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Development of a quantitative approach using surface-enhanced Raman chemical imaging: First step for the determination of an impurity in a pharmaceutical model☆

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ABSTRACT

This publication reports, for the first time, the development of a quantitative approach using surface-enhanced Raman chemical imaging (SER-CI). A pharmaceutical model presented as tablets based on paracetamol, which is the most sold drug around the world, was used to develop this approach. 4-Aminophenol is the main impurity of paracetamol and is actively researched in pharmaceutical formulations because of its toxicity. As its concentration is generally very low (<0.1%, w/w), conventional Raman chemical imaging cannot be used.

In this context, a SER-CI method was developed to quantify 4-aminophenol assessing a limit of quantification below its limit of specification of 1000 ppm. Citrate-reduced silver nanoparticles were used as SERS substrate and these nanoparticles were functionalized using 1-butanethiol. Different ways to cover the tablets surface by butanethiol-functionalized silver nanoparticles were tested and a homogeneity study of the silver nanoparticles covering was realized. This homogeneity study was performed in order to choose the best way to cover the surface of tablets by silver colloid. Afterwards, the optimization of the SER-CI approach was necessary and different spectral intensity normalizations were tested. Finally, a quantitative approach using SER-CI was developed enabling to quantify 4-aminophenol from 0.025% to 0.2% in paracetamol tablets. This quantitative approach was tested on two different series of tablets using different batches of silver nanoparticles.

1. Introduction

During the last decade, Raman imaging has taken an important place in the analysis of pharmaceutics [1,2]. Raman imaging enabled the assessment of active pharmaceutical ingredient (API) solid-state within pharmaceutical sample [3–5], the study of distribution of different constituents in pharmaceutical formulations [6–9] and the quantitative distribution analysis of API in a pharmaceutical device [10]. It has also been used to estimate the particle size of the API in a nasal spray formulation [11] and to detect counterfeit medicines [12].

The keen interest for this technique can be explained by the possibility to obtain simultaneously a visual representation of a sample enabling the evaluation of the molecules distribution within it using spatial information while allowing their identification and quantitation using spectral information [13]. Furthermore, this technique keeps the main advantages of classical Raman spectroscopy including non-invasive, non-destructive and solvent free technique. Moreover, the sample preparation is negligible. Regarding tablets, it involves only the milling of tablet surface before the Raman imaging to obtain a flat sample surface. However, Raman imaging also presents limitations namely its lack of sensitivity and the appearance of fluorescence which can limit its pharmaceutical applications [14]. For example, using Raman imaging, the analysis of tablet surrounded by a coloured coating or comprising coloured constituents is difficult due to the fluorescence and the detection of low-dose constituent such as an impurity is not possible due to the weak sensitivity of this technique. One way to circumvent these limitations is surface-enhanced Raman scattering (SERS) which enables to dramatically increase the Raman

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scattering of molecules which are adsorbed or very close to rough metallic surfaces [15]. Therefore, SERS enables to obtain spectral information of very low dosed components allowing qualitative and quantitative analysis while reducing the fluorescence effect on spectra. Gold and silver nanoparticles are mainly used as SERS substrate because of the ease to implement their synthesis and the possibility to easily functionalize their surface using different molecules. Some SERS pharmaceutical applications based on the quantitative determination or detection of low-concentrated analytes are found in the literature [16–19]. Regarding surface-enhanced Raman chemical imaging (SER-CI), it results from a combination of the advantages of SERS and chemical imaging. However, the number of publications based on this technique in the pharmaceutical field is very limited [20]. This phenomenon could be explained by the well-known stability and reproducibility problem of SERS and also by the difficulty to obtain a homogeneous colloid covering of samples surface before SER-CI analyses.

In this context, the possibility to develop a quantitative approach using SER-CI on a pharmaceutical model was studied. The model paracetamol/4-aminophenol presented as tablets was chosen for several reasons. First, 4-aminopenol (4-AP) is the main impurity of paracetamol, the most sold drug around the world, coming from its degradation during the storage or from its synthesis and which might be present in pharmaceutical formulations at low concentration. This impurity is actively researched because of its toxicity including nephrotoxicity and hepatotoxicity [21]. The limit of specification of 4-AP in paracetamol tablets is 1000 ppm or 0.1% (w/w) to ensure the drug safety according to the British Pharmacopoeia [22]. Conventional Raman imaging is not able to detect this impurity within paracetamol tablets at its limit of specification. Therefore, SER-CI might be interesting in this pharmaceutical case to enhance the response of 4-AP. Secondly, 4-AP presents an amine group in its structure which is very interesting for SERS analyses. Indeed, 4-AP can be adsorbed on metallic surface thanks to its amine group and thus its Raman scattering can be selectively enhanced which is not the case for the other constituents of the paracetamol tablets [23]. In this context, spectra obtained by SER-CI of paracetamol tablets will be dominated by the SERS response of 4-AP. It is therefore possible to consider the selective detection of this molecule in presence of a large amount of API or excipients. Finally, this pharmaceutical model is well known by our research group as it has been considered in a previous work based on quantitative SERS in aqueous medium [16].

