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Quantification of low drug concentration in model formulations with multivariate analysis using surface enhanced Raman chemical imaging

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ABSTRACT

This paper reports the application of surface enhanced Raman chemical imaging (SER-CI) as a potentially non-destructive quantitative analytical method for the investigation of model pharmaceutical formulations containing the active pharmaceutical ingredient (API) in low concentrations (0.5–2%). The application of chemometric techniques for processing the spectra enables the determination of API distribution in products of different concentrations. In addition, the applied multivariate curve resolution can be proper method to identify unexpected contaminants in illicit drugs. The drastic Raman signal enhancement in the presence of silver nanoparticles provides significantly improved calibration accuracy and, at the same time, radically decreased image acquisition time compared to conventional Raman chemical imaging.

1. Introduction

Raman chemical imaging (R-CI) and its specialized versions such as hyperspectral stimulated Raman scattering microscopy and coherent anti-Stokes Raman scattering microscopy have become important imaging techniques for quantitative analysis [1–3]. However, in spite of these improvements, some typical disadvantages of Raman spectroscopy, such as low sensitivity and long image acquisition time still limit the applicability of R-CI. Taking advantage of the powerful signal enhancing behavior of metal (primarily silver and gold) substrates, surface enhanced Raman spectroscopy (SERS) can offer a solution for the aforementioned difficulties [4–12]. The benefits of SERS can be utilized in quantitative analytical method development as well [13–15], for instance, in the case of pH sensors [16], nucleotide chemistry [17] or marine applications [18]. The combination of R-CI with SERS, called surface enhanced Raman chemical imaging (SER-CI), has also started to gain serious attention in biotechnology and nanotechnology [19–21]. In our previous work [22] the potential of SER-CI was demonstrated in the investigation of drug distribution in tablets, where radical decrease was reached in the acquisition time using SER-CI. Furthermore, the spatial distribution of the active pharmaceutical ingredient (API) could be revealed well below the detection limit of R-CI and thus the characteristics of different manufacturing technologies could be identified at very low API concentrations. However, the results could not provide any quantitative or even semi-quantitative information about the API content. Moreover, to the best of our knowledge, there is only one publication regarding the possible application of SER-CI for quantitative analysis of an active component in pharmaceutical products [23]. There the authors applied a univariate approach without deeper statistical analysis. Therefore, further studies were required to develop a quantitative multivariate technique, supported by detailed statistical calculations, to estimate the amount of the API using SER-CI. Furthermore, tablets can be examined owing to applied chemometric method without any previous knowledge about Raman or surface-enhanced Raman spectra of the components such as suspect, illegal or counterfeit products. For instance, some curve resolution methods may tackle these challenges.

Although R-CI is a widespread technique in pharmaceutical technology [24] and it can also be used to get quantitative information about pharmaceutical products, this requires extremely

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long acquisition times, making it hardly applicable for daily pharmaceutical practice. Moreover, in the case of low (<2%) drug concentrations, accuracy problems can easily occur with the traditional approach (unless the API is of very strong Raman scattering character), making the quantification difficult or impossible at such low concentrations.

This study intends to offer a solution to the aforementioned problem by making SER-CI capable of providing appropriately accurate quantitative information based on spatial API distribution maps, while the drastic reduction of image acquisition time is still maintained. In addition, statistical calculations serve for making SER-CI capable to achieve much higher accuracy and applicability than R-CI at low drug concentrations. According to our hopes the applied partially destructive method can be replaced a truly non-destructive technique with further improvements, where the silver particles will be embedded in a thin transparent film allowing their fully removal from the sample surface after SER-CI.

2. Materials and methods

2.1. Preparation of model formulations

Lactose monohydrate (LMH) was purchased from Sigma–Aldrich. The active pharmaceutical ingredient is referred to as API instead of its original name due to IPR (industrial protection of rights) reasons. Model mixtures were prepared by blending API and LMH in a mortar to ensure homogeneous drug distribution. Model formulation samples weighing 400 mg each were prepared in a Manfredi 0057C00 type KBr disk press. API contents were 0.25%, 0.5%, 1%, 1.5% and 2% in mass ratio.

2.2. Preparation of SERS colloid

Ag nanoparticles were prepared by Lee and Meisel's method [25], which is widely used in SERS studies to synthesize silver substrates [26–30]. 90 mg of silver-nitrate (Reanal Ltd.) was dissolved in 500 ml of double distilled water. The solution was heated to boil and 10 ml of 1% trisodium-citrate (Sigma–Aldrich) aqueous solution (also made with double distilled water) was added dropwise under vigorous stirring. Boiling was continued for 10 min. Upon completion of the reaction, a greenish-gray colloidal solution was obtained.

