Rapid monitoring of antibiotics using Raman and surface enhanced Raman spectroscopy

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Comparatively few studies have explored the ability of Raman spectroscopy for the quantitative analysis of microbial secondary metabolites in fermentation broths. In this study we investigated the ability of Raman spectroscopy to differentiate between different penicillins and to quantify the level of penicillin in fermentation broths. However, the Raman signal is rather weak, therefore the Raman signal was enhanced using surface enhanced Raman spectroscopy (SERS) employing silver colloids. It was difficult by eye to differentiate between the five different penicillin molecules studied using Raman and SERS spectra, therefore the spectra were analysed by multivariate cluster analysis. Principal components analysis (PCA) clearly showed that SERS rather than the Raman spectra produced reproducible enough spectra to allow for the recovery of each of the different penicillins into their respective five groups. To highlight this further the first five principal components were used to construct a dendrogram using agglomerative clustering, and this again clearly showed that SERS can be used to identify which penicillin molecule was being analysed, despite their molecular similarities. With respect to the quantification of penicillin G it was shown that Raman spectroscopy could be used to quantify the amount of penicillin present in solution when relatively high levels of penicillin were analysed (>50 mM). By contrast, the SERS spectra showed reduced fluorescence, and improved signal to noise ratios from considerably lower concentrations of the antibiotic. This could prove to be advantageous in industry for monitoring low levels of penicillin in the early stages of antibiotic production. In addition, SERS may have advantages for quantifying low levels of high value, low yield, secondary metabolites in microbial processes.

Introduction

The ability to control a bioprocess is paramount for product yield optimization, and it is imperative that the concentration of the fermentation product, the determinand, is assessed accurately.[1] The development of such monitoring methods is driven by economic and ecological needs.[2] Further, in medicine and biotechnology there is a continuing need to find new pharmaceuticals, and hence to develop rapid and efficient methods for the screening of large numbers of microbial cultures for the production of biologically active metabolites.[3] In addition, the increasing rise of multiple drug resistance bacteria and fungi has necessitated screening for new antibiotics, which are often important secondary microbial metabolites.[4]

Many spectroscopic studies of fermentation broths, in particular those using near-infrared detection, have concentrated on measurements of biomass[5] and nutrient supply[6] but comparatively few have attempted to gain qualitative or quantitative information on the product, unless it results from relatively non-complex chemical processes.[7] In this study we aim to quantify microbial secondary metabolites in broths using Raman spectroscopy and surface enhanced Raman spectroscopy (SERS).

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As an analytical tool, Raman spectroscopy has many advantages over infrared spectroscopy and other techniques, which make it attractive for the analysis of bioprocesses.[8] The Raman measurements are non-destructive, non-intrusive and require little or no sample preparation. In addition, the low levels of interference that arise from water allow analytes to be detected in aqueous solution with minimum interference.[9] Our own microbiological studies using Raman spectroscopy have been very useful for the identification of bacteria[10–12] and for the analysis of fermentations.[8,13]

Raman spectroscopy measures inelastic collisions between molecules and light. The collisions cause an exchange of energy, which is recorded as a wavelength shift from the incident radiation and illustrated as a Raman spectrum. The spectrum is a vibrational signature of a molecule,[14] which can be considered as a highly characteristic and structurally sensitive fingerprint. Raman spectra are acquired by irradiating a sample with an intense laser source. The interaction of laser light with the molecule produces scattering of three types, namely Rayleigh, Stokes and anti-Stokes Raman scattering. Most of the radiation is scattered elastically (Rayleigh scattered) and provides no vibrational information. However, a very small fraction of the photons ($\sim$1 in 10^6) are scattered inelastically as the molecule undergoes a vibrational transition.[15] When there is a transfer of energy from the photon to the analyte due to the creation of a molecular vibration, the result is termed Stokes scattering; by contrast, when there is a

net gain of energy from the analyte to the photon the result is termed an anti-Stokes scattering.

This relatively weak Raman Effect can be greatly enhanced, by up to 10^6, when the analyte of interest is adsorbed onto or in close proximity to a roughened surface of a suitable metal such as gold, silver or copper. This technique is known as surface enhanced Raman spectroscopy (SERS) and offers several advantages over conventional Raman scattering. A major analytical advantage of SERS is the dramatic increase in signal intensity,[16] allowing an extended concentration range at lower concentrations to be studied, with detection limits considerably lower than those offered by resonance Raman scattering. This increase in sensitivity allows lower laser powers. Indeed, single molecule detection using SERS has been reported.[17] This increase in sensitivity allows lower laser powers to be utilised, resulting in less chance of thermal degradation of the sample. Fluorescence of the analyte or other material on the surface may also be reduced,[18] which is often a problem in Raman scattering.

