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Short Communication

Towards the development of a rapid, portable, surface enhanced Raman spectroscopy based cleaning verification system for the drug nelarabine

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Abstract

Objectives Cleaning verification is a scientific and economic problem for the pharmaceutical industry. A large amount of potential manufacturing time is lost to the process of cleaning verification. This involves the analysis of residues on spoiled manufacturing equipment, with high-performance liquid chromatography (HPLC) being the predominantly employed analytical technique. The aim of this study was to develop a portable cleaning verification system for nelarabine using surface enhanced Raman spectroscopy (SERS).
Methods SERS was conducted using a portable Raman spectrometer and a commercially available SERS substrate to develop a rapid and portable cleaning verification system for nelarabine. Samples of standard solutions and swab extracts were deposited onto the SERS active surfaces, allowed to dry and then subjected to spectroscopic analysis.
Key findings Nelarabine was amenable to analysis by SERS and the necessary levels of sensitivity were achievable. It is possible to use this technology for a semi-quantitative limits test. Replicate precision, however, was poor due to the heterogeneous drying pattern of nelarabine on the SERS active surface. Understanding and improving the drying process in order to produce a consistent SERS signal for quantitative analysis is desirable.
Conclusions This work shows the potential application of SERS for cleaning verification analysis. SERS may not replace HPLC as the definitive analytical technique, but it could be used in conjunction with HPLC so that swabbing is only carried out once the portable SERS equipment has demonstrated that the manufacturing equipment is below the threshold contamination level.
Keywords active pharmaceutical ingredient; cleaning verification; drying patterns; nelarabine; surface enhanced Raman spectroscopy

Introduction

Cleaning verification is the process by which the cleanliness of pharmaceutical manufacturing equipment is confirmed. The process of cleaning verification is typically conducted by swabbing the spoiled manufacturing equipment, extracting residue from the swab and then subjecting the extract to analysis. High performance liquid chromatography (HPLC) is typically used for analysis of residues.[1–3] The principal disadvantage associated with HPLC analysis is the length of time involved in running the necessary samples. Adequately representing the cleanliness of the equipment train can result in the analysis of 70–80 swabs. Choice of swabbing locations must recognise the fact that contamination levels will differ between easy to clean flat stainless steel surfaces and difficult to clean areas such as curved pipes, valves and ‘dead legs’.[4]

Sensitive detection of an active pharmaceutical ingredient is necessary for the analysis of cleaning residues. Previous work carried out in our laboratory has shown that surface enhanced Raman spectroscopy (SERS) can meet the sensitivity requirements for cleaning verification samples. The level to which equipment must be cleaned is an area where no firm rules exist. Regulatory requirements are such that responsibility lies with the manufacturer to validate its cleaning process and decide upon the required cleaning limits. When calculating the limit to which equipment must be cleaned, three formulae are often considered. (i) The 10 ppm formula. This calculation allows no more than 10 ppm of the previous product

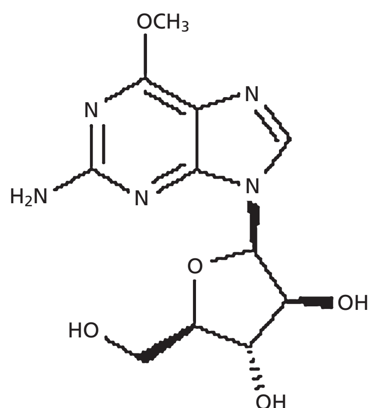

Figure 1 Structure of nelarabine.

entry into the maximum potential daily dose of the next product.[4] (ii) The acceptable daily intake formula. This calculation allows no more than the previous product entry into the product to be manufactured than the acceptable daily intake would allow. This acceptable daily intake can be calculated by employing the 8-h acceptable worker exposure limit.[4] (iii) The 1/1000 of therapeutic level formula. This formula allows no more than one thousandth of the previous compound's minimum daily therapeutic dose entry into the maximum therapeutic dose of the next compound to be manufactured.[4]

The most stringent cleaning limit is chosen routinely and in the case of the drug investigated in this study, nelarabine, the final requirement of a finalised cleaning verification system is 5 ng/cm^2. Nelarabine (Figure 1) is a nucleoside analog used in the treatment of certain leukaemias. It is an extremely potent drug and this accounts for the low cleanliness threshold of 5 ng/cm^2.

