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TOWARDS A SPRAY-COATING METHOD FOR THE DETECTION OF LOW-DOSE COMPOUNDS IN PHARMACEUTICAL TABLETS USING SURFACE-ENHANCED RAMAN CHEMICAL IMAGING (SER-CI)

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KEYWORDS: Surface-enhanced Raman scattering (SERS) - Spray-coating - Metallic colloidal suspension - Silver nanoparticles - Chemical imaging - Pharmaceutical tablets

ABSTRACT

Surface-enhanced Raman chemical imaging (SER-CI) is a highly sensitive analytical tool recently used in the pharmaceutical field owing to the possibility to obtain high sensitivity along with spatial information. However, the covering method of the pharmaceutical samples such as tablets with metallic nanoparticles is a major issue for SER-CI analyses due to the difficulty to obtain a homogeneous covering of tablet surface with the SERS substrates. In this context, a spray-coating method was proposed in order to fully exploit the potential of SER-CI. A homemade apparatus has been developed from an electrospray ionization (ESI) probe in order to cover the pharmaceutical tablets with the colloidal suspension in a homogeneous way. The silver substrate was pulled through the airbrush by a syringe pump which was then nebulized into small droplets due to the contact of the solution with the gas flow turbulence. A robust optimization of the process was carried out by adjusting experimental parameters such as the liquid flow rate and the spraying time. Besides, the performances of this spraying technique were compared with two others covering methods found in the literature which are drop casting and absorption coating. A homogeneity study, conducted by SER-CI and matrix assisted laser desorption/ ionization mass spectrometry imaging (MALDI-MSI) applied to the different covering techniques was performed.

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The influence of the metallic nanoparticles deposit on soluble compounds was also investigated in order to highlight the advantages of using this new spray coating approach.

Introduction

In recent years, surface-enhanced Raman chemical imaging (SER- CI) has emerged as a sensitive imaging technique in pharmaceutical sciences [1,2]. SER-CI combines the advantages of SERS adding a spatial dimension which makes it possible to identify the presence of active pharmaceutical ingredients (API) or impurities in very low concentrations while visualizing their distributions. This technique improves the sensitivity of conventional Raman imaging and significantly reduces the image acquisition time with the enhancement of the signal intensity.

However, the applications of SER-CI in the pharmaceutical field remain limited, especially due to the difficulty to obtain a homogeneous layer of metallic nanoparticles onto the sample surface [3]. From the reviewed literature, the most common covering method used on tablets is a drop casting deposition due to its simplicity and its fast implementation [4,5]. This method consists in depositing a droplet of metallic nanoparticles on the tablet surface. However, the colloidal suspension is spread over the surface before to be absorbed in the pharmaceutical formulation, which prevents a homogeneous distribution of nanoparticles over the entire surface of the sample. This phenomenon known as the coffee-ring effect, is the result of the accumulation of nanoparticles at the edges of the droplet [6]. The formation of a ring of concentrated nanoparticles induces remarkable variations of the SERS signal intensity and reproducibility issues which limits its application as covering method for SER-CI in the pharmaceutical field. In this context, deposition techniques based on nebulization such as spray-coating were investigated. Spray-coating is a very promising deposition technique used in a wide variety of applications such as agriculture, graphic art and industrial spraying [7]. More recently, it has demonstrated to be an attractive coating method for the manufacturing of organic electronic and optoelectronic thin film devices [8–11] but also as a procedure for SERS substrates preparation [12–15].

In this paper, a spray-coating method using a homemade apparatus was applied to control the deposition of SERS nanoparticles on pharmaceutical samples. To the best of our knowledge, such an application has never been reported using direct spraying deposition method on tablets, in order to improve the SERS-CI measurements. The device used is composed of a pair of coaxial tubes. The

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colloidal suspension is pulled through the inner tube from a syringe pump and the outer tube is connected to a source of high pressure gas. The nebulizing gas breaks the suspension at the nozzle of the air gun creating a flow of tiny droplets which are deposited onto the sample surface. The spray-coating device is easy to implement and the entire surface of the tablet may be covered by the SERS substrate in a homogeneous way. This covering method may greatly improve the potential of SER-CI and could be a suitable technique for quantitative analyses of low drug concentrations or impurities in solid dosage forms.

We investigated the performances of the technique after the optimization of the spraying setup parameters, including the deposition time and the liquid flow rate, by a design of experiments. The homogeneity of the covering was characterized by SER-CI and matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) analyses. We also compared this covering method to common coating methods found in the literature and already used for SERS applications. In addition, the effects of the coating on the migration of soluble compounds present in the pharmaceutical formulations were evaluated. This phenomenon can disturb the spatial characteristics of the sample surface and therefore prevents accurate quantitative information from SER-CI analyses.

Materials and method

CHEMICALS AND REAGENTS

Silver nitrate (extra pure), acetonitrile hypergrade for LC-MS LiChrosolv®, water for LC-MS LiChrosolv® and trifluoroacetic acid (TFA) were obtained from Merck (Darmstadt, Germany). Trisodium citrate (anhydrous, 98%) was purchased from Acros Organics (Morris Plains, USA). Lactose monohydrate, D-mannitol (98%), 4-aminophenol (purity > 99%), 2,5-dihydroxybenzoic acid (DHB), P14R MALDI-MS standard and α-cyano-4-hydroxycinnamic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). A mixture of paracetamol/poly- vinylpyrrolidone 3% (paracetamol/pvp) was kindly provided by S.M.B. Technology (Marche-en-Famenne, Belgium).

PREPARATION OF SILVER NANOPARTICLES

SILVER NANOPARTICLES SYNTHESIS

The synthesis of silver nanoparticles (Ag Nps) was based on the method described by Lee and Meisel

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[16]. Forty-five milligrams of silver nitrate were dissolved in 250 mL of ultrapure water generated from a Milli-Q system (Millipore, Bellirica, MA, USA). The solution was heated to boil and 5 mL of a solution of 1% trisodium citrate was added dropwise under stirring with a Teflon-coated magnetic stir bar at 750 rpm. The resulting suspension was kept on stirring at reflux for one hour and turned into a greenish grey color after a few minutes.