2.3. Raman instrumentation

For each mapping experiment, Raman imaging spectra were collected using a Jobin Yvon Labram instrument attached to an Olympus BX-41 microscope. The samples were illuminated with frequency-doubled Nd-YAG laser (532 nm). An objective of 50× magnification was used for optical imaging and spectrum acquisition. The outer surface of the model formulations was investigated in every imaging experiment without any sample preparation process.

Before SER-CI analysis, four types of R-CI reference map series were taken from each sample. The first type "background" reference series used the same imaging parameters as SER-CI analyses (see later) to ensure that no signals of the API (or the excipient) are detectable without SERS. Such "background" maps are not presented in the paper, as they only consisted of noise.

The other three types of reference (R-CI) images were obtained by setting high enough acquisition times to detect the signals of the API, in an attempt to reveal the distribution of API without SERS. Acquisition times for a spectrum were 0.8 s (method I), 3 s (method II) and 10 s (method III); and twenty such spectra were accumulated and averaged at each pixel to get proper signal-to-noise ratio. The step size between neighboring pixels was increased to 200 μm along both axes to avoid the sampling error. As a compromise between map size and overall imaging acquisition time, the measured area on the sample surfaces was 31 pixel × 31 pixel and acquisition of each map took 4.2 h (method I), 16 h (method II), and 53 h (method III). When applying 10 s acquisition time, the confocal hole was set to 500 μm to avoid unnecessary signals from the neighboring pixels.

For SER-CI analysis, SERS colloid was dropped on top of the samples and, after drying, mapping was performed on their surface (i.e. without any further sample preparation to avoid alteration of the sample structure). In this case, spectrum acquisition time was 0.5 s per pixel and only 1 spectrum was taken at each point without any multiple accumulations or averaging, to avoid degradation of the colloid system. For these images, step size of 50 μm was used between neighboring pixels to achieve high spatial resolution, and the investigated area was 49 pixel × 49 pixel. The average particle size of API was found below 50 μm due to fragmentation during the grinding that can be seen on the microscopic images of Figure SM-1. Consequently, one particle was not measured more than once and the adjacent pixels were independent. The overall acquisition time for each SER-CI and "background" image (without SERS effect) was only 20 min.

For SER-CI analysis the laser power was decreased to 10% of its original value with an intensity filter for the same goal (to avoid damage to colloids), while full power (~50 mW) was used for the R-CI investigations. The spectrograph was set to provide a spectral range of 400–1835 cm^{-1} and 3 cm^{-1} resolution.

2.4. Data analysis

R-CI and SER-CI maps were processed with the same multivariate curve resolution-alternating least squares (MCR-ALS) method we applied in our previous work [22]. The developed multivariate approach using MCR-ALS enables to assess the Raman maps for any SERS-active ingredient. The method was applied for the API through similar steps in this paper as those shown for acetylsalicylic acid in our previous study [22]. As the details are thoroughly discussed in the referred paper, the description of this chemometric technique and the detailed procedure for the spectral preprocessing and the evaluation of R-CI and SER-CI images are presented in the electronic supplementary material (ESM). It has to be noted that the iterative curve resolution methods, in general, give no unique solution. Experimental errors and uncertainties in data may lead to indeterminacy in concentrations and spectral profiles called rotational ambiguity. The problem persists even when concentrations are known in calibration and quantitative model building [31]. Generally, the width of feasible bands can be restricted applying proper constraints according to our previous knowledge about the nature of studied systems. Many authors have already reported about these constraints [32]. In our work, setting non-negativity for spectra and concentrations, and normalization of spectra were used to reduce ambiguities. The band boundaries of the feasible regions were investigated and indicated with MCR-BANDS algorithm [33] and are shown in the ESM. However, even MCR-BANDS may give improper bands as reported in the literature [34]. Further relevant publications are cited in the supporting material as references SR1–SR19, and also in the present manuscript as Refs. [37–55].

In the course of the quantitative evaluation of R-CI and SER-CI results, the calibration point belonging to the model formulation with 0.25% API content was taken out from the quantitation process, because preliminary SER-CI investigations indicated that linear correlation primarily existed only between 0.5% and 2% API content.

Statistical investigations were performed on the calibration datasets to compare the linear regression models. The sum of squares due to lack-of-fit (SSLF) and residual sum of squares (RSS)