Penicillins belong to the β-lactam class of antibiotics. They are produced industrially in large fermenters by *Penicillium chrysogenum*, the fermentations of which are reasonably well characterised and are industrially important systems.[3] In a previous study we used the pyrolysis mass spectrometry (PyMS) technique, with multivariate calibration and artificial neural networks (ANNs) to effect the rapid prediction of the amount of penicillin in fermentor broths containing *P. chrysogenum*.[19] However, this method is thermally destructive and not generally suitable for on-line analyses. The aim of the present study was to investigate the ability of Raman spectroscopy for the quantitative analyses of penicillins.

Materials and methods

Raman and SERS spectroscopy

The Raman instrument used to collect the spectra was a dispersive Renishaw Raman probe system 100 with holographic notch filters and a deep depletion CCD (Renishaw plc., Old Town, Wootton-under Edge, UK). The EIC fibre optic probe, encased in a protective coating, utilizes micro-optic components for both delivering the laser excitation source to the sample and for collection of scattered light, resulting in a compact probe head that is fibre optically coupled to the laser source and the spectrograph. Dichroic and edge filters are used to separate out the excitation and scattered light, using backscattering sampling geometry. The resulting cable has a diameter of 0.25″ and is fairly flexible, making this an ideal instrument for monitoring samples up to 5 m away from the instrument.

The fibre optic diode laser used here provided excitation at 785 nm with $\sim$7 mW power at the sample. This wavelength produces spectra with low background noise compared with the Raman signal of the analytes in the sample, making Raman a promising tool for the rapid, non-invasive and multi-parameter analysis of aqueous biological systems.[20]

The machine was routinely calibrated with 100% ethanol for the C–C–O vibration at 882 cm^{-1}, prior to taking spectra of the samples. Generally, 330 μL of each sample (see below) was pipetted into a 96 well plastic plate (Sigma–Aldrich, Poole, UK). The laser was then focused onto the sample at a distance of 10 mm between the probe and the meniscus of each sample, the optimum distance based on the observed Raman signal, and spectra collected for 10s with a single accumulation.

For SERS the silver colloid employed was prepared using a modified Lee and Meisel method.[21,22] An aggregating agent (1 M NaCl) was added to the colloid, causing the silver nanoparticles to clump together. Generally, 170 μL of each sample (see below), 170 μL of silver colloid and 100 μL of 1 M NaCl were added together and mixed thoroughly. A 330 μL aliquot was removed and pipetted into a well of a 96 well plate, and the spectra collected as detailed above from each sample for 10 s with a single accumulation.

Systems studied

Three experimental systems were studied, which are briefly detailed below.

Experiment 1. Five replicates were prepared of each of the five penicillins studied: Penicillin G, Penicillin V, ampicillin, carbenicillin and 6-aminopenicillanic acid (Sigma–Aldrich). All penicillins were analysed at a concentration of 62.5 mM in distilled water, equivalent to 22.3 mg mL^{-1} for Penicillin G, which is within the range of a standard industrial fermentation. Both Raman and SERS spectra were collected.

Experiment 2. Solutions of penicillin G, in the range from 2–1000 mM (0.7128–356.4 mg ml^{-1}), were used to investigate the limits of detection using Raman spectroscopy and SERS. Penicillin solutions (1 M) were prepared in distilled water and 2-fold dilutions were made from the stock solution, down to 2 mM, and replicated 10 times. Both Raman and SERS spectra were collected.

Experiment 3. A complex medium (lactose, 70 g; sucrose, 10 g; $(NH_4)_2SO_4$, 7 g; K_2HPO_4, 1.6 g; $FeSO_4{\cdot}7H_2O$, 0.04 g; $MgSO_4{\cdot}7H_2O$, 0.1 g; KCl, 0.5 g; $CaCl_2$, 0.04 g; $MnSO_4{\cdot}H_2O$, 0.02 g; $ZnSO_4{\cdot}7H_2O$, 0.02 g; $CuSO_4{\cdot}5H_2O$, made up to 1 L of distilled water and steam sterilized at 15 psi for 15 min) used to culture *Penicillium chrysogenum* was spiked with different concentrations of penicillin G, ranging from 0–130 mM (0–45 mg mL^{-1}). These penicillin concentrations were chosen as they were below the concentrations of what appears to be the saturation point of the colloid (*i.e.*, above monolayer coverage of the penicillin on the colloid as determined from experiment 2, and see below). SERS spectra were collected as previously described.

Data capture and analyses

The GRAMS WiRE software package (Galactic Industries Corporation, Salem, NH, USA) running under Windows 95 was used for instrument control and data capture. Spectra were collected over 100–3000 wavenumbers with 1735 data points. The data were displayed as the number of Raman scattered photons against Raman shift in wavenumbers (cm^{-1}). The spectral data were extracted into multi-text files (ASCII data) and imported into Matlab version 5.0 (The MathWorks Inc., Natwick, MA, USA) for data analysis.