The colloidal suspension was then cooled down to room temperature and stored in a fridge until it was used. Before each experiment, the suspension was centrifuged for 20 min at 6000 rpm. The supernatant was removed in order to concentrate the nanoparticles by a factor of 10. Three batches of Ag Nps were synthesized and concentrated in the same way.

CHARACTERIZATION OF SILVER NANOPARTICLES

The characterization of the Ag Nps was carried out by UV–visible spectroscopy. The measurements were recorded with an Agilent 8453 UV–visible spectrophotometer from Agilent Technologies (Santa Clara, CA, USA) using a 10 mm quartz cuvette (Hellma Analytics, Mullheim, Germany) from 300 to 800 nm with 1 nm spectral resolution. The size and the zeta potential of the nanoparticles were determined with a Zetasizer Nano ZSP (Malvern Instruments, Malvern, England) using a capillary cell DTS 1070 (Malvern Instruments, Malvern, England). For both techniques, the metallic nanoparticles were diluted 10 times in ultrapure water. The diluted solution was then sonicated for 2 min in a Branson 2510MT ultrasonic cleaner (Branson Ultrasonics, Banbury, CT, USA) before measurements. The nanoparticles were also observed by Transmission Electron Microscopy (TEM). The TEM images were obtained with a JEOL 1400 Plus Scanning transmission electron microscope (JEOL, Tokyo, Japan) with an accelerating voltage of 120 kV using SightX-Viewer Ver.1.2.3.537, JEOL Tem Imaging Viewer. The samples for TEM analysis were prepared from one drop of the nanoparticles suspension deposited on a Formvar/Carbon coated copper grid and dried at room temperature before being imaged. The TEM images were then processed in ImageJ (National Institutes of Health, Bethesda, MD, USA) in order to determine the nanoparticles mean diameter.

PREPARATION OF PHARMACEUTICAL TABLETS

All the tablets ended with a mass of 200 mg, a diameter of 10 mm and a thickness of 2 mm, after having been compressed with a SPECAC (Slough, England) press system under 1 t. Paracetamol was chosen as pharmaceutical model in our study because it is a popular drug sold all around the world and is well known by our research group due to previous work based on the SERS quantification of

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its main impurity, 4- aminophenol (4-AP) [5,17]. Lactose monohydrate and D-mannitol were also selected as soluble compounds (solubility around 100 mg mL^{-1} in water at room temperature) in order to study if the effect of the covering method on the dissolution of the studied surface which can create a slight migration of the chemical compounds which composed it.

Prior to performing the MALDI-MSI acquisitions of the covered tablets, a homogeneous coating of a MALDI matrix was deposited by sublimation [18]. DHB was chosen as matrix material to highlight the mass spectra of silver and paracetamol components. Matrix deposition was performed by sublimation at a fixed vacuum of $1.6 \cdot 10^{-3}$ mbar. DHB matrix was deposited over 4.5 min at 140 °C according to Thomas et al. [19]. Under these conditions, the matrix layer was homogeneous over the entire tablet surface.

To ensure a clear understanding, the preparation of each tablet related to the experiments present in this paper is summarized in the Supplementary material (Table S-1).

COVERING METHODS

The tablet surfaces were covered by concentrated nanoparticles using three different ways and the homogeneity of the coatings was compared using different approaches.

DROP CASTING

Drop casting consisted in dropping 50 µL of the concentrated Ag Nps with a Rainin E4 XLS electronic pipette (Mettler-Toledo, Colombus, OH, USA) directly on the top of the tablet surface as shown in Fig. 1a.

ABSORPTION COATING

Absorption coating was performed by depositing 50 µL of the concentrated Ag Nps on a microscope slide and then by laying the tablet onto the drop as shown in Fig. 1b. The Ag Nps were absorbed by capillarity on the top of the tablets.

SPRAY-COATING

A spraying method was developed in order to deposit the concentrated Ag Nps on the tablet surface. Fig. 2 shows a schematic description of the deposition system developed for this study. The device was composed of a 250 µL gas tight syringe (SGE Analytical Science, Victoria, Australia) mounted on a Harvard model PHD 2000 infusion pump (Harvard Apparatus, Inc., Holliston, MA). The colloidal

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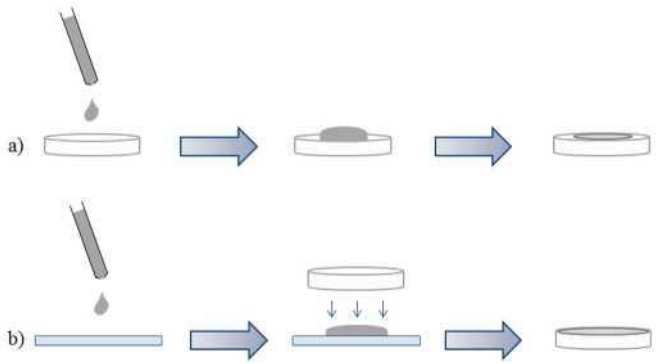
suspension was infused through a 100 cm length peek tube (Waters, Milford, USA) with an internal diameter (i.d.) of 0.127 mm from the syringe to an Electrospray Ionization (ESI) probe. The ESI probe was collected on a Q-Tof Premier Mass Spectrometer (Waters, Milford, USA) and includes a pair of coaxial tubes. The colloidal suspension was injected through the inner tube which is a stainless-steel capillary tube (185 mm length and 0.127 mm i.d.), and the outer tube was connected to a source of high pressure gas. An inert gas, nitrogen in this study (Air liquide, Paris, France) was used as the nebulizing gas to create a flow of small droplets. The gas pressure was fixed at 2 bars in order to achieve a dense spray of droplets.