SERS is a spectroscopic technique based on the phenomenon of Raman scattering. Raman spectroscopy utilises a source of monochromatic light and is a rapid non-destructive spectroscopic technique with the disadvantage of poor sensitivity. The poor sensitivity of Raman spectroscopy is associated with the fact that Raman scattering is an inherently weak process with approximately only 1 in 10^8 incident photons undergoing a Raman scattering event. SERS has the advantage of being a highly sensitive spectroscopic technique capable of reaching the same sensitivity as fluorescence based techniques.[5] SERS predominantly involves the use of silver or gold structures to produce an enhancement of signal. Nano structured surfaces or gold colloids can be used to produce the SERS enhancement. There are two mechanisms at the basis of the SERS enhancement: (i) enhancement of the electromagnetic field produced at the metal surface; and (ii) chemical enhancement resulting from an adsorption of the molecule onto the gold surface.[6] The exact nature of the SERS phenomenon and the relative importance of both mechanisms is still under debate but it is becoming increasingly apparent that the electromagnetic mechanism plays the major role in providing the enhancement of the Raman signal.[6] Several studies have been published over a wide range of fields that report the sensitive detection of various chemical entities with SERS.[5,7,8] In addition, SERS substrates are improving and this is increasing the possibilities for quantitative measurements.[9,10]

Previous work in our laboratory with a standard laboratory Raman microscope has demonstrated the possibility of using SERS for cleaning verification.[11] The aim of this study was to develop a portable cleaning verification system for nelarabine. The portable Raman instrument was small, able to be carried, could be connected to a laptop computer and it was possible to shield the laser. This meant that it was possible to set up in the manufacturing environment and acquire SERS spectra from collected samples.

Materials and Methods

Nelarabine was provided by GlaxoSmithKline (Barnard Castle, UK). The EZ Raman M Analyzer was purchased from EnWave Photonics (Irvine, CA, US). Klarite substrates were purchased from D3 Technologies (Glasgow, UK). Methanol was purchased from Fisher (Loughborough, UK) and Millipore water was used throughout the study.

Samples were prepared by depositing 5–0.5-μl aliquots of nelarabine solution onto the SERS active Klarite surface and then allowing the drop to evaporate to dryness. Klarite slides were placed on a hot plate set at 70°C in order to speed up evaporation times. Once dry, the sample was analysed spectroscopically using either a Horiba LabRam BX40 Olympus Raman microscope (Stanmore, UK) or the portable EZ Raman M Analyzer. When using the Raman microscope, a laser power of 20 mW was employed in conjunction with a 633-nm laser wavelength and an accumulation time of 10–30 s with a total of 3–5 accumulations. Using the portable EZ Raman M Analyzer, accumulation times of 20 s were employed in conjunction with a 300-mW, 785-nm laser. Spectra were collected between 250 and 2000 cm^{-1}. With the EZ Raman M Analyzer, consistency of sampling was produced by fixing the laser probe into a laboratory clamp in a way that allowed the Klarite slide to be consistently manoeuvred directly beneath the laser.

Simulation of cleaning verification was carried out by contaminating a 10×10-cm stainless steel coupon. Nelarabine solution (1.0 ml) was spread across the steel surface and allowed to dry. The contaminated area was subsequently wiped with a moistened swab from which either samples of solution were taken directly using a pipette or were subjected to an extraction protocol. All contaminations and the resulting swabbings were carried out by one individual in order to minimise error due to the swabbing process. Extraction of nelarabine from contaminated swabs was carried out in a sealed plastic bag and by squeezing the swab inside the bag.

Results

Raman and SERS spectra of nelarabine

The Raman spectrum of nelarabine was initially collected from its powder using a Raman microscope (Figure 2). The Raman spectrum of nelarabine revealed prominent peaks at the following wavenumbers: 784, 848, 1332, 1386, 1520 and 1616 cm^{-1}.

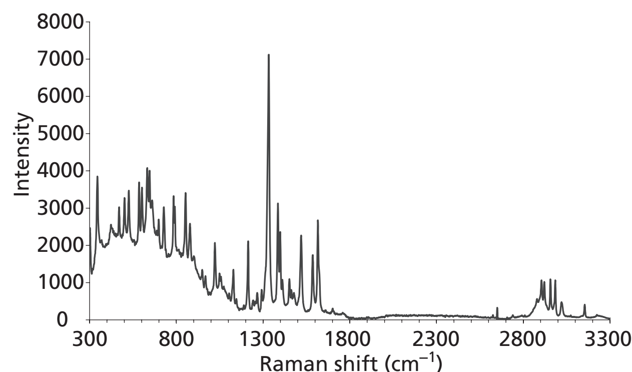

Figure 2 Raman spectrum of nelarabine powder.