Prior to conducting data analyses the data were pre-processed as follows: (1) the spectra were smoothed using a mean average filter of 9 bin width ($=\sim 13$ cm^{-1}); (2) the spectra were scaled so that the laser line (at ~ 250 cm^{-1}) was equal to one; and finally (3) no spectral correction has been made prior to data analysis for contributions to the Raman signal generated by the Raman fibre optic probe or the sample container vessel.

Multivariate statistical analyses were carried out using cluster analyses including principal components analysis (PCA) and hierarchical cluster analysis (HCA), as detailed in ref. 10. Briefly, for experiment 1 PCA was computed using the NIPALS algorithm and resulted in scatter plots where the first principal component (PC1) accounted for 48.2% of the total explained variance, whilst PC2 = 27.5%. For HCA the dendrogram was constructed using an agglomerative algorithm[23] computed on the Euclidean distances in the first 5 PCs (which accounted for 93.9% of the explained variance).

In order to quantify the penicillin concentration in experiments 2 and 3 the area under specific Raman peaks was calculated using an integration routine: the start and end bins of the chosen peaks were determined by eye, a line was draw from where these wavenumbers intersected the Raman spectrum, and the area above this line and under the peak calculated and plotted against the known (spiked) concentrations.

Results and discussion

Raman and SERS spectra collected from structurally similar penicillin molecules

The chemical structure of the β-lactam penicillin consists of two rings and a side chain, as depicted in Fig. 1. In the core penicillin structure there is a thiazolidine ring which is derived using a non-ribosomal synthetic route from valine and cysteine.[3] The other ring is a four-membered β-lactam, which is responsible for the effect of penicillin antibiotics on bacteria by inhibiting the cell wall synthesis of bacterial cells.[3] The side chain (R) is variable and is attached to the β-lactam ring by a peptide bond. This side chain adds chemical variability to the various types of penicillin, which in turn has an effect on different bacterial infections.[24] Four out of five of the penicillins studied have a benzene ring attached to this side chain (Fig. 1). Penicillin G is the main industrially produced penicillin and derivatives (so called third generation penicillins) of this are used for therapy.

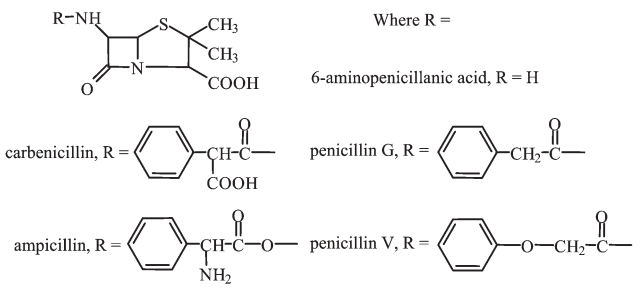

Fig. 1 Generalised penicillin structure showing the β-lactam and thiazolidine rings derived from two amino acids (valine and cysteine). The various side chains at R of five types of penicillin are illustrated: note the lack of the benzene ring in the 6-aminopenicillanic acid.

In infrared studies of ampicillin[25] it was found that the carbonyl vibration (C=O stretch) on the β-lactam ring is constrained and so absorbs IR radiation at 1767 cm^{-1}, rather than at ~ 1660 cm^{-1}. The Raman shift of this functional group may be used for identification and quantification purposes, of the different penicillins. However, for 10 s collects this was barely visible in the raw Raman spectra (Fig. 2A). The characteristic Raman active vibration for the phenyl ring at 1005 cm^{-1} was observed in all penicillins tested, apart from 6-aminopenicillanic acid which does not contain this ring (Fig. 2A).

SERS spectra of the same penicillins gave much more information rich spectra over the same 10 s accumulation period (Fig. 2B). However, more striking were the clear spectral differences between the various penicillins. It was particularly interesting to note the large difference between penicillin G and V, especially since these only differ by an ether bond between the benzene ring and the CH_2 of the side chain. By contrast, it was very difficult to differentiate by eye between the SERS spectra of carbenicillin, ampicillin and penicillin V.

The surface enhancement due to SERS is clear but it is small compared with values of 10^{-6} for molecules such as pyridine.