Fig. 1*. a) Drop casting method: a droplet of concentrated metallic nanoparticles is simply drop onto the tablet surface. b) Absorption coating: the concentrated colloidal suspension is at first deposited on a glass slide and*



then absorbed by capillarity onto the tablet surface.

A design of experiments was conducted to determine the optimum parameters of the spray-coating system with JMP Pro 12 software (SAS Institute, Cary, NC, USA). A central composite design at three levels was used and the experiments with the different parameters are presented in Table S-2 (Supplementary material). The center point was performed in triplicate and the other points were measured once. The factors taken into account were the liquid flow rate and the spraying time. The aim was to determine the settings which minimize the heterogeneity of the covering and maximize the SERS signal intensity of the target compound. This optimization was carried out on tablets comprising one half of paracetamol/pvp and one half of 4-AP as shown in Table S-1 (Supplementary material) with the same batch of Ag Nps. Eleven SER- CI maps were then acquired and processed in order to determine the two independent responses per samples.

Once the settings are optimized, the droplet size was estimated using a Keyence VHX-2000F microscope with a 200× magnification (Keyence, Courbevoie, France) directly on a sprayed

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microscope slide after drying.

INSTRUMENTATION

RAMAN INSTRUMENTATION

Optimization of the spray-coating system.

During the optimization of the homemade spraying system, eleven mappings related to the eleven experiments were performed with a dispersive spectrometer, the Raman Station 400 F (Perkin Elmer, Waltham, MA, USA) equipped with a two-dimensional CCD detector (1024 × 256 pixel sensor) operating at - 50 °C. The wavenumber range was 400–3000 cm^{-1} with a resolution of 2 cm^{-1}. The laser excitation wavelength used was 785 nm with a power of 100 mW at the sample. One scan of 1 s exposure time was accumulated for each spectrum. The step size between two consecutives pixels was set at 200 µm with a total area of 52 × 52 pixels. The images were acquired with the Spectrum Image v6.1 software (Perkin Elmer, Waltham, MA, USA).

Study of the coffee-ring effect.

The study of the coffee-ring effect was realized with the same Raman spectrometer as reported previously. The studied spectral range was also fixed at 400 – 3000 cm^{-1} with a spectral resolution of 2 cm^{-1}. The laser excitation wavelength used was 785 nm with a power of 100 mW at the sample. One spectrum was acquired per pixel. The acquisition time was set at 1 s and the step size between each pixel set at 100 µm with a total area of 104 × 104 pixels.

Study of the soluble compounds migration.

The analyses were performed with a LabRAM HR Evolution system (Horiba Jobin-Yvon, Lyon, France) in order to reach a high spatial resolution. The microscope was equipped with a two-dimensional Newton 970 front- illuminated EMCCD detector (1600 × 200 pixel sensor). An objective of 10 × magnification and a 532 nm laser (Oxxius, Lannion, France) with a power of 50 mW were used for mapping measurements. A 300 gr/mm grating fixed at 1950 cm^{-1} (315–3310 cm^{-1}) was used to perform the mappings with a single acquisition of 500 ms (SWIFT™ mode). The confocal slit-hole was fixed at 200 µm. A 10 µm step size between each pixel was used in order to achieve high spatial resolution mappings and images of 1030 × 151 pixels were acquired with the LabSpec 6 software (Horiba Jobin-Yvon, Lyon, France).

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Fig. 2. *Schematic diagram of the homemade spray-coating apparatus developed for this study*

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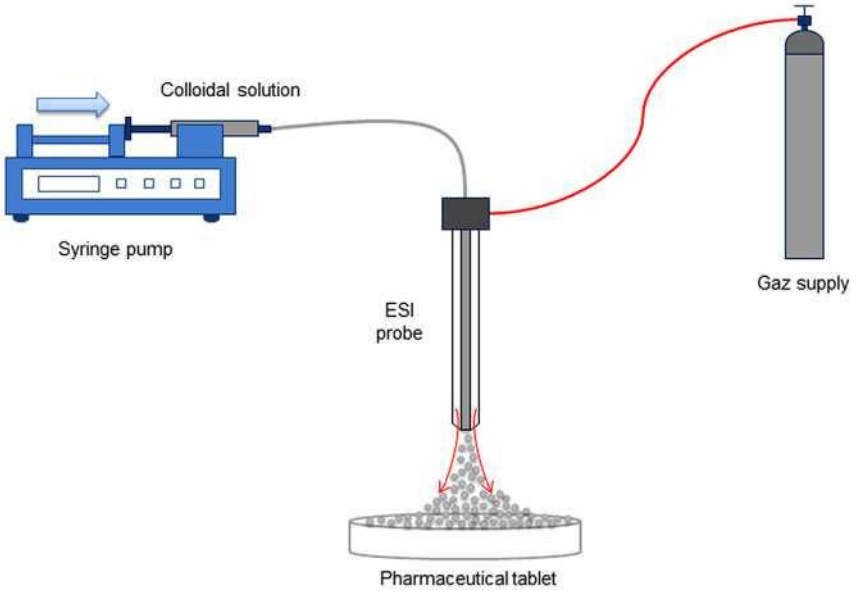

MALDI-MS INSTRUMENTATION

Data were acquired from a MALDI SYNAPT HDMS G1 (Waters, Milford, USA) in a fully automated mode driven by the MassLynx v4.1 software (Waters, Milford, USA). This system is equipped with a Nd:YAG UV laser at 355 nm wavelength operating at 200 Hz with a power of 100 µJ. The experiments were performed in the positive-ion mode in a mass range between 50 and 500 Da with a spatial resolution at 100 µm. The laser energy parameter in MassLynx software, corresponding to a dimming factor of laser power, was set to 200 (approximately 40 µJ). Calibration was achieved on P14R fragments in MS/MS mode (10 nMol of P14R in a 3.6 mg mL^{-1} solution of α-cyano- 4-hydroxycinnamic acid in 2:1 (v/v) CH_3CN/H_2O: 0.1% TFA).