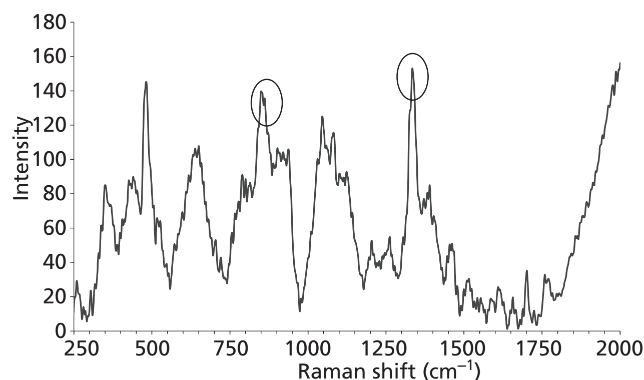

Figure 3 SERS spectrum of 30 μg/ml nelarabine. Peaks at 844 and 1332 cm^{-1} (circled) were particularly prominent and were diagnostically useful in the rest of the study.

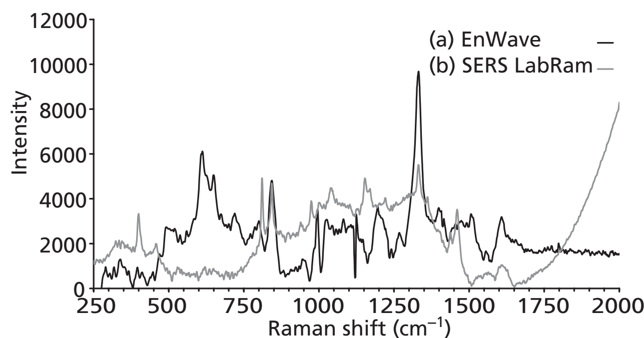

Figure 4 SERS spectrum of 25 μg/ml nelarabine taken with the portable instrument and SERS spectrum of 1.0 mg/ml nelarabine taken on the Raman microscope. 0.5 μl aliquots.

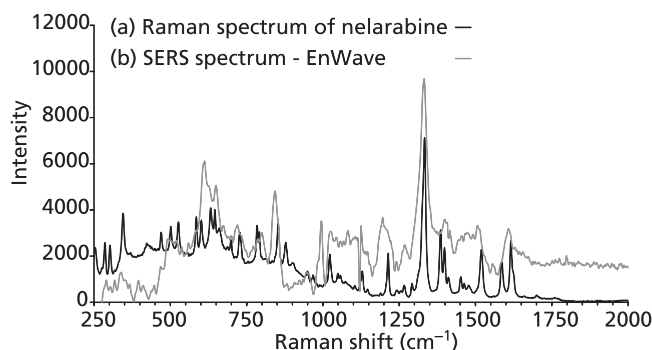

Figure 5 SERS spectrum of 25 μg/ml nelarabine taken with the portable instrument and a Raman spectrum of solid nelarabine taken on the Raman microscope.

The SERS spectrum of a compound can differ from its Raman spectrum and so next a SERS spectrum of 0.1 mM nelarabine was acquired. The sample was prepared by depositing a 0.5-μl drop of 0.1 mM nelarabine solution onto a Klarite slide and allowing it to evaporate to dryness. Once dry, analysis was carried out on the Raman microscope.

Figure 3 represents an example SERS spectrum for nelarabine and it showed good agreement with the ordinary Raman spectrum. Two peaks, at 844 and 1332 cm^{-1}, were particularly prominent in the SERS spectra and proved to be diagnostically useful in the rest of the study.

Having established Raman and SERS spectra for nelarabine using the Raman microscope, the portable Raman spectrometer was employed in order to obtain nelarabine spectra. The SERS spectrum of nelarabine was then compared with the bulk Raman spectrum. Nelarabine was tested initially on the Raman microscope during the initial phase of the project. Once SERS detection looked promising, the portable spectrometer was purchased. Spectra differ between the two instruments because of different collection optics and the instruments being equipped with lasers of different wavelengths. The laboratory Raman microscope possessed a 633-nm laser whereas the portable EnWave instrument had a 785-nm laser.

