Fast spatially resolved surface-enhanced Raman spectrometry on a silver coated filter paper using charge-coupled device detection

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Abstract

This paper describes the performance of surface-enhanced Raman spectroscopy (SERS) on a silver-coated filter paper using a charge-coupled device (CCD) detector. It is demonstrated that spatially resolved SER spectrometry with satisfactory spectral resolution can be performed with a low integration time. When the analytical information is distributed in space, CCD-SERS provides an effective solution for rapid analysis. For instance, binary mixtures separated by paper chromatography on a silver-coated filter paper can be studied in situ by SERS. The effect of binning on the spatial resolution and on the analytical figures of merit is discussed. Binning improves the sampling efficiency and improves the limits of detection by two orders of magnitude.

Keywords: Raman spectrometry; Charge-coupled device (CCD)

1. Introduction

Advances in Raman spectrometry have been related to instrumental development, mainly in the areas of the excitation sources and detection systems. The need of Raman spectrometry for detectors with high responsivity, low readout noise, large dynamic range, low dark count rate and a linear response is well known. For many years, photomultiplier tubes (PMTs) have been used almost exclusively for detection of photons in spite of being single-channel detectors with a relatively poor quantum efficiency.

Although former multichannel detectors did not offer the necessary characteristics to be competitive with the PMT, recent technological advancement has made available to the analytical chemist new types of multichannel detectors, such as photodiode arrays (PDAs) and charge-coupled devices (CCDs), with improved sensitivity and dynamic range performance. PDAs have a large readout noise and a significant dark current, and consequently they are not suitable for the low photon fluxes involved in Raman spectroscopy. CCDs are thus the detectors of choice, in particular if the multiplex advantage can be fully exploited. That results in lower limits of detection, more rapid analysis, the opportunity to use lower power, less expensive lasers and because of their broad spectral response, CCDs have few restrictions on the choice of laser wavelength for Raman...
excitation. The performance of CCDs in a variety of experimental situations has been discussed [1–10]. Several authors have reported Raman spectroscopy using CCD detectors with UV, visible and near-IR excitation [11–16]. The capability of CCDs for low integration times often eludes problems with sample photodecomposition. This advantage has been exploited for the analysis of light-sensitive biological samples [12,17]. However, the main characteristic of CCDs derives from their two-dimensional configuration, which permits the spatial information imaged on the spectrograph entrance slit readily to be investigated. High spatial resolution allows CCDs to analyze easily light transmitted through or reflected by spatially heterogeneous samples such as photovoltaic cells or chemical sensors [18] or complex samples by plasma emission spectrometry [19–21]. Researchers have reported simultaneous spectral and spatial information using imaging CCD detectors [10,22,23]. Bowden and co-workers studied the spatial distribution of Raman spectra of poly(vinyl chloride) sheets degraded by chemical dehydrochlorination using microline focus spectrometry [24]. CCDs have been evaluated for simultaneous detection of distinct spectral processes by connecting multiplex fiber optics at the slit of the spectrograph, transferring the image of the fibers to the CCD, and dividing the detector into different regions, one for each process being monitored [25,26].

In recent work, we reported the performance of a CCD-based spectrometer for luminescence spectrometry of plant pigments [23]. In this work, surface-enhanced Raman spectrometry on a silver-coated filter paper substrate is demonstrated. Using simple excitation-collection optics, it is demonstrated that spatially resolved SER spectrometry with satisfactory spectral resolution can be achieved. Integration times as small as 120 s permit the acquisition of 128 spectra with acceptable signal-to-noise ratios. When the analytical information is distributed in space, CCD-SERS provides an effective means of rapid analysis. For instance, binary mixtures separated by paper chromatography on a silver-coated filter paper can be studied in situ by SERS. The effect of binning on the spatial resolution and on the analytical figures of merit is discussed. Binning improves the sampling efficiency and improves the limits of detection by two orders of magnitude.