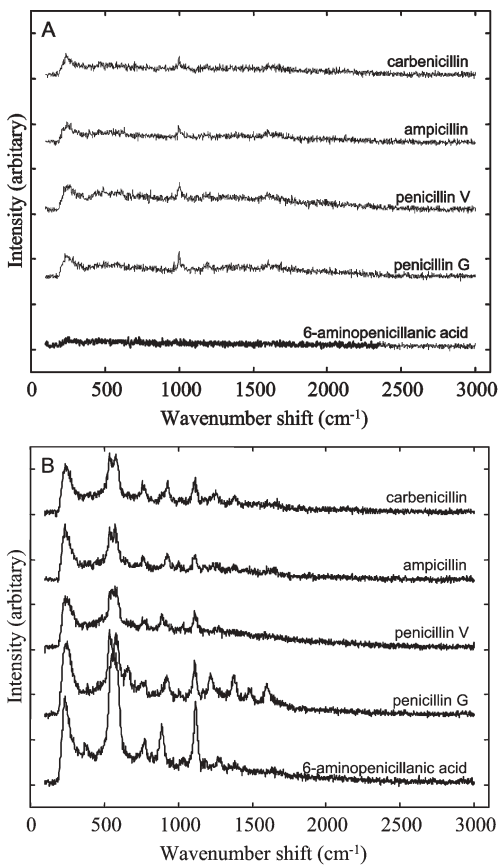

Fig. 2 (A) Raman and (B) SERRS spectra of the five different penicillin molecules (all at 62.5 mM). All spectra were collected for 10 s with a single accumulation. The baselines of the spectra are flattened for illustration purposes only and it should be noted that when carrying out the data analysis no baseline correction was carried out.

The likely reason for this is that there is poor adsorption of the penicillin onto the surface. Nevertheless, the enhancement is sufficient to improve the signal to noise ratio over Stokes Raman scattering, indicating potential advantages for low level *in situ* determination.

Therefore, the Raman and SERS data were analysed with a chemometrics cluster method to determine whether these spectra could be used to differentiate the different types of penicillin. The smoothed and scaled data were used to carry out PCA and HCA on the different penicillins as detailed above. The results are shown in Figs. 3A and 3B, respectively. PCA of the Raman spectra show that only 6-aminopenicillanic acid can be separated from the other penicillins. By contrast, PCA on the SERS spectra clearly show that each of the different penicillins cluster separately into five groups. To highlight this further the first five PCs were used to construct a dendrogram using agglomerative clustering (Fig. 3C), which again clearly shows that SERS can be used to identify which penicillin molecule was being analysed, despite their molecular similarities.

Quantification of penicillin G using Raman spectroscopy and SERS

The Raman and SERS spectra of penicillin G are shown in Fig. 4A and 4B, respectively. Whilst the normal Raman scattering is relatively information-poor, since it is dominated by the benzene ring at 1005 cm^{-1}, in addition to the 1005 cm^{-1} band, the SERS spectrum has many more enhanced bands. The question arises as to whether any of these vibrations are significantly reproducible so that they can be considered to have a quantitative response to the level of penicillin G. Therefore, the peak areas of these peaks were calculated (as described above) and plotted against the penicillin G concentration.

Given the nature of the investigation it was perhaps not surprising that generally high concentrations of penicillin G were quantifiable using Raman compared with SERS, whilst at lower antibiotic levels the SERS responses were more quantifiable. Tables 1 and 2 summarise the results of this investigation for Raman and SERS spectroscopies, respectively. The tables contain details of each of the Raman and SERS enhanced peaks, the potential origin of these vibrations, the details of the integrations, and whether the peak was quantitative or not. The peaks that were considered quantitative are highlighted with asterisks in Fig. 4. Below is a brief discussion of the most notable findings.

The main Raman band from the benzene ring at 1005 cm^{-1} was typical of the other quantifiable peaks at 580 cm^{-1} and 1191 cm^{-1}. At concentrations above 50 mM these peaks gave quantitative results (Fig. 5A). Note that the number of Raman scattered photons is not linear and starts to level off at higher levels of penicillin G.

When the SERS peak at 1606 cm^{-1} was plotted against the concentration of penicillin G (Fig. 6) good quantifiable results could be obtained but at much lower concentrations compared with Raman. The inset in this figure also shows that the response is not linear, but there is a high degree of reproducibility since the standard deviation error bars are small. At higher concentrations (>100 mM penicillin G) the peak area decreased, for this and other SERS active vibrations. It is likely that this is the point at which the colloid surface has become saturated with penicillin molecules and an over-aggregation and precipitation are reducing the signal.