2. Materials and methods

2.1. Chemicals and reagents

Silver nitrate ($AgNO_3$, extra pure) was obtained from Merck (Darmstadt, Germany). Trisodium citrate (anhydrous, 98%) and 1-butanethiol (BT) (purity > 99%) were obtained from Acros Organics (Morris Plains, USA). 4-aminophenol (purity > 99%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). A mixture of paracetamol/polyvinylpyrrolidone 3% (paracetamol/pvp) was kindly provided by S.M.B. Technology (Marche-en-Famenne, Belgium). Ultrapure water was generated from a Milli-Q system (Millipore, Bellirica, MA, USA).

2.2. Preparation of butanethiol-functionalized silver nanoparticles

2.2.1. Silver nanoparticles synthesis

Silver nanoparticles (Ag Nps) were synthesized according to the method described by Lee and Meisel [24]. All glassware (three-neck round bottomed flask of 500 mL, stirrer and condenser) was rigorously cleaned with freshly prepared aqua regia ($HCl:HNO_3$, 3:1, v:v) and thoroughly rinsed with ultrapure water. Silver nitrate (45 mg) was dissolved in 250 mL of ultrapure water and heated to boiling in a Drynsyn heating block (Asynt, England) fitted with a probe of temperature. Then, 5 mL of a solution of trisodium citrate 1% was added using a dosing device (Dosimat, Metrohm AG, Herisau, Switzerland) set at a dropping rate of 5 mL min^{-1}. The addition of trisodium citrate, which is used as a reducing agent, was realized under stirring and boiling. The resulting solution was kept on boiling for 1 hour while refluxing and then cooled down to room temperature. A heating block and the dosing device were used to improve the repeatability of the synthesis from batch to batch. Two batches of Ag Nps were synthesized (one per series).

2.2.2. Silver nanoparticles characterization

To characterize the synthesized Ag Nps, visible absorption spectra were recorded with a Lambda 40 spectrophotometer (Perkin Elmer, MA, USA) from 300 to 800 nm using a 1 cm quartz cuvette. To determine the mean diameter of Ag Nps, the photon correlation spectroscopy (PCS) using a high performance particle sizer (HPPS) instrument (Malvern instruments Ltd, Malvern, England) was used. Measurements were made at 25 °C with a fixed angle of 90° and the results were expressed as the average nanoparticles hydrodynamic diameter (nm). Viscosity and refractive index of pure water were used. The system was calibrated with an aqueous polystyrene dispersion of particles with a 100 nm diameter. For both techniques, samples of Ag Nps were prepared by solving 400 μL of the colloidal suspension in 3600 μL of ultrapure water. Resulting solutions were sonicated for 2 min before measurements.

2.2.3. Functionalization of silver nanoparticles surface by 1-butanethiol

To functionalize the silver nanoparticles surface, a solution of butanethiol (BT) was prepared at a concentration of 16.8 mg mL^{-1} and 500 μL of this solution were added in a centrifuge tube with 10 mL of the Ag Nps suspension. The resulting solution was shaken for 2 min before being centrifuged for 20 min at 6000 rpm. Nine millilitres of the supernatant were removed in order to concentrate the silver nanoparticles functionalized with butanethiol (BT-functionalized Ag Nps). The residue was suspended and sonicated for 2 min before its use.

2.3. Preparation of the pharmaceutical model for SER-CI analyses

2.3.1. Preparation of paracetamol/4-aminophenol tablets

Appropriate amounts of the mixture paracetamol/pvp and 4-AP were grinded in a mortar with a pestle to obtain a final concentration of 1% (w/w) of 4-AP. Different amounts of this mixture were weighted and grinded with paracetamol/pvp to constitute five different blends comprising 0.025%, 0.05%, 0.1%, 0.15% and 0.2% (w/w) of 4-AP. From each blend, tablets with a diameter of 13 mm were prepared using a tablet hydraulic press (Perkin Elmer). Three tablets for each concentration level were necessary to develop the quantitative SER-CI approach. This procedure was realized two times on different days and the SER-CI analyses were performed using two different batches of Ag Nps.

Tablets comprising only the mixture paracetamol/pvp were also pressed and used to carry out a homogeneity study of the silver colloid covering.

The surface of tablets was prepared before colloid covering using the pharmaceutical milling system EM Rapid equipped with a Tungsten Carbide miller (Leica Microsystems GmbH, Wetzlar, Germany). The tablets were stuck on a microscope slide and the outer of each one was milled to obtain a flat surface.