The MS datasets were manually selected and aligned from an optical image of the tablet glued on a homemade plate with conductive tape from Shimadzu (Kyoto, Japan). MALDI Imaging Pattern Creator v1.1.0.0 software from Waters Corporation was employed for this step.

DATA ANALYSIS

The entire surface of each tablet was imaged and all the data processing was carried out on Matlab R2015a software (The Mathworks, Natick, MA, USA).

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SER-CI ANALYSIS

Considering the spherical geometry of the samples which did not cover the entire scanned square image, the area around the sample composed of highly noisy spectra, was removed. The region of interest (ROI) was selected manually by eliminating the desired area on the score image of the first Principal Component (PC) of a Principal Component Analysis (PCA) model. Once the background removed, each SER-CI map was transformed in a bi-dimensional matrix and was pre-processed separately using the Asymmetric Least Squares (AsLS) algorithm [20] to correct baseline variations with parameters of λ value of 10^5 and p value of 0.001.

Optimization of the spray-coating system.

On the 4-AP side of the dual zone tablet, a univariate analysis was applied based on the measurement of the peak intensity at 474 cm^{-1} which characterized the SERS response of the target compound. On the spectra acquired from the other side composed of paracetamol/pvp, the relative standard deviation (RSD) of the peak intensities at 922 cm^{-1} from citrate present around the Ag Nps surface were calculated. Univariate analysis of the mapping data was performed using the Trendtool analysis option of PLS Toolbox 8.5.1 (Eigenvector Research Inc., Wenatchee, WA, USA) running on Matlab R2015a.

Study of the coffee-ring effect.

The SER-CI maps were compiled in one image and a K-means algorithm was applied. This clustering technique was performed in order to highlight several classes in the mapping acquisition where a pixel is assigned to only one class. This algorithm principle is to segment the data in K clusters chosen as input by the operator for their representativeness [21]. In this case, the number of clusters was fixed at 3.

Study of the soluble compounds migration.

Univariate analysis of the mapping acquisitions before and after covering was performed. The area of the peak situated at 872 cm^{-1} related to D-mannitol was calculated in order to get the distribution map of the soluble compound.

MALDI-MSI ANALYSIS

Each mass spectrum was acquired into centroid mode. This acquisition mode reduces each Gaussian mass peak to only two values, a *m/z* ratio and an associated intensity. In addition, all *m/z* values were rounded to the nearest tenth Dalton to reduce again the data size. ROI selection was

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also applied in order to focus on the tablet pixels.

Besides the data reduction, a tiny shift of the mass spectrometer calibration during MALDI-MSI acquisition has been observed, leading to a slight spectral variations of few parts per million (ppm) of the m/z value in each spectrum. These sources of variability distorted the multivariate statistical analysis algorithm results of the MS dataset and were therefore removed. According to Gut et al. [22], a weighted average process was applied on the two dimensional matrix to avoid this phenomenon. A fixed size window was moved through the mass spectrometry dimension to compute the weighted average of *m/z* value per intensity of each peak.

From the mapping of covered tablets, the intensities of the peak at *m/z* = 106.9 related to the $[Ag]^+$ ion were measured after normalization by the intensity of the peak situated at *m/z* = 137 which comes from DHB matrix, $[DHB - H_2O + H]^+$, to avoid the variability caused by surface roughness. All computations were processed in Matlab R2015a (The Mathworks, Natick, MA, USA).

Results and discussions

CHARACTERIZATION OF SILVER NANOPARTICLES

UV–visible spectroscopy was used to characterize the synthesized Ag Nps. The UV–visible spectrum gives information about the repeatability of nanoparticles synthesis through the position of the peak related to the maximum absorption wavelength of the colloidal suspension as shown in Fig. S-1 (Supplementary material). The full width half maximum (FWHM) can also be used to estimate the nanoparticles size distribution, a larger peak being the result of a greater size distribution [23]. The mean maximum absorption of the three different batches was 406 ± 2 nm and the FWHM was 89 ± 6 nm. Dynamic Light Scattering (DLS) and Laser Doppler Electrophoresis (LDE) were also used to measure respectively the mean size and the zeta potential of the colloidal suspension. The average diameter obtained by DLS was 55 ± 7 nm and the average zeta potential of the nanoparticles measured was - 49.8 ± 0.6 mV. The latter value provides information about the stability of the nanoparticles, which must have enough positive or negative charges on their metallic surface in order to maximize the electrostatic repulsion between them [24]. TEM images were also acquired in order to compare and validate the fast and simple characterization methods presented previously. The images demonstrated the homogeneous size distribution of the Ag Nps and also their spherical shape (Supplementary material, Fig. S-1). The diameter measurement of the nanoparticles obtained

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on TEM images was 52 ± 8 nm and confirmed the results from the DLS measurements.

DEVELOPMENT OF A SPRAY-COATING METHOD

The SER-CI approach is limited in the pharmaceutical field due to the difficulty to cover homogeneously the tablets with the SERS substrates. No real strategies were explored until now to achieve this goal. In this context, a spray-coating method was developed using a homemade spraying deposition system. This technique is notably used in MALDI-MSI for the matrix deposition onto the sample [25]. It is also applied to the fabrication of thin film solar cells [26,27]. The covering process is simple. A colloidal suspension of metallic nanoparticles is sprayed on the tablet surface in small droplets produced by the nebulization of the suspension. A syringe pump controls the flow rate of the silver colloids suspension through an ESI probe capillary as previously described in Fig. 2. The ESI probe used as an airbrush is limited in operation because the nozzle diameter of the inner capillary tube is fixed and the handheld spray device is heavily operator dependent. Therefore, the skill of the user is important and need some practices in order to perform spray-coating with straight uniform strokes moving across the sample surface. To ensure optimal results, special care in setting the spray-gun motion by the operator is necessary. First, the spraying device should be held perpendicularly to the tablet surface. Secondly, the nozzle to sample distance must be approximately equal from one spray cycle to another, in order to prevent non-uniform coating and excessive overspray. However, any issue during the spraying can be quickly identified and controlled instantly by the operator with a handheld spray device, rather than a fully automated system.