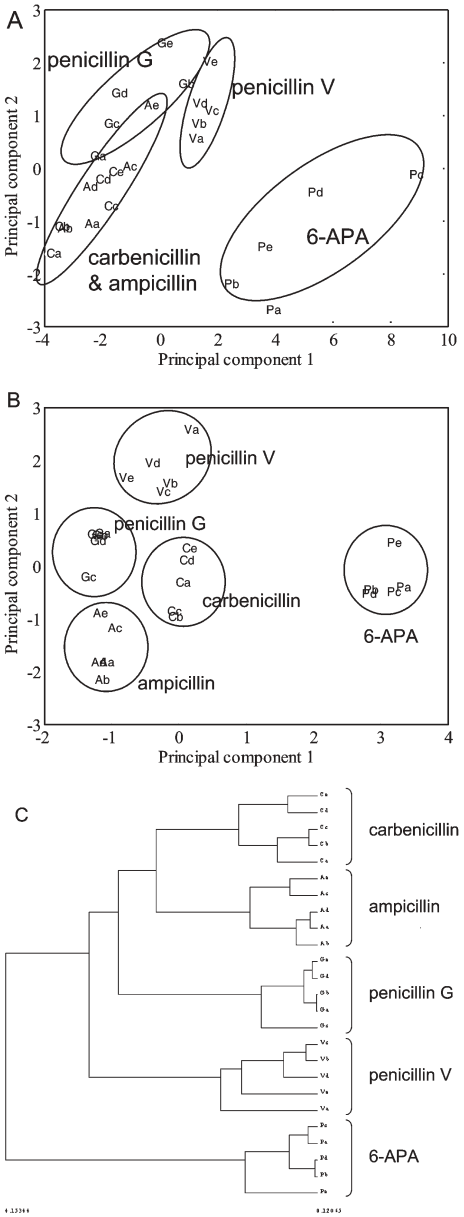

Fig. 3 (A) PCA of Raman spectra showing clustering of 6-aminopenicillanic acid [P] and no clear separation of the four other penicillins. (B) PCA of SERS spectra of the 5 different penicillin molecules. Each penicillin is recovered separately; note 6-aminopenicillanic acid [P] is separated in the first PC, whilst clustering of penicillin G [G], penicillin V [V], carbenicillin [C] and ampicillin [A] are separated in both PC1 and PC2. (C) HCA of the SERS spectra on the first 5 PCs, chosen as these accounted for 93.9% of explained variance. All penicillins were analysed in replicate ($n = 5$), and details of cluster analysis are given in the text. Circles are drawn as a visual aid and have no statistical significance.

Most of the SERS peaks observed were not present in the Raman spectrum of penicillin G (Fig. 4). However, the phenyl ring mode at 1005 cm^{-1} exhibited a quantifiable Raman and

