed an original approach using DoE to optimize SERS analysis. In order to obtain a SERS signal, the control the experimental conditions and thus of aggregation is essential to optimise the signal enhancement. Due to the wild sources of variability in the aggregation parameters such as nanoparticle nature, size or concentration, aggregation agent nature or concentration, analyte concentration and volume ratio between the analyte and the NPs solution, the use of a DoE is essential. To limit the number of experiments in this work, only three optimization factors were considered since the nature and size of the AuNPs and the nature of the aggregating agent were fixed. The work focused on the most important factors for aggregation: the ratio $V_{\text{NPs}} / V_{\text{Nor}}$, the HCl concentration and the gold amount by

synthesis. Two DoE at two extreme concentrations showed that the optimal conditions varied depending on the NOR concentration and showing its role in the aggregation process.

Despite the interest of the DoE to optimize experimental conditions of SERS analysis, this approach was particularly difficult to perform because aggregation conditions depended on optimization parameters. Regarding results, the concentration of AuNPs in suspension probably has a small impact on the SERS intensity comparatively to AuNPs size which was significantly different between the 3 protocols. Since the size decreased with the augmentation of the gold / citrate ratio, the concentration of AuNPs in the suspensions increased (27). To improve the aggregation between the AuNPs and the analyte, an optimization on the size of AuNPs could be realized. The second parameter evaluated by the DoE, the ratio between the volume of AuNPs and the volume of analyte in the sample, also had a small impact on the aggregation and the SERS signal. The last parameter was the HCl concentration which promotes aggregation between nanoparticles and NOR. This parameter is important to control because an excess of HCl leads to a rapid sedimentation and a loss of the SERS intensity, in particular with high concentration of AuNPs. Despite the impact of the contact time between AuNPs and the analyte, this parameter was not integrated as factor in the DoE due to the difficulty to select three levels. Thus, SERS signal was acquired from 0 to 4 min every 8 seconds, 31 SERS spectra were acquired per sample and all times were considered to optimize the contact time. In order to simplify the interpretation of the DoE, one time was selected for each SERS experiment based on the most intensive mean intensity at 1280 cm^{-1} .

In addition to promoting aggregation, the data analysis and more particularly data processing such as pretreatment and outliers' identification constitute one of the main elements to interpret SERS spectra and DoE. The objective after preprocessing is to obtain for one experimental condition a stable evolution of SERS signal over time which corresponds to stable aggregation conditions, developing a quantitative model. Several conditions are necessary: the reproducibility of AuNPs synthesis, the control of aggregation conditions (limitation of saturation phenomenon or sedimentation phenomenon), a good pre-processing of SERS spectra and the withdrawal of outliers before the development of the model.

Finally, to have a SERS detection, the interaction between the NPs and the drug has to be maximal and therefore, the aggregation has to be controlled. This work was based on the optimization of SERS experimental conditions and thus, on the aggregation phenomenon. To obtain a good enhancement of the signal for all samples, robust aggregation is required. Moreover, the study shows that optimal condition varies with the concentration of the drug and therefore, the usefulness of optimizing experimental conditions prior to quantitative analyses according to the concentration range.

5. Conclusion

This study highlights the use of DoE as a powerful tool to optimize SERS signal and SERS sample preparation. The objective of this work was to determine the optimal conditions of analysis in SERS spectroscopy to obtain the most intense enhancement and the optimal experimental conditions to construct quantitative models. Optimal conditions were determined at $20 \mu\text{g.mL}^{-1}$ and $100 \mu\text{g.mL}^{-1}$ in order to consider quantification over this concentration range. To determine these conditions, several conditions such as reproducibility of the synthesis of gold nanoparticle suspensions, optimization of the aggregation of gold nanoparticles and interaction with norepinephrine are necessary.

This article is thus a first indispensable work to define optimized experimental parameters to reduce SERS signal variability and promote sensibility. **Indeed, this study shows the usefulness of a prior optimization of SERS experimental conditions before starting quantitative analyses.**

Acknowledgments :

The authors would like to thank the Laboratory of Pharmaceutical Analytical Chemistry of Liège University, specifically Ph.D. Eric Ziemons and Charlotte De Bleye. **The authors would also like to thank NanoBio-ICMG Platform (FR 2607, Grenoble), specifically Jean-Luc Putaux for the TEM images.** Finally, they are grateful to ARC for the financial support to Marion Berge.

Formatting of funding sources :

This work was supported by the ARC foundation.