Figure 4 shows the SERS spectrum of nelarabine at 25 μg/ml taken with the portable instrument and the SERS spectrum of 1.0 mg/ml nelarabine taken on the Raman microscope. The peak at 1332 cm^{-1} can be seen in both spectra along with an agreement of peaks at 844 cm^{-1}. The sample tested with the portable instrument was more dilute but it produced a much more intense spectral response with greater peak amplitude.

Figure 5 shows the SERS spectrum of nelarabine at 25 μg/ml taken with the portable instrument and a Raman spectrum of solid nelarabine taken on the Raman microscope. The peak at 1332 cm^{-1} (CH deformation) was observed in both spectra. It can be pointed out that a similar profile of peaks was seen but there were some small shifts in the frequencies at which they occurred, for example, the prominent peak at 844 cm^{-1} (C-O-C stretch) in the SERS spectra of nelarabine was found at 848 cm^{-1} in the Raman spectrum. Small shifts in frequency between SERS and Raman spectra are normally explained by adsorption of the molecule onto the gold surface, altering the dipole moment and slightly shifting the resulting frequency of the vibration. During the study, other proprietary drugs were investigated and found not to be as SERS active as nelarabine. Based on spectra recorded on plain gold and SERS active Klarite slides (0.1 g/ml *vs* 0.1 μg/ml), given that nelarabine is not in resonance with either laser used in the study and with reference to the product data sheet, we estimate an enhancement factor of 10^5–10^6 for nelarabine using Klarite slides.