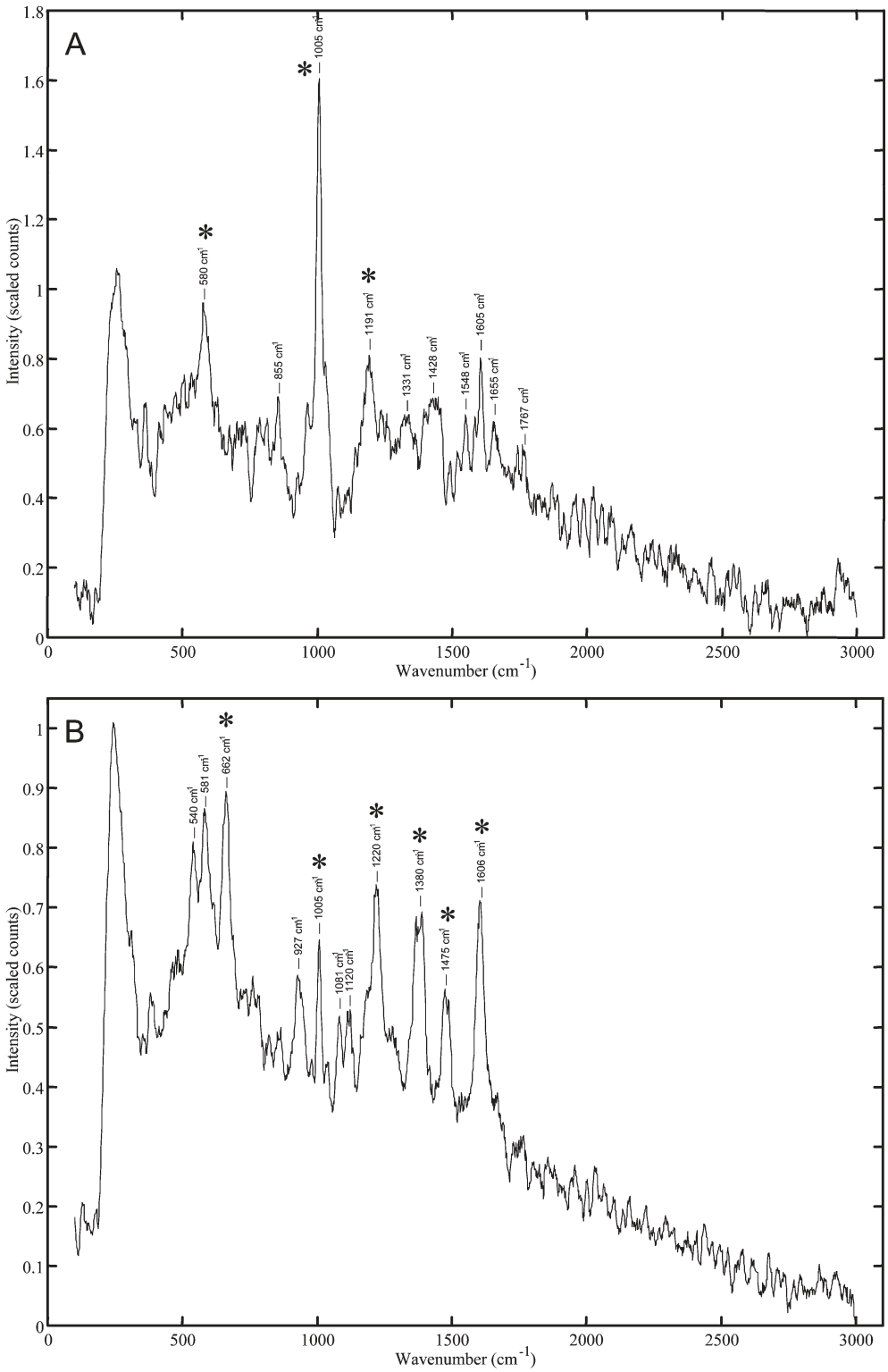

Fig. 4 (A) Raman (60 s collect) and (B) SERS (10 s collect) spectra of Penicillin G (1 M). Asterisks show peaks which are linear with respect to concentration (see ranges and details in Tables 1 and 2).

SERS response (Table 1 and 2). When the area under the peak was plotted against the concentration of penicillin, a combination of SERS enhancement and normal Raman scattering was observed (Fig. 5B). The SERS response gave good quantifiable results at the concentrations below 125 mM. Above this level there was a reduction in the peak area, which increased above 250 mM. It is likely that this is due to the colloid reaching saturation point at the higher concentration (as seen in Fig. 6): after saturation at these higher antibiotic concentrations normal Raman scattering was observed.

Table 1 Raman peaks detected over the range 0.19–1.10 M. Also shown are the integrations and how quantitative these areas are

Peak/ cm^{-1}	Tentative assignment of vibrations[a]	Integration/ cm^{-1}	Quantifiable[b]
580	Ring and –CH deformations Covalent sulfites	550–608	++
855	Cyclopentane	829–868	+
1005	Benzene ring	993–1060	++
1191	$-(CH_3)_2CH$ Sulfates	1152–1219	++
1428	Carboxyl salts	1401–1470	−
1548	–NH deformation vibrations Heterocycles	1533–1567	−
1605	Aromatics Amides Nitrates Carboxyl salts	1595–1620	+
1655	Aromatics Amides Nitrates Amino acids	1629–1682	−
1767	Carbonyl group Lactones (5 membered rings) Cyclic amide (4 membered ring)	1724–1841	−

[a] The source of these vibrations has been assigned using a Raman table of assigned peaks,[26] and linking these to the atoms and structures present in the penicillin molecule. This is by no means a definitive result as, so far as we are aware to date, no data is readily available on the exact vibrations of different parts of the penicillin molecule. [b] ++ = good, + = semi-quantifiable, − not quantitative.

Table 2 SERS peaks detected over the range 40–520 mM. Also shown are the integrations and how quantitative these areas are

Peak/ cm^{-1}	Tentative assignment of vibrations[a]	Integration/ cm^{-1}	Quantifiable[b]
540	Secondary amides Inorganic thiosulfides	528–555	−
581	Ring and –CH deformations Covalent sulfites	567–620	−
662	Aromatic compounds	634–700	++
927	Alkanes	901–960	−
1005	Benzene ring	993–1027	++
1081	Aromatics Linear alkyl ethers (–C–O stretching) $-CH_2NH_2$ vibrations	1068–1093	−
1120	Secondary alcohols	1100–1134	−
1220	Aromatics	1152–1244	++
1380	–CH–C $-CH_3$ deformations $-OH_2$ deformation Amides	1328–1411	++
1475	Alkanes $-CO_2$ stretch Amino acids	1436–1511	++
1606	Aromatics Amides Nitrates Carboxyl salts	1578–1629	++

[a] The source of these vibrations has been assigned using a Raman table of assigned peaks,[26] and linking these to the atoms and structures present in the penicillin molecule. This is by no means a definitive result as, so far as we are aware, to date no data is readily available on the exact vibrations of different parts of the penicillin molecule. [b] ++ = good, + = semi-quantifiable, − not quantitative.

It is clear from the above that the Raman and SERS responses were quantitative for penicillin G at levels found in fermentation broths. However, all these experiments were conducted in distilled water and the next stage was to ascertain if the SERS response for penicillin G could be detected in a complex fermentation broth.