Bibliography

- [1] L. Lê, M. Berge, A. Tfayli, P. Prognon, E. Caudron, Discriminative and Quantitative Analysis of Antineoplastic Taxane Drugs Using a Handheld Raman Spectrometer, *BioMed Res. Int.* 2018 (2018) 1–7. <https://doi.org/10.1155/2018/8746729>.
- [2] L.M.M. Lê, M. Berge, A. Tfayli, J. Zhou, P. Prognon, A. Baillet-Guffroy, E. Caudron, Rapid discrimination and quantification analysis of five antineoplastic drugs in aqueous solutions using Raman spectroscopy, *Eur. J. Pharm. Sci.* 111 (2018) 158–166. <https://doi.org/10.1016/j.ejps.2017.09.046>.
- [3] L. Lê, M. Berge, A. Tfayli, A. Baillet Guffroy, P. Prognon, A. Dowek, E. Caudron, Quantification of gemcitabine intravenous drugs by direct measurement in chemotherapy plastic bags using a handheld Raman spectrometer, *Talanta.* 196 (2019) 376–380. <https://doi.org/10.1016/j.talanta.2018.11.062>.
- [4] J. Cailletaud, C. De Bleue, E. Dumont, P.-Y. Sacré, L. Netchacovitch, Y. Gut, M. Boiret, Y.-M. Ginot, Ph. Hubert, E. Ziemons, Critical review of surface-enhanced Raman spectroscopy applications in the pharmaceutical field, *J. Pharm. Biomed. Anal.* 147 (2018) 458–472. <https://doi.org/10.1016/j.jpba.2017.06.056>.
- [5] A. Haddad, M.A. Comanescu, O. Green, T.A. Kubic, J.R. Lombardi, Detection and Quantitation of Trace Fentanyl in Heroin by Surface-Enhanced Raman Spectroscopy, *Anal. Chem.* 90 (2018) 12678–12685. <https://doi.org/10.1021/acs.analchem.8b02909>.
- [6] C. Zhang, S. Han, H. Zhao, S. Lin, W.-L.-J. Hasi, Detection and Quantification of Bucinnazine Hydrochloride Injection Based on SERS Technology, *Anal. Sci.* 34 (2018) 1249–1255. <https://doi.org/10.2116/analsci.18P158>.
- [7] Pilot, Signorini, Durante, Orian, Bhamidipati, Fabris, A Review on Surface-Enhanced Raman Scattering, *Biosensors.* 9 (2019) 57. <https://doi.org/10.3390/bios9020057>.
- [8] M. Fleischmann, P.J. Hendra, A.J. McQUILLAN, RAMAN SPECTRA OF PYRIDZNE ADSORBED AT A SILVER ELECTRODE, *Chem. Phys. Lett.* 26 (n.d.) 4.
- [9] L.F. Sallum, F.L.F. Soares, J.A. Ardila, R.L. Carneiro, Optimization of SERS scattering by Ag-NPs-coated filter paper for quantification of nicotinamide in a cosmetic formulation, *Talanta.* 118 (2014) 353–358. <https://doi.org/10.1016/j.talanta.2013.10.039>.

