

## Surface-Enhanced Hyper-Raman Spectra and Enhancement Factors for Three SERS Chromophores. SEHRs Spectra on Ag Films at Pulse Energies below 2 pJ

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Surface-enhanced Raman scattering (SERS) is demonstrating potential as a sensitive, vibrationally specific probe for chemical and bioanalytical sensing and imaging applications.<sup>1–5</sup> The two-photon analogue, surface-enhanced hyper-Raman spectroscopy (SEHRs, Figure 1),<sup>6–16</sup> has been reported for only a small number of organic chromophores, and there have been few comparative studies of spectra, enhancement factors, and wavelength dependences. While SEHRs is normally considered a weak process requiring high laser powers to observe, the use of far-red or near-IR excitation coupled with blue–green detection in SEHRs has the potential advantages of greater depth of penetration into biological and other “real-world” samples, improved selectivity, and reduced photochemical damage. Similar advantages lead to the choice of multiphoton-excited fluorescence over linear fluorescence for certain applications.<sup>17,18</sup>

Here we examine three chromophores that exhibit relatively strong and stable SERS and SEHRs spectra in Ag colloidal suspensions: rhodamine 6G (R6G), crystal violet (CV), and 1,1'-diethyl-3,3,3',3'-tetramethylindocarbocyanine iodide (TMICCI) (Figure 2). The Ag colloids were prepared by the standard citrate reduction method.<sup>8,19</sup> The spectroscopic setup utilized a mode-locked Ti:sapphire laser (82 MHz, 1–2 ps pulses) for hyper-Raman excitation and argon ion or He–Ne lasers for linear Raman excitation, coupled to a modified J–Y Raman microscope with a CCD detector. Figure 3 shows representative hyper-Raman spectra in methanol solution and in aqueous Ag colloidal suspension. The surface-enhanced and homogeneous solution spectra are very similar (as often observed with SERS of large molecules<sup>20</sup>), although the SEHRs spectra are slightly broader, probably reflecting heterogeneity of the surface binding sites. These, and the corresponding SERS spectra (not shown), agree fairly well with most of those previously published.<sup>9,11,12,21</sup> Note the different concentrations and scaling factors for the surface-enhanced and unenhanced spectra.

Table 1 summarizes the SEHRs enhancement factors (lower limits assuming all chromophore is bound to the surface) for the three chromophores at two excitation wavelengths. The average enhancement factors, which may conceal large heterogeneities among different surface binding sites,<sup>22</sup> are  $\sim 10^3$  for all three chromophores. Comparable factors of  $10^3$ – $10^4$  are obtained for SERS of TMICCI at 458 and 633 nm. Silver should provide little electromagnetic field enhancement at the incident wavelength in the hyper-Raman experiments, and we might expect smaller overall enhancement factors than in SERS, where the fields at both the incident and scattered wavelengths can be strongly enhanced. However, the nonlinear nature of the SEHRs process further amplifies even a small enhancement of the incident field. Additionally, aggregation of the particles can shift the electromagnetic field enhancements to much longer wavelengths.<sup>23,24</sup> Changes in the one- and/or two-photon transition strengths or energies in the surface-bound molecules may influence the experimental enhancement

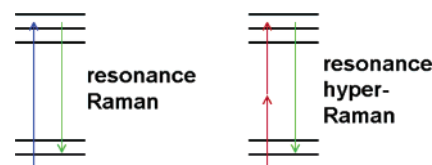


Figure 1. Resonance Raman and hyper-Raman processes.

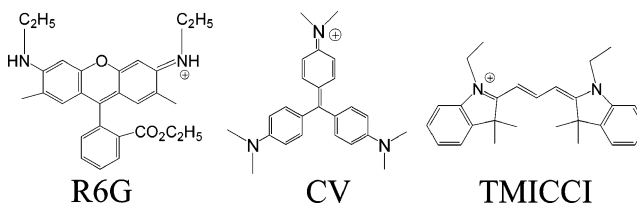


Figure 2. Structures of the three chromophores examined.

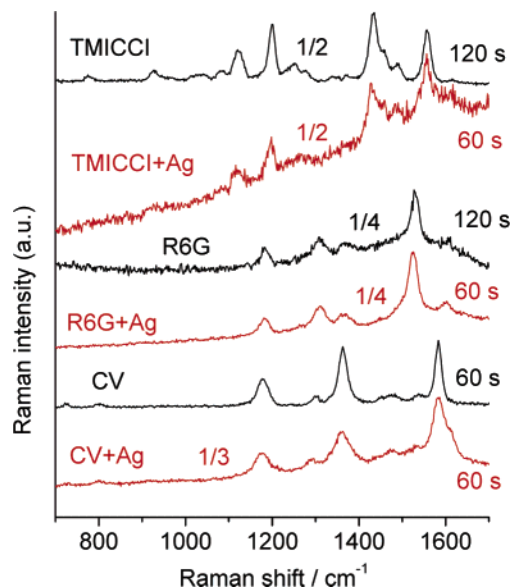
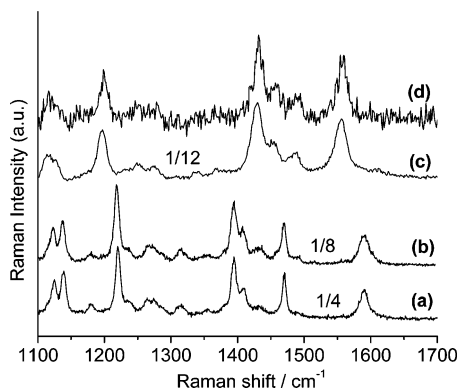


