

# Subnanogram Detection of Dyes on Filter Paper by Surface-Enhanced Raman Scattering Spectrometry

*Sir:* Filter paper has been used extensively as rigid supports for the identification of compounds at subnanogram levels by fluorescence and room-temperature phosphorescence (RTP) techniques (1). There is, however, a limitation of those useful techniques; i.e., they can only be used for compounds which fluoresce and/or phosphoresce, restricting these techniques to the analysis of aromatic compounds. Unfortunately most of the aromatic hydrocarbons absorb in the same region so that the selective excitation of one component in a mixture can be difficult, if not impossible (2). Furthermore, fluorescence and phosphorescence spectra tend to be structureless and so do not provide much information on fluorophores. It is, therefore, very desirable to find a new detection technique for compounds adsorbed on filter paper that would complement fluorescence and RTP.

Vibrational spectra provide abundant information on the structure of molecules compared to fluorescence and phosphorescence. Presently, however, it is not possible to obtain a high-quality infrared absorption spectrum of a compound adsorbed on filter paper. While the Raman scattering technique is considered promising because of its independence from moisture and background absorption, it is not practical as an analytical method because Raman cross sections are normally too weak to be detected. Any technique capable of enhancement and, hence, detection of the Raman scattering of compounds adsorbed on filter paper at nanogram or subnanogram levels would, therefore, be a major contribution to analytical methodology.

Raman scattering enhancements can sometimes occur when molecules are adsorbed on specific metal surfaces. These remarkable enhancements, which are frequently 5 or 6 orders of magnitude higher than those in the absence of metal surface, have been observed for molecules adsorbed on rough Au, Ag, and Pt electrode surfaces, on Au, Ag, and Pt deposited surfaces, and also on surfaces of gold, silver, and platinum colloidal hydrosols (3). The latter type provides a convenient system for surface-enhanced Raman scattering (SERS) since they can be easily prepared (4, 5). The disadvantage of the colloid system is its tendency to coagulate. Although stabilizers such as poly(vinyl alcohol), poly(vinylpyrrolidone), and sodium dodecyl sulfate have been used (6-8), the use of such stabilizers could result in interferences as reported recently (8). Such considerations prompted initiation of the present study to find a simple alternative method for using SERS as an analytical method to determine compounds at very low concentrations. It will be demonstrated in this paper that silver colloidal hydrosols stabilized by filter paper supports (cellulose, glass, and quartz fibers) enhance the Raman scattering of dyes adsorbed onto them and that this enhancement is dependent on the difference in the average distance between the dye and silver particles induced by filter paper fibers and, therefore, on the type of filter paper that is used. With this technique, subnanogram amounts of various dyes, i.e., triphenylmethane, cyanine, and azo dyes, were detected with a He-Ne laser of only 3 mW.

## EXPERIMENTAL SECTION

Crystal violet (tris[*p*-(dimethylamino)phenyl]carbonium chloride) was purchased from Aldrich and used as received. 3,3'-Diethylthiacarbocyanine chloride and 1,1',9-trimethyl-2,2'-cyanine perchlorate were from the same source as cited in ref 9 and was a gift from the Royal Institution, London, England. Methyl Red (2-[[*p*-(dimethylamino)phenyl]azo]benzoic acid) (Fischer) was used without further purification. Pallflex tissue

Table I. Surface-Enhanced Raman Scattering Limits of Detection (LOD) for Various Dyes

dye	$\epsilon$ (632.8 nm), $M^{-1} cm^{-1}$	limits of detection, ng
Crystal Violet	10303	0.500
1,1',9-trimethyl-2,2'-cyanine perchlorate (TMCP)	1379	7
3,3'-diethylthiacarbocyanine chloride (DECC)	1250	15
Methyl Red (MR)	13	240

quartz, type QAO 2500, was obtained from Pall Corp., Putnam, CT. The 0.33-mm chromatographic cellulose paper (3MM) and glass fibers (GF/B, GF/C, GF/D, and GF/F) were products of Whatman. All other chemicals were of reagent grade.

