A note on distance dependence in surface enhanced Raman spectroscopy

Feng Ming Liu a, P.A. Köllensperger b, Mino Green a,*, A.E.G. Cass b, L.F. Cohen c

a Department of Electrical and Electronic Engineering, Imperial College London, Exhibition Road, London SW7 2BT, United Kingdom
b Institute of Biomedical Engineering, Imperial College London, Exhibition Road, London SW7 2BT, United Kingdom
c Department of Physics, Imperial College London, Exhibition Road, London, SW7 2BT, United Kingdom

Received 20 July 2006; in final form 18 August 2006
Available online 26 August 2006

Abstract

It is appreciated that the surface enhanced Raman signal falls away rapidly from the substrate surface that provides the dominant electromagnetic enhancement. As many bio-molecules of interest are large, it is important to establish for any practical surface the usable distance from the surface at which molecules can still be detected. Using monolayer ssDNA arrays with three adenine bases close to the surface, or far from the surface, a two-point distance dependence for our toroidal shaped random array of silver structures has been performed. The results show enhancement at 30 nm out is definitely viable, making SERS and large bio-molecule compatible.

1. Introduction

We are investigating the use of Surface Enhanced Raman Scattering (SERS) as an analytical technique for, inter alia, the tag free detection of oligonucleotides [1]. We use functionalized arrays of probe ssDNA chosen to detect their complementary sequences by showing characteristic changes in the SERS spectrum. The SERS is obtained from structured silver surfaces with a non-resonant enhancement (measured using pyridine) [2] of ca. 10^8. Raman scattering is a result of the interaction of the substrate plasmon field and the molecules field, and since the intensity of the electromagnetic field above the substrate falls off with the distance normal to the surface there, is a distance dependence for SERS. Here we have undertaken to obtain a measure of this distance dependence for our system using particular probe molecule structures. These probes are single strand 45 mer DNA molecules attached to the silver surface via a short hydrocarbon chain (–CH_2–)_6 and an anchoring sulphur bond (from thiol group). The ssDNA molecule is either attached (via the 3’ position) as: Ag–S–(CH_2)_6–AAA–(GCT)_14 for what we call the near measurement or Ag–S–(CH_2)_6–(GCT)_14–AAA for the far measurement. We use the signal from the GCT repeats as our reference signal and compare this with the signal from the three As. The small difference (ca. 6%) in reference signal arising from the difference in vertical displacement of the (GCT) chain due in one case to the AAA, is neglected. The SERS substrate, whose preparation and properties have been described elsewhere [1–3] consists of silver structures made in wells of side-wall SiO_2 on top of a silicon wafer. The silver structures are like strings of beads squashed together and resemble toroids, as can be seen from the electron micrograph in Fig. 1.

A considerable number of publications have appeared pertaining to the dependence of the SERS intensity as a function of the distance of the scattering centre above the surface [4–7]. There is, however, no consensus about the functional relation as in real systems it is a highly case dependent issue. We regard the current experimental work as an empirical study designed to test the limits of the usefulness of our substrates in the analytical applications of SERS for larger molecules interacting with functionalized surfaces.
2. Experimental

The SEM of the silver toroid array (Fig. 1) was taken using a LEO 1450, while the high resolution image was obtained using a LEO 1525.

Adsorption of anchor–linker–ssDNA is from an aqueous solution of 0.1 M TEAA (triethyl ammonium acetate) buffer. The amount of adsorbate (anchor–linker–ssDNA) in solution is about five times more than the amount required to form a monolayer on the silver substrate, and should therefore be sufficient to ensure vertical alignment. The establishment of an isotherm (Raman intensity vs ssDNA probe molecule concentration) [1], showing an excellent plateau, is consistent with the proposed behaviour of these layers. The high ionic strength of the solution gives a small Debye length, ca. 1 nm, which screens the negatively charged back-bone of the nucleic acid leading to a monolayer concentration of about $2 \times 10^{13}$ molecules cm$^{-2}$. The degree of Manning counter ion condensation during monolayer formation (chemisorption) cannot be too excessive because the monolayer formed has a surface concentration that is consistent with the presence of an excluding space charge region [3]. The ‘near’ and ‘far’ type monolayers, having been formed on (different chips) of structured silver SERS substrate over an 18 h period (cf. [1]), are washed with buffer solution (pH 7.4) and surfactant (to remove any non-attached ssDNA material). There were three washes, namely, $2 \times$ SSPE + 0.2% SDS, $2 \times$ SSPE, and finally $0.2 \times$ SSPE. The multiplier in front of the initials indicates the concentrations of the components, viz., $1 \times$ SSPE corresponds to 0.15 M sodium chloride, 0.01 M sodium hydrogen phosphate and 0.001 M Na$_2$EDTA, and e.g. $0.2 \times$ would indicate five times lower concentrations. Na$_2$EDTA is the disodium salt of ethylene diamine tetraacetic acid, and SDS is sodium dodecyl sulphate. The Debye length for the final conditions would be about 2 nm (cf. 1 nm above).

The Raman spectra of these two kinds of test substrate were measured using a confocal Raman microscope (Renishaw 2000) with a 20× objective, of focal length 35 mm, and N.A. 0.35. The exciting radiation used was the 633 nm line of a He–Ne laser. The beam spot power was 0.2 mW, 10% of the original intensity, and the diameter of the Gaussian beam was 3.8 μm. The data were recorded without polarization analysis, and sampling times were 3 s. The relative humidity inside the Raman cell was controlled to be ~93%; this was done by the appropriate mixing of gas streams of dry N$_2$ with N$_2$ saturated in water vapour (see Fig. 2).