Plenty of parameters are able to influence the spray quality and should be considered. Namely, the type of metallic colloids (metal and concentration), the liquid flow rate and the spraying time. The concentration of the colloidal suspension must be taken into account because the physical properties of the solution being sprayed have an effect on the flow and can increase the viscosity of the solution which can proportionally influence the droplet size [9]. In addition, a high amount of aggregated nanoparticles can also clog the capillary of the spray-gun. In another way, the density of metallic nanoparticles should be sufficient to generate an intense SERS signal [4]. A trade-off must therefore be done and consequently the suspension was concentrated by a factor 10. Considering the other parameters, the spraying time and the liquid flow rate could also affect the nanoparticles deposition.

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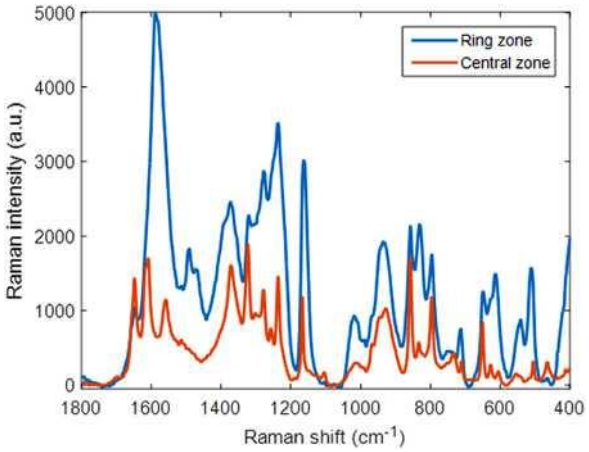
Fig. 3. *Representation of median SERS spectra collected in the ring zone (blue) and in the central zone (orange) of paracetamol/pvp tablets coated with the drop casting method. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)*



A central composite design was thus carried out implicating these spraying parameters in order to find the optimal conditions leading to a high repeatability of the signal and to a strong SERS enhancement of a target compound. In this context, the spraying time was chosen from 2.5 to 7.5 min which is a reasonable time to hold the spray-gun by the operator and the flow rate was selected from 5 to 15 µL min^{-1}. Above that flow rate, too many droplets can be accumulated on the sample surface and disturbed the surface homogeneity. The mappings were performed on tablets comprising one half of paracetamol/pvp and one half of 4-AP, covered by concentrated metallic nanoparticles. On the spectra acquired from the paracetamol/pvp area, the RSD of the citrate band intensities, located at 922 cm^{-1}, adsorbed on Ag Nps surface was calculated for each condition as first response. The RSD value should be as small as possible in order to obtain a covering with a high homogeneity. This citrate band is related to the distribution of the nanoparticles and as a consequence to the coating of the tablet [17]. Moreover, the peak intensity situated at 474 cm^{-1}, which characterized the SERS signal intensity of 4-AP was measured as second response. This latter should be maximized in order to have a strong signal enhancement. Results coming from both responses of the design of experiments are presented in Fig. S-2 (Supplementary material).

The optimum parameters were obtained with a 10 µL min^{-1} flow rate and a spraying time of 5 min which is a good compromise between high repeatability and strong signal enhancement. Increasing the flow rate exponentially augments the SERS signal intensity due to the high density of Ag Nps. The signal reached a maximum around 10 µL min^{-1} and remained stable before declining. Beyond 5

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min of spraying, the number of spray cycles increased and a large amount of silver colloids was delivered on the sample surface leading to a longer evaporation time which disturbed the homogeneity of what had previously been deposited. This heterogeneity could also be explained by the fact that the operator has to hold the spray-gun during a long time.

Under the optimal conditions, we estimated the corresponding droplets size by optical microscopy. One spray cycle was applied to a microscope slide and was then observed with a digital microscope. Fig. S-3 (Supplementary material) shows the droplets impact on the glass slide with an estimation of the average size diameter around 99.9 ± 10.5 µm.

CHARACTERIZATION OF THE COVERING METHODS

In this part, three coating techniques were compared: drop casting, absorption coating and spray-coating (see Section 2.4). The quality and the reproducibility of theses covering methods were evaluated notably by the study of the coffee-ring effect and the impact of the nanoparticles deposit on tablets comprising water-soluble compounds.

STUDY OF THE COFFEE-RING EFFECT

SER-CI analysis.

The tablets covered by Ag Nps were obtained by three different methods: drop casting, absorption coating and spray-coating. The drop casting method, illustrated in Fig. 1a, is very simple, fast and easy to implement but leads to a poor uniformity of the coating. When a droplet of the colloidal suspension is deposited on a tablet surface, the absorption and the evaporation of the solvent from the droplet leave a visible non-concentric ring on the surface [6]. This ring pattern results from the coffee-ring effect carrying the nanoparticles with a capillary flow to the pinned edge of the drying droplet. This nanoparticle transport mechanism is induced by the differential evaporation rates at the edges and at the center of the drop [8]. Consequently, the colloids are highly concentrated at the edge of the covering. This phenomenon perturbs the SERS measurements during the SER-CI analysis, resulting in remarkable variations in the SERS signal intensity which is higher in the ring zone than in the central zone. To illustrate this phenomenon, Fig. 3 displays the SERS spectra of paracetamol/pvp in the two different zones which clearly demonstrates an increase of the signal intensity and the appearance of anomalous Raman bands, notably at 1580 cm^{-1}. These bands are related to the sodium citrate added in excess during the Ag Nps synthesis and adsorbed onto the Ag Nps surface. This covering method is also not suitable for the coating of a large area because the

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substrate is not distributed totally on the surface before absorption. For a more reproducible coating that avoids an accumulation of the nanoparticles at the edge of the droplet, two others methods were considered: an absorption coating and a spray-coating method. In the first method, a drop of the colloidal suspension is placed on a microscope glass slide before putting a tablet on it. The drop is then absorbed by capillarity as it is shown in Fig. 1b [5]. In the second method, a spray generated by the system described previously was used.

