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Methodologies for assessment of limit of detection and limit of identification using surface-enhanced Raman spectroscopy

Enrico Massarini[a,c], Pär Wästerby[b], Lars Landström[b], Christian Lejon[b], Olof Beck[c], Per Ola Andersson[b,*]

[a] Department of Public Health, Section of Legal Medicine, University of Rome "Tor Vergata", 00133 Rome, Italy
[b] Swedish Defence Research Agency, CBRN – Defence and Security, Cementvägen 20, SE-90182 Umeå, Sweden
[c] Department of Laboratory Medicine, Section of Clinical Pharmacology, Karolinska University Hospital, SE-141 82 Stockholm, Sweden

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ABSTRACT

Here we rationally evaluate surface-enhanced Raman spectroscopy (SERS) substrates in terms of limit of detection (LOD), limit of identification (LOI) and dynamic range for ten common narcotic drug analytes. The drugs were amphetamine, cocaine, methadone, diazepam, methylphenidate, oxazepam, tramadol, morphine, buprenorphine and 6-monoacetylmorphine. A Raman microscope system was complemented with portable instrumentation, both in conjunction with commercial SERS substrates, and, by vibrational peak assignments, the functionality of substrates and pureness of samples was ensured. The dynamic range is explored qualitatively by concentration series measurements, where the Langmuir adsorption isotherm provided good fits. Moreover, an output fit parameter, the inverse of Langmuir constant, was found to roughly scale with LOD and can therefore be helpful in SERS substrate evaluations. Four different statistical methodologies were tested to estimate LOD: (i) a general formula to calculate a one-sided prediction interval for the mean value of blanks (LOD_B), (ii–iii) calculated from a one-sided prediction interval (at significance level 0.05) of a linear regression line, where the obtained limit of detection in the signal domain was sometimes outside the linear concentration range, which is why the corresponding concentration was calculated from (ii) a linear calibration curve (LOD_{LR}) and (iii) a non-linear calibration curve (LOD_{NR}), and (iv) using receiver operating characteristic (ROC) curves to estimate LOD_{ROC}. Here, a new optimization approach was introduced for LOD_{ROC} estimation, based on interpolation and thus better suited to handle a few data points spanning a large concentration range. LOI was assessed by discriminant analysis of partial least squares (PLS-DA) classification for seven of the drug compounds using PLS-DA, and the extracted LOIs were found to be higher than the LODs and were varying with respect to accuracy of the model which is strongly correlated to the probability of false positive detection that can be accepted.

1. Introduction

The phenomenon of surface enhanced Raman scattering (SERS) was discovered during the seventies [1–3] by studying Raman signals from roughened silver electrodes, and afterwards the SERS mechanism has been widely debated and examined both experimentally and theoretically [4,5]. The enhancement relative normal Raman scattering varies a lot and depends on the local electromagnetic field enhancement induced by resonant coupling between the surface plasmons in the metallic nanostructures and incident laser light, as well as the "chemical enhancement". Factors that influence the strength of local field enhancement are many, e.g., geometry and distribution of nanostructures and the optical properties of the material (e.g., Cu, Ag, Au). Normally, the structures are optimized for laser excitation in the visible or NIR wavelength region. The "chemical enhancement", which arises from species that are chemisorbed to the surface and become a charge-transfer complex, can nowadays be carefully modeled [6]. For both mechanisms the proximity of the analyte to the surface is fundamental, meaning that the substrate-molecule interaction is another key parameter to consider. So called single molecule detection (SMD) was reported for the first time 1997 [7,8]. Here, the molecules should preferably be situated at the junction (hot spots) of nanoparticle aggregates and/or organized arrays, and a lot of attention has been focused on SMD utilizing lithographic nanofabrication techniques [5,9], but recently, increased interests has raised on SERS measurements

* Corresponding author. Tel.: +46 90106842; fax: +46 90106800.
E-mail address: perola.andersson@foi.se (P.O. Andersson).

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on ensemble average enhancement. The corresponding substrates could be fabricated by less costly approaches [9,10], which also looks more promising to be incorporated in qualitatively and quantitative analytical applications [5]. Lately, researchers have, in addition to sensitivity and reproducibility, started to investigate parameters that are of more concern in analytical set-ups, i.e. ease of handling, long shelf time and/or regeneration, dynamic range and cost [11,12]. Today, SERS based applications are performed in a broad range of disciplines, e.g., in areas of medical diagnostics [13], food safety [14], environmental monitoring [15], security and defence [12]. As the number of analytes increases, it is appropriate to introduce selectivity in the SERS methodology, which commonly is supported by powerful algorithms and chemometric based analyses. A remaining challenge is to introduce sufficient selectivity to resolve signals from an interfering matrix, as for example for drugs in saliva [16–18] or antibiotic substances [19] and nicotine [20] in urine. The first step towards this goal is to obtain SERS substrates that are giving rise to reliable, reproducible and satisfying detection limits in the analytical set-up. There are numerous of different versions of SERS substrates that can be fabricated and few are commercial available. It is not obvious to deduce which fabrication procedure to choose and subsequently which SERS substrate is optimal for respective application. During the last years, different research groups have started to discuss how to evaluate SERS substrates from a standardized and analytical point of view [21–23], thus determining analytical and spectroscopic figures of merit.