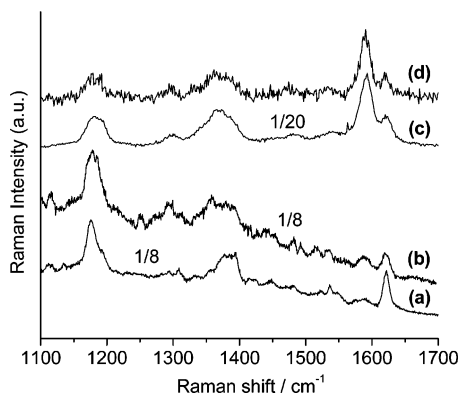
Figure 3. Solution phase (methanol, black) and surface-enhanced (Ag colloidal suspension, red) hyper-Raman spectra of the three chromophores (848 nm, 230 mW). Concentrations for all chromophores are  $10^{-6}$  M in Ag colloidal suspension and  $10^{-3}$  M in methanol solution. Accumulation times and scaling factors are indicated in the figure. Spectra are vertically offset by arbitrary amounts.

factors, although the linear absorption spectra of these molecules do not appear severely perturbed by silver.

We have also examined both SERS and SEHRs spectra of these chromophores on Ag colloids prepared as for the aqueous suspension experiments but then deposited as films on polylysine-coated glass slides. Figures 4 and 5 compare the SERS and SEHRs spectra, both in aqueous colloids and as films, for TMICCI and CV (similar data were obtained for R6G, not shown). The SEHRs spectra are



**Figure 4.** Spectra of TMICCI,  $10^{-6}$  M. (a) SERS in aqueous colloidal suspension,  $100 \mu\text{W}$ ,  $633 \text{ nm}$ ,  $20 \text{ s}$ . (b) SERS on Ag film,  $14 \mu\text{W}$ ,  $633 \text{ nm}$ ,  $2 \text{ s}$ . (c) SEHRS in aqueous colloidal suspension,  $140 \text{ mW}$ ,  $926 \text{ nm}$ ,  $60 \text{ s}$ . (d) SEHRS on Ag film,  $140 \mu\text{W}$ ,  $926 \text{ nm}$ ,  $20 \text{ s}$ . Spectra are scaled by the indicated factors and offset vertically.



**Figure 5.** Spectra of CV. (a) SERS in aqueous colloidal suspension,  $10^{-7}$  M,  $100 \mu\text{W}$ ,  $633 \text{ nm}$ ,  $20 \text{ s}$ . (b) SERS on Ag film,  $10^{-7}$  M,  $14 \mu\text{W}$ ,  $633 \text{ nm}$ ,  $2 \text{ s}$ . (c) SEHRS in aqueous colloidal suspension,  $10^{-6}$  M,  $140 \text{ mW}$ ,  $926 \text{ nm}$ ,  $60 \text{ s}$ . (d) SEHRS on Ag film,  $10^{-6}$  M,  $140 \mu\text{W}$ ,  $926 \text{ nm}$ ,  $20 \text{ s}$ . Spectra are scaled by the indicated factors and offset vertically.

**Table 1.** Hyper-Raman Surface Enhancement Factors (aqueous Ag colloidal suspension versus methanol solution) for the Specified Lines at the Indicated Excitation Wavelengths

molecule	Raman shift ( $\text{cm}^{-1}$ )	927 nm	849 nm
CV	1583	$15 \times 10^3$	$2.5 \times 10^3$
R6G	1523	$2.5 \times 10^3$	$1 \times 10^3$
TMICCI	1435	$0.5 \times 10^3$	$0.6 \times 10^3$

broader than those of the SERS mainly because of the greater line width of the pulsed laser source. The three chromophores studied here, which are nominally nonpolar or only weakly polar, exhibit significantly different SERS and SEHRS spectra consistent with previous reports.<sup>9,13</sup> In contrast, the highly dipolar “push–pull” molecules we have studied previously show very similar intensity patterns in the linear Raman and hyper-Raman spectra, both with and without surface enhancement.<sup>8,25,26</sup> This may indicate (near)-resonance enhancement from the B- and/or C-term mechanisms in these more symmetric molecules, rather than the A-term enhancement that dominates in highly polar systems.<sup>27</sup>

The Ag films are heterogeneous and exhibit bright spots under white light or laser illumination. On these spots, good quality SERS spectra can be obtained in less than 10 s using  $10\text{--}20 \mu\text{W}$  of cw power from a He–Ne laser at  $633 \text{ nm}$ , and SEHRS spectra are obtained in 20 s with average powers below  $200 \mu\text{W}$  from the Ti:sapphire laser at  $830\text{--}930 \text{ nm}$  (see legends to Figures 4 and 5). Previous SEHRS studies used average powers of  $100 \text{ mW}$  or greater;  $140 \mu\text{W}$  of laser power at  $82 \text{ MHz}$  corresponds to  $1.7 \text{ pJ}$  of energy per pulse, or about  $1 \text{ W}$  of average power during the  $1\text{--}2 \text{ ps}$  pulse duration. These results suggest that, particularly if substrates can be optimized to maximize the electromagnetic field enhancements at both the incident and scattered wavelengths, SEHRS detection could be feasible with inexpensive, relatively low peak power laser sources.

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**Supporting Information Available:** Complete experimental procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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