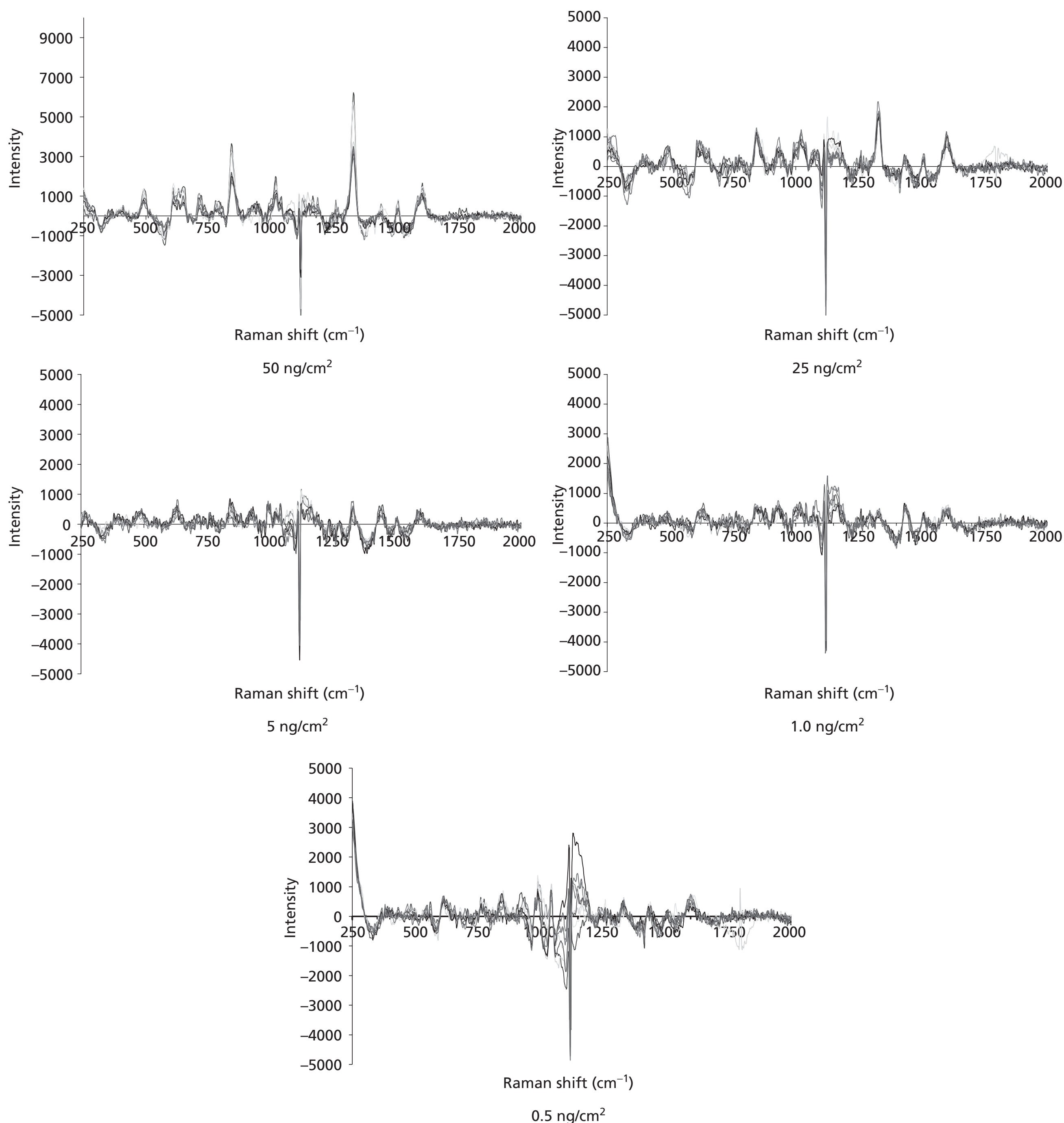

Figure 6 SERS spectra of nelarabine solutions at 50, 25, 5, 1 and 0.5 μg/ml. $n = 8$ series. Spectra background corrected using the proprietary EnWave software.

Figures 4 and 5 demonstrated that SERS spectra could be acquired for nelarabine on the portable instrument and that these spectra compared well with the bulk Raman spectrum of nelarabine. Having obtained Raman and SERS spectra for nelarabine using Klarite substrates in conjunction with both the Raman microscope and portable instrument, the possibility of carrying out cleaning verification analysis for nelarabine was investigated.

Cleaning verification with nelarabine

SERS spectra were recorded for the detergents Tepol, Spiriclens and Antichlor. Raman activity was noted but it was found that none of the cleaning agents produced peaks that interfered with the SERS signal of nelarabine, particularly the peak at 1332 cm^{-1}. A clean stainless steel coupon was sampled using the swabbing protocol. The resulting Raman spectrum

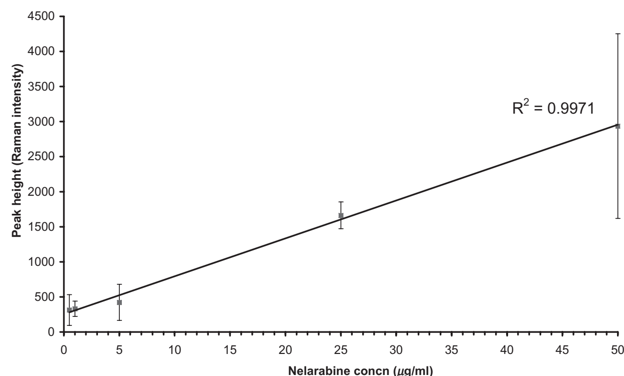

Figure 7 Peak height at 1332 cm^{-1} against nelarabine concentration. $n = 8$; bars represent SD.

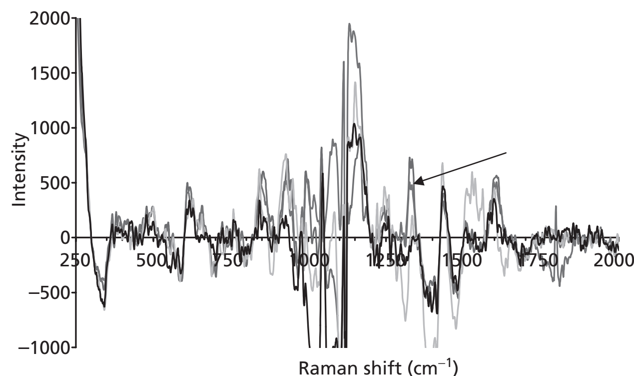

Figure 8 SERS spectra of nelarabine solutions at 1, 0.75, 0.25 and 0.5 μg/ml. $n = 5$. The arrow points to the peak of interest at 1332 cm^{-1}, the intensity of which decreased as nelarabine concentration decreased.

gave no characteristic peaks in the spectral range used in these experiments.

A series of optimisation experiments were conducted to look at aliquot size and drying time and it was found that SERS spectra were best collected from 5.0-μl aliquots of solution dried on a heating block set at 70°C. Drying the sample was necessary in order to reduce analysis times and the use of a heat block was found to be superior to the use of a hairdryer.