To characterize sensor systems or analytical tools in general, key parameters to be determined are related to the sensitivity and specificity (or selectivity) in form of limit of detection (LOD) and limit of identification (LOI), respectively. In the literature varying statistical and chemometric methodologies are represented for this purpose and it has been argued that they result in different limits [24] and hence it is not straightforward to draw conclusions about the best methodology or sensor performance. In real world applications, background signals could vary a lot, as well as the number and origin of interfering substances, which should also be considered in an evaluation study. However, with respect to SERS substrate evaluation, receiver operating characteristic (ROC) protocols have recently been introduced [22]. In the spirit of the above, we have herein evaluated commercial SERS substrate using drug substances which could be considered both as model compounds and as chemicals of high relevance to trace in more or less complex surroundings [25]. SERS signals were recorded at various drug concentrations and by introducing chemometric analyses based on partial least squares discriminant analysis (PLS-DA), a model is built to elucidate the potential of identification. Furthermore, different methodologies to achieve values of LOD [24] are performed and discussed. Especially, in order to estimate LOD based on ROC curves a new optimization strategy is presented. Another helpful component is fitting of non-linear data with the Langmuir isotherm to transform data from signal to concentration regime and to generate a fitting parameter that scales with LOD trends of the drugs. Finally, it is worth to point out that the resulting data and limits are not universal but are strongly associated to instrumental and experimental conditions applied, thus herein one should focus the attention on the methodology procedures rather than specific data.

2. Materials and methods

2.1. SERS substrates and chemicals

Commercial SERS surfaces were used in this study: Klarite® 313 (Renishaw Diagnostics, Ltd). These substrates have been well characterized elsewhere and the enhancement effect is well documented [26]. Briefly, the active surface consists of equally spaced μm-sized inverted square-based pyramids (apex pit angle of 70.5°) etched in silicon with a gold layer deposited onto the surface. Within the pyramids, the gold film has a nanostructured roughness of about 20 nm.

For the preparation of solutions of illicit drugs at different concentrations, the following commercially available stock solutions were used: cis-Tramadol HCl (Cerilliant, 1.0 mg/mL in methanol), methylphenidate HCl (Cerilliant, 1.0 mg/mL in methanol), cocaine (Cerilliant, 1.0 mg/mL in acetonitrile), oxazepam (Cerilliant, 1.0 mg/mL in methanol), diazepam (Cerilliant, 1.0 mg/mL in methanol), 6-monoacetylmorphine (6-MAM) (Cerilliant, 1.0 mg/mL in acetonitrile), buprenorphine (Cerilliant, 100 μg/mL in methanol), morphine (Sigma 98 μg/mL in 0.1% formic acid), rac-amphetamine (LGC, 1.0 mg/mL in methanol) and rac-methadone (LGC, 1.0 mg/mL in methanol). Dilutions of samples down to 100 ng/mL for amphetamine, methadone and cocaine, and to 1 μg/mL for the other drugs, were made in HPLC grade methanol. The samples of amphetamine, cocaine and methadone were of 7 concentrations (0.1, 0.3, 0.5, 1.5, 10.5, 100 and 1000 μg/mL), 6-MAM, buprenorphine and morphine of only three (1.5, 10.5 and 100 μg/mL), and the rest were of four concentrations (1.5, 10.5, 100 and 1000 μg/mL). The final concentrations were never confirmed by a separate technique. The molecular structures of the ten compounds are found in Fig. 1.

A volume of 5 μL was pipetted onto the surfaces (this volume ensured complete surface coverage over the whole Klarite substrate of 10 mm × 6 mm size) after which the solvent was left to evaporate, leaving adsorbed analyte on the substrates with a 4 mm × 4 mm SERS active area. The measurements started within 60 s after the evaporation was completed.

2.2. Instrumentation

A confocal Raman microscope (LabRam HR800UV, Horiba Jobin Yvon) equipped with DuoScan™; two mirrors in the laser path attached to piezocrystals which allow deflection of the laser beam through the objective, was used to acquire the SERS spectra. To obtain an average of the SERS signal over a larger area, a macrospot of 400 μm × 400 μm, acquired through the 10× objective (NA = 0.25) and the DuoScan™ facility, and the spectral recording is the average of ten accumulations with each of 5 s integration time. Furthermore, three to six replicative measurements were done at different spots on the surface by moving the *x*-*y* positioning stage mounted below the microscope objective. The average of the replicates were calculated when LOD was evaluated. Continuous wave 785 nm laser light was applied as excitation source for all the measurements, and the Raman scattered light was collected in backscattering geometry and dispersed by a 600 grooves/mm grating (blazed at 750 nm) and collected with an Andor Newton thermoelectrically cooled (−65 °C) EM-CCD camera with the gain set to zero. The confocal hole was set to 500 μm. With these instrumental parameters the spectral resolution is around 6–7 cm^{-1}. Spectra were collected between 200 and 1800 cm^{-1} and the overall system response was taken into account by correcting acquired spectra relative measurements performed on a NIST standard reference material (SRM2241). Degradation of drug molecules upon laser illumination were always checked by repeated accumulation of Raman signal from a single spot on sample surface. The portable Raman instrumentation, First Defender RMX (Thermo Fisher Scientific Inc.), named FD-RMX, was used with a fixed integration time (2, 5 or 10 s) and with output power of 54, 108 or 240 mW of the 785 nm laser beam focused on the SERS sample via optics with a working distance of 5 mm. The laser spot diameter on the SERS substrates was approximately 150 μm and the spectral resolution was about 10 cm^{-1} according to the