2. Experimental

2.1. Instrumentation

Samples were excited with the 488-nm line of an argon-ion laser (Coherent, Model Innova 70-5). The laser power on the filter paper substrate was 15 mW. The laser beam was defocused with a 15-mm focal length biconvex quartz lens. The defocused beam was collimated in the vertical direction and focused in the horizontal direction to a microline using a 17-cm focal length cylindrical lens (Fig. 1a). A spatial filter was placed between the cylindrical lens and the sample holder. In this configuration, all the CCD was illuminated. Collection was performed with a glass biconvex lens with a focal length of 25.4 mm.
and f-number of 1. A long-pass glass filter (Ealing Electro-Optics, part number 35-5545) was placed between the collection lens and the slit of the spectrograph to remove the excitation light from the scattered radiation. The filter transmits 85% of the scattered Raman light at a Raman shift of > 1100 cm\(^{-1}\). A sample holder for the solid substrate was designed and constructed in our laboratory. The sample holder consisted of an aluminum frame with a rectangular section removed for sample placement. A rectangular window of 1.5 cm \(\times\) 0.5 cm limited the exposed substrate area (Fig. 1b). The sample was placed approximately 11.5 cm from the collection lens, with the distance from the entrance slit to the lens being 5 cm. Thus the optical magnification was ca. 0.5. Right-angle geometry was used for Raman sampling. The scattered light was collected onto the entrance slit of a triple indexable grating spectrograph (Acton Research Co., SpectraPro 275, Cze- ny-Turner; F/3.8) fitted with three gratings of 300, 600 and 1800 grooves mm\(^{-1}\). The 1800 grooves mm\(^{-1}\) grating was employed in this study, with a linear reciprocal dispersion of 1.5 nm mm\(^{-1}\) at the focal plane. A solid-state two-dimensional charge coupled device (CCD) (EG&G PAR, Thomson CSF, THX-31159A) was used in this study. The CCD consists of 512 \(\times\) 512 elements each being 19 \(\mu m\) \(\times\) 19 \(\mu m\). The active area is 9.7 mm \(\times\) 9.7 mm. This system has a quantum efficiency of 32% at 550 nm. The CCD was cooled to \(-70^\circ\) C by a Peltier system. When cooled to \(-70^\circ\) C, this detector exhibits a dark current of 10 photoelectrons per pixel per second and a readout noise of 4–5 electrons per scan. Wave-length calibration of the detector system was conducted with a mercury pen lamp. Operation of the detector was controlled by a personal computer with the OMA Spec 4000 software. The spectrograph was connected to the controlling PC by a conventional IEEE-488 general-purpose interface bus (GPIB).

2.2. Chemicals and procedure

Analytical-reagent grade chemicals and distilled, deionized water were used throughout. \(p\)-Aminobenzoic acid (PABA) was obtained from Fisher; acridine, quinacrine and 9-aminoacridine hydrochloride monohydrate (AA) were purchased from Sigma. All were used as methanolic solutions. Whatman No. 1 filter paper was used as a support for SERS-active silver. The procedure for the preparation of the substrate was as follows: the filter paper was immersed in aqueous 0.1 M silver nitrate. The wet filter paper containing silver ions was sprayed with 0.2 M sodium tetrahydroborate solution in a vertical fashion from a distance of about 20 cm for a few seconds. A nebulizer was used for spraying. This treatment turned the white paper black when wet or greenish brown when dry. For a SERS measurement, a small portion of wet coated paper was cut to fit the cell holder and 0.5–3 \(\mu l\) of the analytes were added. For quantitative analysis the filter paper was cut in E-shaped profiles to avoid the spreading of the sample spot (Fig. 1b). Samples were added at the center of each arm of the E-shaped substrates. All SER spectra were recorded when the paper was still wet.

3. Results and discussion

3.1. Spectral characteristics and spatial distribution

Acridine, quinacrine, 9-aminoacridine and \(p\)-aminobenzoic acid were used as model compounds for SERS on the silver-coated filter paper. The corresponding SER spectra are shown in Fig. 2. A volume of 3 \(\mu l\) of 1000 \(\mu g\) ml\(^{-1}\) methanolic solutions of the analytes were applied to the substrate with a Hamilton microsyringe. For these measurements all the CCD output was binned. It is known [27] that the laser beam induces a local drying of the paper, in such a way that the SER spectrum is progressively lost, while the background gradually increases. However, the ability of CCDs to use short integration times avoids or minimizes photothermal effects on the substrate. The spectra in Fig. 2 were acquired using an integration time of only 40 s and thus photothermal effects are not expected to contribute to the spectral profiles. The SERS bands of these compounds on the silver-coated paper filter agree well with those reported on colloidal silver [28–30].