3. Results and discussion

The results for three sets of experiments are shown in the Table 1. The bead radius, $R$, was obtained as $(\text{o.d.}–\text{i.d.})/4$, where o.d. is the external diameter of a torus and i.d. the internal diameter. The mean $R$ so obtained was $0.087 \pm 0.006$ μm. The mean o.d. was $0.508 \pm 0.031$ μm: (cf. Fig. 1). In our model we suppose that the ssDNA molecule is configured so that it is at right angles to the plane of the silver surface. Our reason is that the phosphodiester backbone is a string of negative charge compensated by the counter ion space charge. The negative charge repulsion is by no means completely screened by the counter charge since the Debye screening length, ~1.8 nm, for the solution...
conditions (0.03 M NaCl) used here is significantly larger than the inter-phosphorus (P–P) distance (max. 0.76 nm). Fig. 3 shows the supposed back-bone structure.

The relation often cited for the distance effect, a simple power law, e.g. Ref. [4], is that for the SERS intensity $I$ from a single spherical metal particle of radius $R$ and the adsorbate distance above its surface, namely

$$I = I_0 \left[ \frac{R}{R + x} \right]^r$$  \hspace{1cm} (1)

$R$ is the effective radius of the silver bead, $x$ is the distance above the surface of the group interacting with electromagnetic field. The value $I_0$ is the integrated SERS intensity for the three A (adenine) bases divided by $I_0$ which is the intensity at $x = 0$. But in the substrates, we use here there is an obvious range of structures, cf. Fig. 1, indeed it is this range that leads to the combination of spatial uniformity (over the relevant length scale of the probe) and high enhancement.

Applying Eq. (1) directly to our case, we are comparing $I$ for the 3As near the surface and far from the surface, i.e. $I_n$ and $I_f$ corresponding to $x_n$ and $x_f$. Thus,

$$I_n/I_f = \left[ \frac{(R + x_f)}{(R + x_n)} \right]^r$$  \hspace{1cm} (2)

The chains here are composed of several parts, namely the silver–sulphur bond and the hydrocarbon chain, the 14×GCT chain, and the group of three adjacent As. This is depicted diagrammatically in Fig. 4. The silver–sulphur covalent bond length is taken to be 0.26(4) nm. The linker of S(CH$_2$)$_6$O–P is 0.93(6) nm, giving a total anchor/linker length of 1.20 nm. The inter-base separation is taken to

<table>
<thead>
<tr>
<th>Run No.</th>
<th>45AAA (near): $I_n$</th>
<th>AAA45 (far): $I_f$</th>
<th>Experiment ($I_n/I_f$)$_\lambda$</th>
<th>$r$ (Eq. (1)) max. base sep. 0.765 nm</th>
<th>$r$ (Eq. (1)) base sep. 0.6 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.83</td>
<td>0.21</td>
<td>3.95</td>
<td>4.49</td>
<td>5.51</td>
</tr>
<tr>
<td>2</td>
<td>0.56</td>
<td>0.14</td>
<td>4</td>
<td>4.53</td>
<td>5.56</td>
</tr>
<tr>
<td>3</td>
<td>0.53</td>
<td>0.19</td>
<td>2.8</td>
<td>3.36</td>
<td>4.13</td>
</tr>
</tbody>
</table>

Calculated $r$.
be the fully extended chain length, 0.765 (cf. Fig. 4), so that
the average length of the three adenines is $1.5 \times 0.765 = 1.48$ nm. With these values, $x_n = (1.20 + 1.48) = 2.68$ nm; and $x_f = (1.20 + 32.13 + 1.48) = 34.81$ nm. Using $R = 87$ nm (given above) we obtain $I_n/I_f = [(87 + 34.81)/(87 + 2.68)]^r$ and $r$ is given as maximum $r$ in the Table 1. A smaller inter-base separation might arise, due to a number of causes, e.g. single strand base stacking [8], ion (Manning) condensation [9], chain bending [10]. A value of 0.6 nm is taken to be the lower bound. In this case we would have, $x_n = (1.20 + 0.9) = 2.10$ nm; and $x_f = (1.20 + 25.2 + 0.9) = 27.30$ nm; and with the same selected metallic radius, $I_n/I_f = [(87 + 27.30)/(87 + 2.10)]^r$. The values of the index $r$, obtained from Eq. (2), are shown in the Table 1. A value of $r = 12$, for a spherical particle, has been proposed on theoretical grounds by Kneipp et al. (cf. Ref. [6]). It is anticipated that in a complex array structure working to provide an EM enhancement over many different length scales, the value for $r$ will deviate significantly from the single sphere case. Indeed for our structures, assuming a single power law dependence is applicable we find that $r \sim 4.6$. The most valuable result from the present work is not this finding however but that by extrapolation from the empirically derived functional form we can estimate that the useful working distance of our substrates is approximately 45 nm.

4. Conclusion

The distance dependence for monolayers of tethered ssDNA in SERS from our structures is such as to give strong signals from chemical groups that are as much as 30 nm (and by extrapolation up to ~45 nm) from the silver surface. This sets better defined limits to the use of SERS as a bio-analytical tool for a range of applications. This does not address the question of single molecule detection, heterogeneity and ‘hot-spots’.

Acknowledgements

The authors thank the Engineering and Physical Sciences Research Council for a grant (EP/C516036/1) in aid of this research.

References