As a preliminary observation, some visual differences between the three covering methods were obvious (Fig. 4). Notably, the formation of the ring pattern resulted from the coffee-ring effect in drop casting deposition. To further prove this point, a homogeneity study of these techniques was performed with the use of the K-means algorithm on a compiled image. The results are displayed in Fig. 4. The number of clusters was fixed at 3 in order to highlight the paracetamol/pvp tablet without any silver colloids coverage, the coffee-ring effect and a spreading phenomenon of the colloidal suspension. As shown at the bottom left of the K-means results in Fig. 4, the green class pixels allow to distinguish the paracetamol/pvp tablet without coating due to the poor area coverage with the drop casting method. The coffee-ring effect is emphasized by the blue class which clearly demonstrates the accumulation of Ag Nps along the edges of the dried droplet. This phenomenon is also visible on the edge region of the tablet covered by the absorption coating method. However, the main area of the tablet surface in the bottom center of Fig. 4 is represented by the red class. This latter corresponds to a spreading phenomenon of the nanoparticles with a SERS signal intensity lower than the intensity present at the edge of silver colloids covering as discussed previously. This variability of the signal intensity between those two classes is related to the Ag Nps concentration on the tablet surfaces. The red area is less concentrated in nanoparticles than the blue one. Concerning the spraying technique at the bottom right of the K-means image, the tablet surface is almost entirely dominated by the blue class. This spraying method reveals a much higher homogeneity of the coating. Moreover, the SERS signal intensity is equivalent to the one observed in the coffee-ring area.

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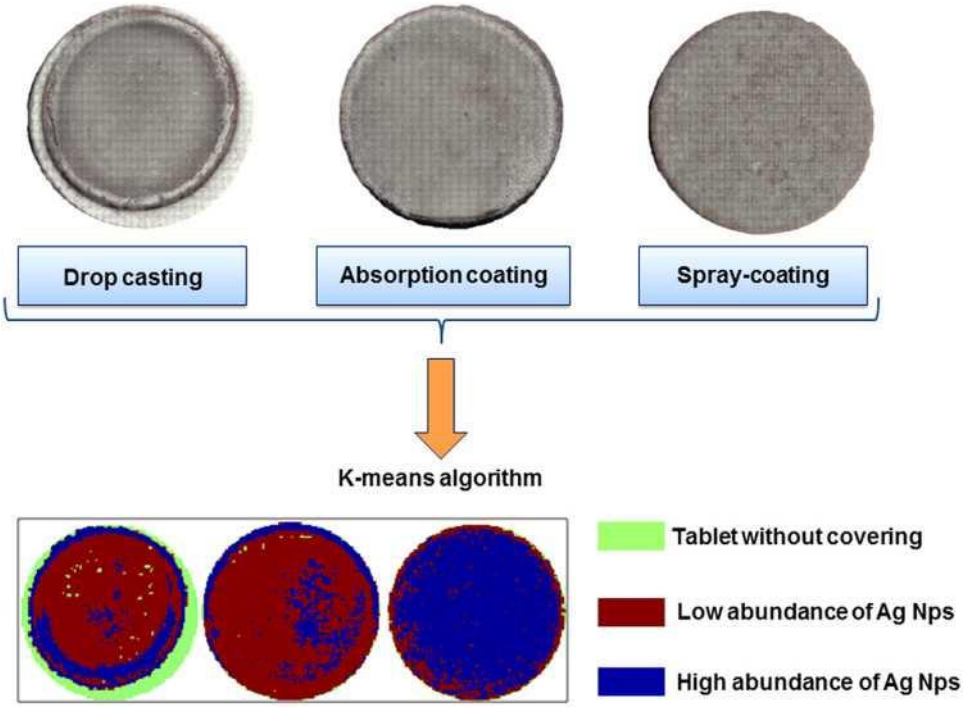
Fig. 4. *Application of the K-means algorithm on a SER-CI compiled image of tablets covered by three different coating methods, namely drop casting, absorption coating and spray-coating technique from left to right.*



Taking into account these results, the spray-coating method seemed to be a judicious method to cover tablets. A large area of the sample is covered, the nanoparticle deposition is uniform and the SERS signal intensity acquired can be significantly improved due to the concentration of Ag Nps.

MALDI-MSI ANALYSIS

The homogeneity of the Ag Nps covering was also characterized by MALDI-MSI. This imaging technique allows the localization of ions on the sample surface and highlights the ionization of Ag Nps. This approach has already been reported in the literature, for the analysis of the molecular composition of latent fingermarks [28,29]. Consequently, we focused on the $[Ag]^+$ ion distribution in this study. In the same way, as discussed in the previous section, the three covering methods were compared to visualize the $[Ag]^+$ ion distribution related to the silver colloids deposition. This approach was performed through a univariate analysis of the peak of the silver ion at m/z = 106.9 as shown in Fig. S-4 (Supplementary material). All the MALDI images acquired were normalized by the peak of the $[DHB - H_2O + H]^+$ fragment at m/z = 137 presented in Fig. S-4 (Supplementary material), enabling the comparison and a good reproducibility of all mass spectra contained in the same acquisition.