The use of a CCD for SERS on the paper filter support permits the study of the spatial distribution of the analyte on the substrate. Fig. 3 shows spatially resolved SER spectra of a spot of 9-aminoacridine. For the acquisition of this figure, 85 SER spectra were acquired, using 5-fold binning in the slit dimen-
Fig. 2. SER spectra of acridine, quinacrine, 9-aminoacridine and p-aminobenzoic acid on silver-coated filter paper. Solution volumes of 3 μl of 1000 μg ml⁻¹ were added to the substrate. The integration time was 40 s and laser power was 15 mW. All CCD output was binned.

3.2. Increasing sampling efficiency

Monochannel detectors in combination with conventional spectrometers allow Raman analysis of the sample under the laser beam. In filter paper SERS, diffusion of the sample on the substrate is substantial due to the porous nature of the support, as discussed above. Consequently, if a focused laser beam is used, the illuminated area is small and the sampling efficiency of the analyte molecules is poor. The situation may partially be solved by handling the substrate, by using microline excitation, and by optimising the detector readout mode.

To decrease sample diffusion, silver-coated filter
papers were cut in E-shaped profiles and samples were applied on each arm of the E, as shown in Fig. 1b. In combination with microline excitation, this approach allows simultaneous analysis of up to three samples and, because a single substrate is prepared, it avoids signal variability due to differences in substrate SERS activity. Fig. 4 shows spatially resolved SER spectra of 9-aminoacridine on the E-shaped substrate. Solution volumes of 0.5 μl of 2.5 μg ml⁻¹, 10 μg ml⁻¹ and 25 μg ml⁻¹ were applied to each arm. The acquisition time needed for the full figure was only 120 s. Each spectrum in the figure represents 4-fold binning in the slit dimension of the CCD. As shown in the figure the signal increases with increasing 9-aminoacridine concentration, while each sample maintains its own identity during the measurement. Calibration graphs can be constructed from the data in Fig. 4. If the signal from a single spectrum at each concentration is used for this purpose, it is clear that much of the sample-encoded signal is being wasted. CCD readout using binning greatly increases the sampling efficiency. A comparison of the analytical figures of merit using different binning levels is given in Table 1. Although spatial resolution decreases as the number of binned rows increases, binning results in a large increase in sensitivity (slope) with a concomitant decrease in limit of detection (LOD) values, with little effect on the method's precision. It is apparent that the maximum number of binned rows allowing a linear response is given by the physical size of each sample spot on the substrate. In this case it is given by the width of the arms of the E, i.e., 120 rows or about 5 mm. As shown in Table 1, full binning over each sample spot

![Image: Space-resolved SER spectrogram of a spot of 9-aminoacridine on silver-coated filter paper. A Solution volume of 3 μl of 1000 μg ml⁻¹ was added to the substrate. Acquisition time was 120 s. The laser power was 15 mW. The binning was 5-fold in the slit dimension.](image-url)
results in an improvement in LODs by two orders of magnitude.

The analytical figures of merit for acridine, quinacrine, 9-aminoacridine and p-aminobenzoic acid are summarized in Table 2. Limits of detection were calculated at their respective maxima of 1410

Table 1
Effect of binning on the analytical figures of merit for 9-aminoacridine on silver-coated filter paper substrates

<table>
<thead>
<tr>
<th>Binning</th>
<th>r</th>
<th>m</th>
<th>LOD (ng ml$^{-1}$)</th>
<th>LOD (pg)</th>
<th>R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9974</td>
<td>35</td>
<td>510</td>
<td>255</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>0.9995</td>
<td>184</td>
<td>90</td>
<td>45</td>
<td>9</td>
</tr>
<tr>
<td>32</td>
<td>0.9980</td>
<td>782</td>
<td>20</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>120</td>
<td>0.9991</td>
<td>3052</td>
<td>6</td>
<td>3</td>
<td>18</td>
</tr>
</tbody>
</table>

a Correlation coefficient ($n = 5$).
b Slope (relative values).
c Limit of detection (signal-to-noise ratio = 2).
d Relative standard deviation ($n = 6$, concentration = 10 μg ml$^{-1}$).
e No binning, only a row of pixels was read.
Table 2
SERS analytical figures of merit on silver-coated filter paper