- [10] M.B. Mamián-López, R.J. Poppi, Quantification of moxifloxacin in urine using surface-enhanced Raman spectroscopy (SERS) and multivariate curve resolution on a nanostructured gold surface, *Anal. Bioanal. Chem.* 405 (2013) 7671–7677. <https://doi.org/10.1007/s00216-013-7200-y>.
- [11] W. Zhu, B.-Y. Wen, L.-J. Jie, X.-D. Tian, Z.-L. Yang, P.M. Radjenovic, S.-Y. Luo, Z.-Q. Tian, J.-F. Li, Rapid and low-cost quantitative detection of creatinine in human urine with a portable Raman spectrometer, *Biosens. Bioelectron.* 154 (2020) 112067. <https://doi.org/10.1016/j.bios.2020.112067>.
- [12] S.-Y. Long, Z.-P. Chen, Y. Chen, R.-Q. Yu, Quantitative detection of captopril in tablet and blood plasma samples by the combination of surface-enhanced Raman spectroscopy with multiplicative effects model: Detection of captopril in tablet and blood plasma samples, *J. Raman Spectrosc.* 46 (2015) 605–609. <https://doi.org/10.1002/jrs.4698>.
- [13] X. Chen, M. Lin, L. Sun, T. Xu, K. Lai, M. Huang, H. Lin, Detection and quantification of carbendazim in Oolong tea by surface-enhanced Raman spectroscopy and gold nanoparticle substrates, *Food Chem.* 293 (2019) 271–277. <https://doi.org/10.1016/j.foodchem.2019.04.085>.
- [14] L. Mandrile, A.M. Giovannozzi, F. Durbiano, G. Martra, A.M. Rossi, Rapid and sensitive detection of pyrimethanil residues on pome fruits by Surface Enhanced Raman Scattering, *Food Chem.* 244 (2018) 16–24. <https://doi.org/10.1016/j.foodchem.2017.10.003>.
- [15] Q. Zuo, Y. Chen, Z.-P. Chen, R.-Q. Yu, Quantification of Cadmium in Rice by Surface-enhanced Raman Spectroscopy Based on a Ratiometric Indicator and Conical Holed Enhancing Substrates, *Anal. Sci.* 34 (2018) 1405–1410. <https://doi.org/10.2116/analsci.18P342>.
- [16] C. De Bleye, E. Dumont, E. Rozet, P.-Y. Sacré, P.-F. Chavez, L. Netchacovitch, G. Piel, Ph. Hubert, E. Ziemons, Determination of 4-aminophenol in a pharmaceutical formulation using surface enhanced Raman scattering: From development to method validation, *Talanta.* 116 (2013) 899–905. <https://doi.org/10.1016/j.talanta.2013.07.084>.
- [17] L.F. Sallum, F.L.F. Soares, J.A. Ardila, R.L. Carneiro, Determination of acetylsalicylic acid in commercial tablets by SERS using silver nanoparticle-coated filter paper, *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.* 133 (2014) 107–111. <https://doi.org/10.1016/j.saa.2014.04.198>.
- [18] A. Dowek, L.M.M. Lê, T. Rohmer, F.-X. Legrand, H. Remita, I. Lampre, A. Tfayli, M. Lavielle, E. Caudron, A mathematical approach to deal with nanoparticle polydispersity

- in surface enhanced Raman spectroscopy to quantify antineoplastic agents, *Talanta*. 217 (2020) 121040. <https://doi.org/10.1016/j.talanta.2020.121040>.
- [19] J. Turkevich, P.C. Stevenson, J. Hillier, A study of the nucleation and growth processes in the synthesis of colloidal gold, *Discuss. Faraday Soc.* 11 (1951) 55. <https://doi.org/10.1039/df9511100055>.
- [20] W. Haiss, N.T.K. Thanh, J. Aveyard, D.G. Fernig, Determination of Size and Concentration of Gold Nanoparticles from UV–Vis Spectra, *Anal. Chem.* 79 (2007) 4215–4221. <https://doi.org/10.1021/ac0702084>.
- [21] A.S. Moody, B. Sharma, Multi-metal, Multi-wavelength Surface-Enhanced Raman Spectroscopy Detection of Neurotransmitters, *ACS Chem. Neurosci.* 9 (2018) 1380–1387. <https://doi.org/10.1021/acschemneuro.8b00020>.
- [22] A.S. Moody, T.D. Payne, B.A. Barth, B. Sharma, Surface-enhanced spatially-offset Raman spectroscopy (SESORS) for detection of neurochemicals through the skull at physiologically relevant concentrations, *The Analyst*. 145 (2020) 1885–1893. <https://doi.org/10.1039/C9AN01708A>.
- [23] N.Soo. Lee, Y.Zung. Hsieh, R.F. Paisley, M.D. Morris, Surface-enhanced Raman spectroscopy of the catecholamine neurotransmitters and related compounds, *Anal. Chem.* 60 (1988) 442–446. <https://doi.org/10.1021/ac00156a014>.
- [24] M.D. Morris, Resonance Raman spectra of the aminochromes of some biochemically important catechol amines, *Anal. Chem.* 47 (1975) 2453–2454. <https://doi.org/10.1021/ac60364a004>.
- [25] C.H. Munro, W.E. Smith, M. Garner, J. Clarkson, P.C. White, Characterization of the Surface of a Citrate-Reduced Colloid Optimized for Use as a Substrate for Surface-Enhanced Resonance Raman Scattering, *Langmuir*. 11 (1995) 3712–3720. <https://doi.org/10.1021/la00010a021>.
- [26] T. Takenaka, Y. Fujiyoshi, N. Uyeda, Surface enhanced raman scattering of citrate ions adsorbed on gold sol particles, (n.d.) 9.