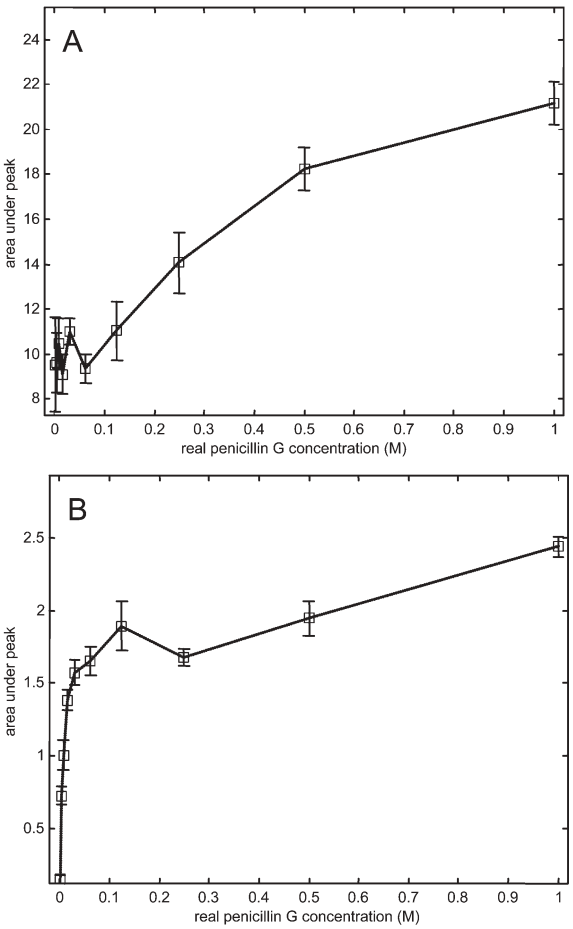

Fig. 5 Plots of the peak areas for Raman (A) and SERS (B) of the benzene ring at 1005 cm^{-1} *versus* the penicillin G concentration. (A) Peak areas from Raman spectra show that 0.05–1 M penicillin G is quantifiable. (B) Peak areas from SERS spectra showing that below 0.1 M penicillin G the SERS response is quantifiable: by contrast above 0.2 M penicillin G a quantifiable Raman response is seen. Data plotted as means with standard deviation error bars.

Quantitative analysis of penicillin G spiked into fermentation broths using SERS

The complex culture medium used contained, amongst other things (full details above), 70 g L^{-1} lactose and 10 g L^{-1} sucrose, 7 g L^{-1} $(NH_4)_2SO_4$ and 1.6 g L^{-1} K_2HPO_4, which will certainly have Raman and SERS peaks. This growth medium was spiked with different concentrations of penicillin G, ranging from 0–45 mg mL^{-1} (0–130 mM). These penicillin concentrations were chosen as they were below the concentrations of what appears to be the saturation point of the colloid and were within the range expected in current production processes.[3]

P. chrysogenum broth spiked with penicillin G gave good quantifiable SERS peaks for the vibrations at 1005, 1220, 1380 and 1606 cm^{-1}, all of which were quantitative when water was used as the medium. Fig. 8 compares the area

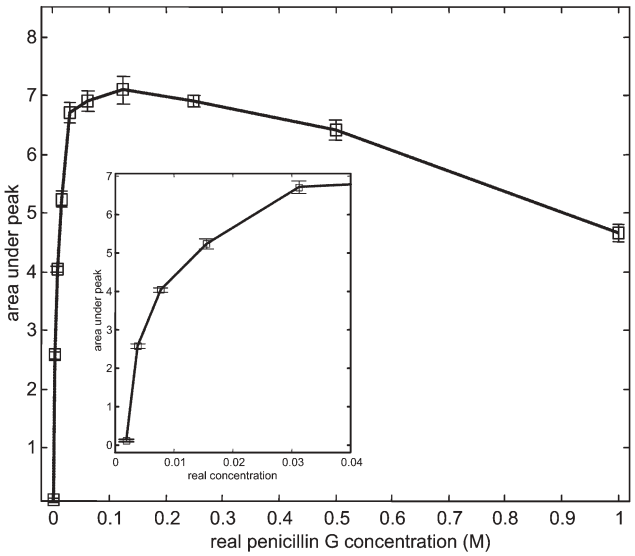

Fig. 6 Plot of the SERS peak area under 1606 cm^{-1} *versus* the penicillin G concentration. The inset shows quantification at low concentrations of penicillin using SERS, whereas at the higher concentrations peak areas appear to decline. Data plotted as means with standard deviation error bars.

under the 1005 and 1606 cm^{-1} peaks *versus* the penicillin G concentration. It should be noted that the spectra of the broth also have peaks around the same areas as some of the penicillin peaks (Fig. 7), which might cause interference. Methods could be employed to subtract the broth spectra from the spiked samples rather than include this contribution, as done here, prior to analyses. The plotted results indicate good correlations between increased levels of penicillin G in broth and SERS peak area. This demonstrates that in this complex media there is little interference from the medium components.