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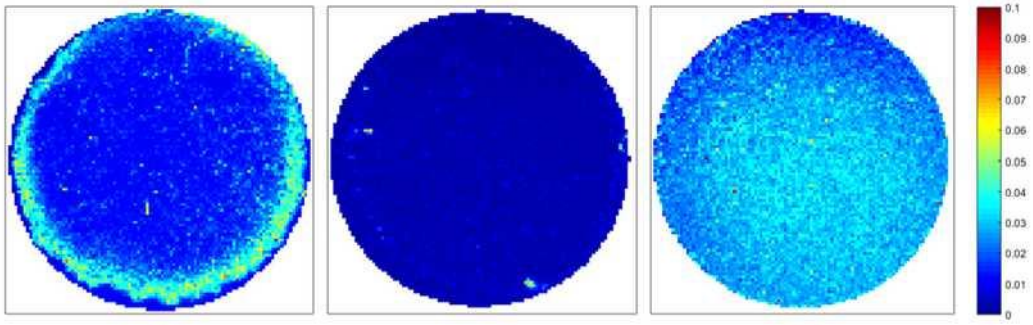
Fig. 5 shows the spatial distribution of the $[Ag]^+$ ion for the three covering techniques. Concerning the drop-casting method, the ring pattern of the coffee-ring effect is clearly apparent highlighting the high concentration of Ag Nps in the ring-zone in contrast to the central zone.

Fig. 5. *Representation of the silver ion distribution analyzed by MALDI-MSI on paracetamol/pvp tablets covered by silver colloids. On the left by drop casting, on the center by absorption coating and on the right by spray-coating method.*



The MALDI image revealed the difference in intensity of the $[Ag]^+$ ion between these two areas and therefore an inhomogeneity of the Ag Nps distribution. This information confirms what we previously discussed for the study of this phenomenon with the use of K-means algorithm in SER-CI images. Regarding the absorption coating, the ion intensity of the silver cluster ion is extremely low under the same analytical conditions which may be explained by a lower concentration of Ag Nps on the direct tablet surface. This makes it difficult to assess the surface homogeneity of the covering which can be explained by the diffusion of silver colloids inside the tablet. In contrast, the analysis by MALDI-MSI of the tablet coated by spray-coating technique on the right of Fig. 5, showed a uniform distribution of the $[Ag]^+$ ion.

Despite the similar amount of metallic nanoparticles deposited on the tablet surfaces before MALDI-MSI acquisitions, the $[Ag]^+$ ion distribution is different from a covering to another. A major amount of Ag Nps is located in the ring zone of the coffee-ring effect for drop casting deposition while a higher concentration is situated inside the tablet for the absorption coating. Hence, a major benefit of using spray-coating is that the majority of the SERS substrate remains homogeneously distributed on the sample surface allowing the whole analysis of the tablet by chemical imaging.

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STUDY OF THE SOLUBLE COMPOUNDS MIGRATION

In addition to homogeneously cover the pharmaceutical samples with the SERS substrates, an important issue in the application of SER- CI is the distortion and the dissolution of the sample in contact with the colloidal suspension. This could be the case notably for highly water soluble compounds, since water is the solvent used for the Ag Nps synthesis. The three different covering methods described previously were performed on tablets in order to investigate the impact of nanoparticles deposition on the migration of soluble compounds constituting the tablets. This study was carried out on dual zone tablets composed of one left half of D-mannitol and one right half of lactose monohydrate. These two compounds were chosen due to their high solubility in water. Owing to the fast acquisition parameter used in order to decrease the high overall image acquisition time, i.e. 500 ms, the signal-to-noise ratio of the lactose spectrum was low and no specific bands were highlighted. On the contrary, the D-mannitol did not require a high acquisition time to reach the appropriate signal-to-noise ratio and to highlight its main Raman band at 872 cm^{-1}. For this reason, the peak area of the band situated at 872 cm^{-1} related to D-mannitol, which is seen in Fig. 6, were calculated in order to follow the distribution map of this compound before and after the covering on each tablet.

Mappings crossing each tablet on the area illustrated by red dashed line rectangles are displayed in Fig. 7. They were performed before and after SERS substrates deposition. Visually, the tablets covered by drop casting and absorption coating are distorted with the formation of many small holes and fine cracks. This difference of surface states after nanoparticles covering can be explained by the porosity of the tablets and the solvent absorption. These covering methods with an important liquid phase deposition resulted in the dissolution of the studied surface and created a slight migration of the chemical compounds which form it. This phenomenon can thus disturb the spatial characteristics of the analyzed sample, leading to reproducibility issues of the SERS signal during the SER-CI analysis. With spray-coating, the spatial characteristics of the surface were unchanged due to the fast evaporation of the solvent in contact with the sample allowing the nanoparticles to remain onto the surface. Moreover, unlike for spray-coating, we observed a deformation of the demarcation line between the two compounds for drop casting and absorption coating which demonstrated a slight migration of the compounds on both sides.

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Conclusion

This work presents an efficient spray-coating method using a homemade system which demonstrated to be an excellent alternative to drop casting deposition of the SERS substrates onto pharmaceutical tablets. This spraying method provides a significant control of the metallic nanoparticles deposit, sprayed over the sample surface in a homogeneous way. The parameters of the system allowing to obtain high repeatability and strong enhancement of the SERS signals have been optimized by a design of experiments. Compared with others covering techniques, the performance of the spraying technique based on specific criteria such as homogeneity, SERS signal intensity and effects of the colloidal suspension on soluble compounds were investigated.

Homogeneity studies conducted by SER-CI with K-means algorithm and MALDI-MSI showed similar results which demonstrated a uniform distribution of the Ag Nps on the sample surface. We found that using spray-coating method, the colloidal suspension was not fully absorbed inside the tablet and remained on the surface which is necessary for SER-CI analyses. Finally, we observed that the technique did not lead to the dissolution or the migration of water soluble compounds that can be contained in a pharmaceutical tablet.