Therefore, 5.0 μl aliquots of nelarabine solutions were deposited onto the SERS active Klarite surface. The following concentrations were used: 50, 25, 5, 1 and 0.5 μg/ml. Accumulation times of 20 s were used to record spectra. With the swabbing method employed, nelarabine solutions of 0.5 μg/ml were equivalent to the contamination threshold of nelarabine of 5 ng/cm^2. Spectra were corrected using an automated, iterative, modified least squares polynomial method present within the proprietary EnWave software. The negative artefact around 1100 cm^{-1} is due to the instrument's grating.

Figure 6 shows SERS spectra from different nelarabine concentrations. It can be seen that reproducibility was good, with consistent baselines and peak heights with similar intensities. Mean peak height was then plotted against nelarabine concentration with the standard deviation calculated from the mean peak height.

Figure 7 shows that using Raman intensity it was possible to construct a linear response curve for the peak at 1332 cm^{-1} and Nelarabine concentration. The R^2 value of 0.9971 indicated a linear relationship between peak height and nelarabine concentration. Replicate precision was not as good as linearity, with an error of approximately 50% associated with the measurements taken at a concentration of 50 μg/ml nelarabine. A 10% error or less is desirable for analytical work of this nature and the error associated with these results means that it would be difficult to confidently assign a concentration to an unknown sample of nelarabine.

Having established the relationship between intensity at 1332 cm^{-1} and nelarabine concentration, it was then decided to investigate low concentrations close to the cleaning threshold of 5 ng/cm^2. Concentrations of 1.0, 0.75, 0.5 and 0.25 μg/ml were investigated. With the swabbing method used, the concentrations were equivalent to contamination levels of 10, 7.5, 5 and 2.5 ng/cm^2.

At lower concentrations of nelarabine, the spectral responses showed high degrees of similarity (Figure 8). The peak of interest at 1332 cm^{-1} was present in the spectrum and its intensity decreased as nelarabine concentration decreased. Intensity at 1332 cm^{-1} was plotted against concentration of nelarabine and linearity was again found to be good (0.9972). However, as depicted in Figure 7, the error associated with measurements was large.

Discussion

Throughout the course of the study it was found that intra-experimental error was low, that is, one dried sample of nelarabine produced highly similar spectra. Inter-experimental error was high, meaning that different analyses of the same solution produced results with significant variation and this error can be attributed to inconsistency of the drying process.

Although linearity was found to be good, it would be necessary to greatly reduce measurement error in order to assign a concentration to an unknown sample of nelarabine. Experiments were conducted where nelarabine was recovered from contaminated surfaces and analysed. In these experiments nelarabine detection was possible but spectral variation was much greater than that observed from standard solutions and this was due to the presence of various cleaning agents and swab fibres in the sample, and also the inherent variation in the swabbing process.

This work points the way towards the potential application of SERS for cleaning verification analysis. Although SERS may not replace HPLC as the definitive analytical technique for cleaning verification, it could certainly be used in conjunction with HPLC so that swabbing is only carried out once the portable SERS apparatus has demonstrated the equipment is below the threshold contamination level.

The major impediment towards quantification of samples is the drying pattern of nelarabine. Drying of the nelarabine aliquot produced a ring-like structure with sparse deposits inside the ring. The drying process was inconsistent, which was reflected in the spectral responses where intensities varied markedly between samples of the same concentration. Further work will look at the possibility of producing a more homogenous drying pattern that could be done by stabilising the

drying process with the use of an 'additive' such as NaCl or using other drying methods such as infrared heating. Another possibility is the use of an 'internal standard', where another compound with a prominent peak is added and used to calibrate the spectral response of nelarabine.

Conclusions

This work showed that it was possible to achieve the low level portable detection of nelarabine from standard solutions. SERS spectra were of high quality and the enhancement factor was high, meaning that good quality spectra were recorded from low concentrations of drug. It was possible to construct linear response curves with good linearity (R^2 values of 0.999 and greater). The standard deviations associated with SERS measurements of nelarabine samples were high, meaning it was difficult to assign concentrations to nelarabine solutions of unknown concentration on the basis of peak intensity at 1332 cm^{-1}. The portable equipment used in the study is suitable for cleaning verification analysis of nelarabine; sensitivity was good and reproducibility of the signal from homogenous samples was also found to be good. Analysis times were very quick, with 20-s accumulation times combined with a 2.5-min drying step. This would make it possible to analyse a series of samples very quickly in the plant environment and would represent a significant step forward in the analysis of cleaning verification samples. The production of user friendly software capable of producing a quick pass or fail response for a plant operative would be a relatively simple exercise and this could be easily combined with the existing system.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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