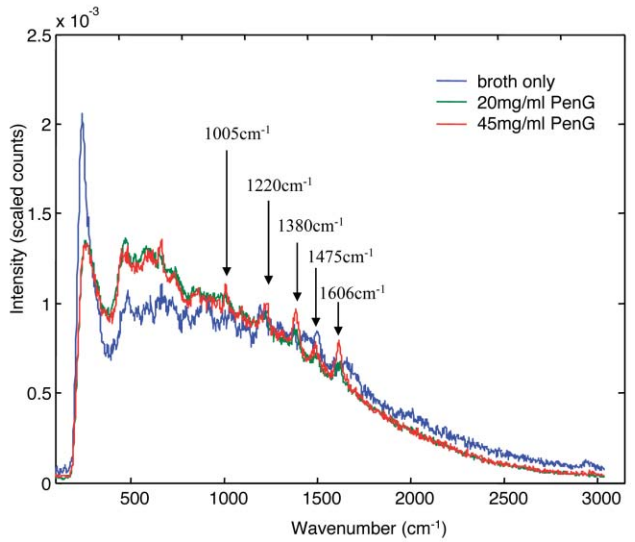

Fig. 7 *Penicillium chrysogenum* broth spiked with Penicillin G: 0 mg mL^{-1} (blue line), 20 mg mL^{-1} (green line) and 45 mg mL^{-1} (red line).

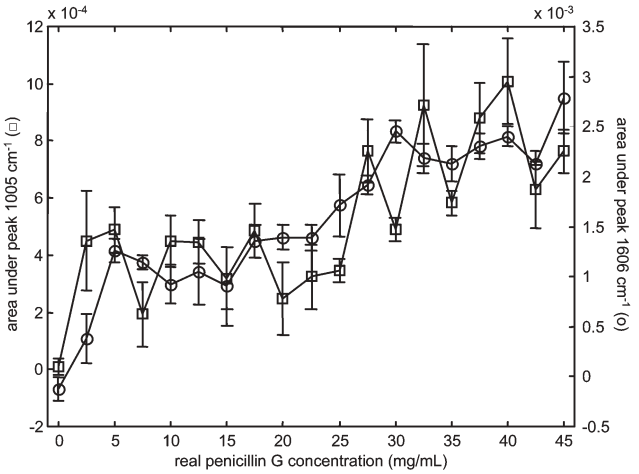

Fig. 8 Area under SERS peaks from spiked broth (averaged data) smoothed and scaled, plotted *versus* real concentration of Penicillin G (0–45 mg mL^{-1}). Quantifiable SERS peaks were detected at 1005 cm^{-1}, depicted (□), and 1606 cm^{-1}, depicted (○). Data plotted as means with standard deviation error bars.

Concluding remarks

The aim of this study was to investigate Raman spectroscopy for the quantitative analysis of penicillin in fermentation broths. Initial work compared Raman and SERS from five different types of penicillin molecules that differed only slightly in their chemical structures (Fig. 1). It was shown that for short collection times (10 s) SERS gave more reproducible spectra (Fig. 2), and in contrast to Raman data PCA plots and dendrograms could clearly be used to identify unequivocally the different penicillins (Fig. 3).

Raman spectroscopy could be used to quantify the amount of penicillin G present in solution when relatively high levels were analysed. The SERS spectra of penicillin G dilutions showed reduced fluorescence (Fig. 4), as well as an improvement in the signal obtained from low concentrations of the antibiotic (Figs. 5–6). This could prove to be advantageous in industry for monitoring low levels of the penicillin, or other secondary metabolites, at early stages in the microbial process of antibiotic production.

Despite the fact that a reproducible SERS signal was seen when broths were spiked with penicillin G (Fig. 8), at this stage it might be considered premature to suggest that SERS could be used to monitor the microbial broth directly on-line for penicillin G. For monitoring penicillin production Raman spectroscopy is more attractive since most modern fermentations have yields up to 50 g L^{-1} penicillin,[3] where Raman signals are strong. In this context, we have shown that this is already possible for industrial bioprocesses producing the secondary metabolite gibberellic acid.[8]

Whilst SERS is considered to reduce fluorescence,[18] some broad fluorescence was seen on the fermentation model (Fig. 7); therefore it would be necessary to filter out the living organism from the medium before analysis in order to obtain good SERS spectra. Note that penicillin is extracellular so no cellular lysis would be needed prior to analysis. Moreover, due

to the poisonous nature of the silver colloid on the microbial process it would also be very unwise to add the colloid into the process plant, but rather to set up an at-line system whereby the growth medium was filtered out of the fermentor and the colloid injected into an off-line monitoring system in order to obtain SERS spectra. Alternatively, one could try gold colloid for enhancement as this is less toxic, although it still requires addition to the fermentation.

In conclusion, we believe that SERS spectroscopy could be of great interest to the process industry in the future for the monitoring of low levels of microbial secondary metabolites. As well as having the potential for monitoring the levels of nutrients in the growth medium it could be used to control the bioprocess during the manufacture of the metabolites and to assess accurately and optimize the product yield by the process plant.

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