In order to cover the samples more efficiently, the spraying system could be improved in future works with an automatic spraying platform controlling the nozzle distance, the nozzle velocity and the spacing between two adjacent spray cycles.

Fig. 6. *Representation of Raman (left) and SERS (right) spectra of lactose (blue) and D-mannitol (orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)*

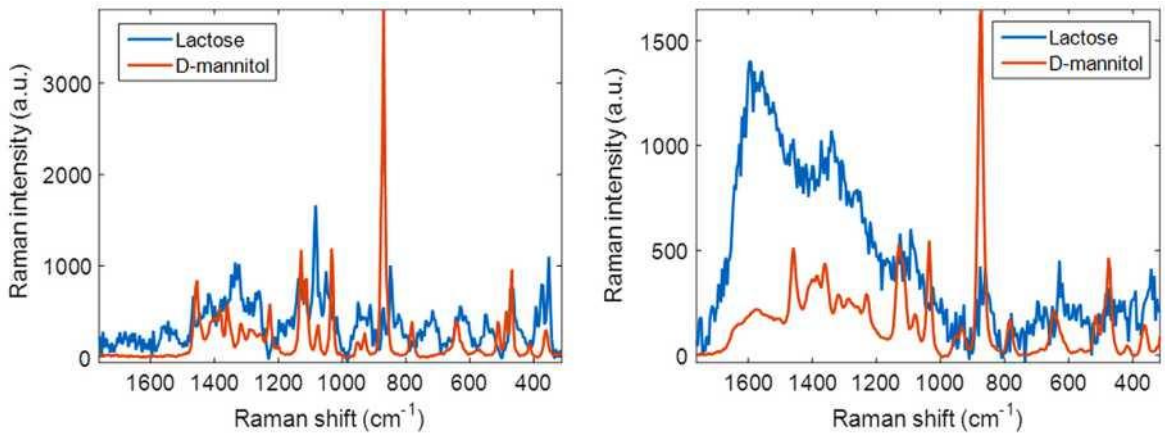

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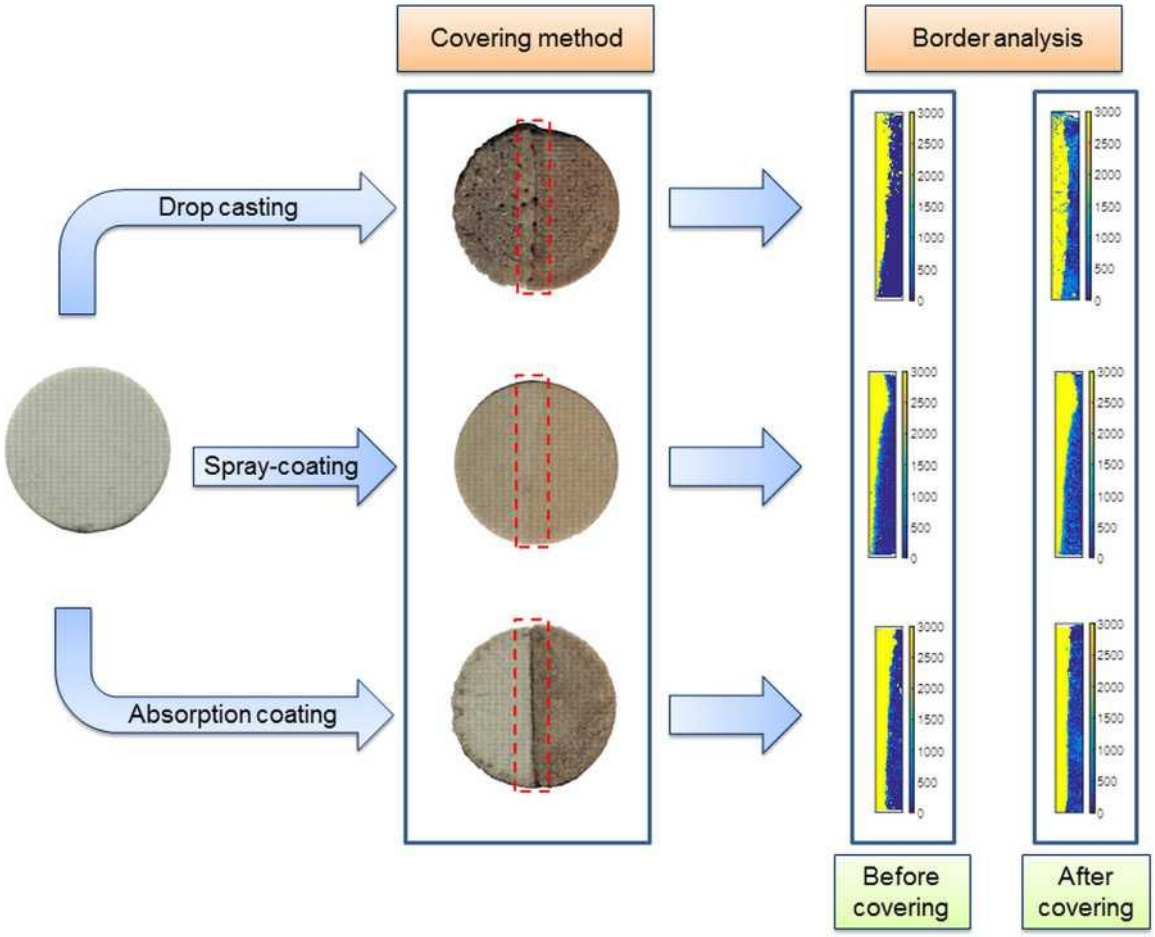
***Fig. 7**. Impact of the covering of Ag Nps on tablets based on water soluble compounds.*



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DECLARATIONS OF INTEREST

None

APPENDIX A. SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2018.06.037.

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