<table>
<thead>
<tr>
<th>Analyte</th>
<th>( r^a )</th>
<th>( m^b )</th>
<th>LOD ((\text{ng ml}^{-1})^c)</th>
<th>LOD (pg)</th>
<th>R.S.D. (d) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acridine</td>
<td>0.9980</td>
<td>230</td>
<td>78</td>
<td>78</td>
<td>18</td>
</tr>
<tr>
<td>Quinacrine</td>
<td>0.9983</td>
<td>2393</td>
<td>7.5</td>
<td>7.5</td>
<td>14</td>
</tr>
<tr>
<td>9-Aminoacridine</td>
<td>0.9991</td>
<td>3053</td>
<td>6</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>( p )-Aminobenzoic acid</td>
<td>0.9870</td>
<td>33</td>
<td>540</td>
<td>560</td>
<td>16</td>
</tr>
</tbody>
</table>

\(^a\) Correlation coefficient \((n = 5)\).
\(^b\) Relative slope (same scale as in Table 1).
\(^c\) Limit of detection.
\(^d\) Relative standard deviation \((n = 6, \text{ concentration } = 10 \ \mu g \ \text{ml}^{-1})\).

cm\(^{-1}\), 1380 cm\(^{-1}\), 1375 cm\(^{-1}\), and 1385 cm\(^{-1}\). To obtain the best results 120-fold binning in the slit dimension was chosen. The integration time was 120 s. The lowest limit of detection was 3 pg for 9-aminoacridine. The relative standard deviations (R.S.D.s) were in the range 8 to 18%.

Fig. 5. (a) SER spectrogram of a mixture of 9-aminoacridine (1 \( \mu g \)) and acridine (2 \( \mu g \)) separated by paper chromatography. The CCD was binned 10-fold in the slit dimension. (b) SER spectrum of the mixture before the chromatographic separation. (c) SER spectra of 9-aminoacridine and acridine after the chromatographic development taken at \( R_F \) values of 0.1 and 0.54, respectively. The integration time was 120 s. The laser power was 15 mW.
3.3. Chromatographic study

The capability for spatial resolution of the present approach was used in combination with planar chromatography for the fast separation and analysis of binary mixtures. The separation of the binary mixtures 9-aminoacridine–acridine and acridine–quinacrine on 4-cm long, 1-cm wide silver-coated filter paper strips was studied. Several solvents were tested before an appropriate one was found. Finally a methanol–water mobile phase (1 + 1, v/v) was used for development. To obtain a minimum spot diameter, the application was repeated at intervals of several seconds, dried with a stream of hot air, until the 3 μl volume of the sample solution had been applied. Rf values were calculated by visualizing the spots under UV radiation after the development of the paper chromatogram. The Rf values obtained were 0.37, 0.48 and 0.30 for 9-aminoacridine, acridine and quinacrine, respectively. Fig. 5a shows the SER spectrogram of a binary mixture of 9-aminoacridine and acridine separated on the silver-coated filter paper. The image was binned 10-fold in the slit dimension. It should be noted that the entire image was acquired in only 120 s. Although the aim of this experiment was not fully to resolve the mixture, three zones are clearly distinguishable in the spectrogram: at Rf values up to ca. 0.2 and from 0.4 to 0.55, 9-aminoacridine and acridine, respectively, were detected. At Rf values from 0.20 to 0.38 9-aminoacridine and acridine are not well separated and the observed spectra contain spectral contribution from both compounds. Fig. 5b shows the SER spectrum of the mixture prior to development and Fig. 5c shows SER spectra of 9-aminoacridine and acridine after chromatographic development, taken at Rf values of 0.1 and 0.54, respectively. Although the chromatographic separation was not ideal, the CCD system gave reasonably good spectra from the edges of the sample spots. The signal-to-noise ratio of the spectra in Fig. 5c could be improved by binning the pixel rows which contained the information from the isolated analytes.

4. Conclusions

It is clearly demonstrated that the combination of SERS with spatially resolved detection could be of great analytical utility in paper chromatography. The relatively low levels of analyte necessary to obtain good quality spectra after a chromatographic separation, which obviously is not optimal, is highly encouraging. If the separation efficiency was increased by using high-performance thin layer chromatography plates, CCD-SERS could be extremely attractive due to the information it can provide at a relatively low cost.

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