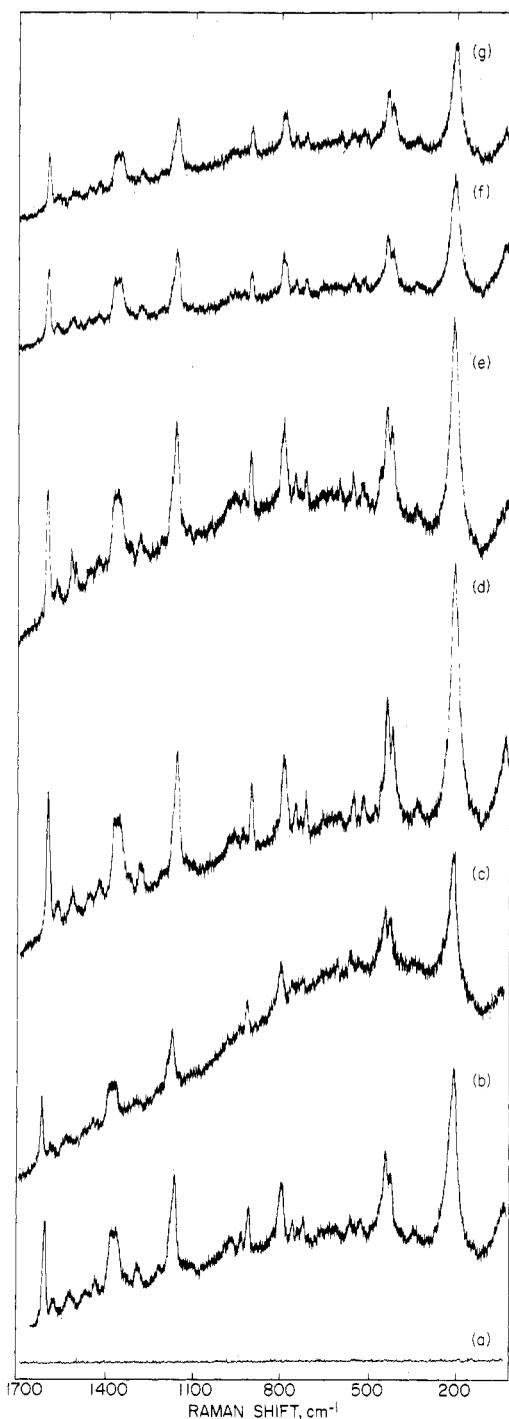
All glassware was cleaned prior to use by sonicating in Alconox aqueous solution. Silver colloidal hydrosols were prepared according to the procedure described earlier (4, 5). Typically 100 mL of  $2.5 \times 10^{-3}$  M of  $AgNO_3$  was added dropwise over a period of 20 min to 300 mL of vigorously stirred ice water cooled  $2 \times 10^{-3}$  M  $NaBH_4$  aqueous solution to yield a brownish yellow colloidal silver solution. Various concentrations of aqueous dye solutions (2.5  $\mu$ L) and silver colloidal (22.5  $\mu$ L) were applied onto filter paper ( $1 \times 1$  cm<sup>2</sup>) by a microsyringe, either as premixed mixture or separately, and their Raman spectra were taken when they were still wet.

Raman spectra were taken with a Hughes Model 3324H-PC He-Ne laser as an excitation source. The laser intensity, after passing the beam through a 6328-Å interference filter to remove unwanted plasma lines, was 3 mW. The unfocused laser beam was parallel to the slit of the double monochromator (Spex Model 1403). The Raman scattered light (90°) was collected by a 6:1 90° off-axis ellipsoidal mirror focused onto the slit of the monochromator and was detected by a dry ice cooled EMI S-20 extended photomultiplier and a conventional photon counting device. Generally the monochromator slits were set at 5 cm<sup>-1</sup>, integration time was 1 s, and scanning speed was 1 cm<sup>-1</sup>/s.

## RESULTS AND DISCUSSION

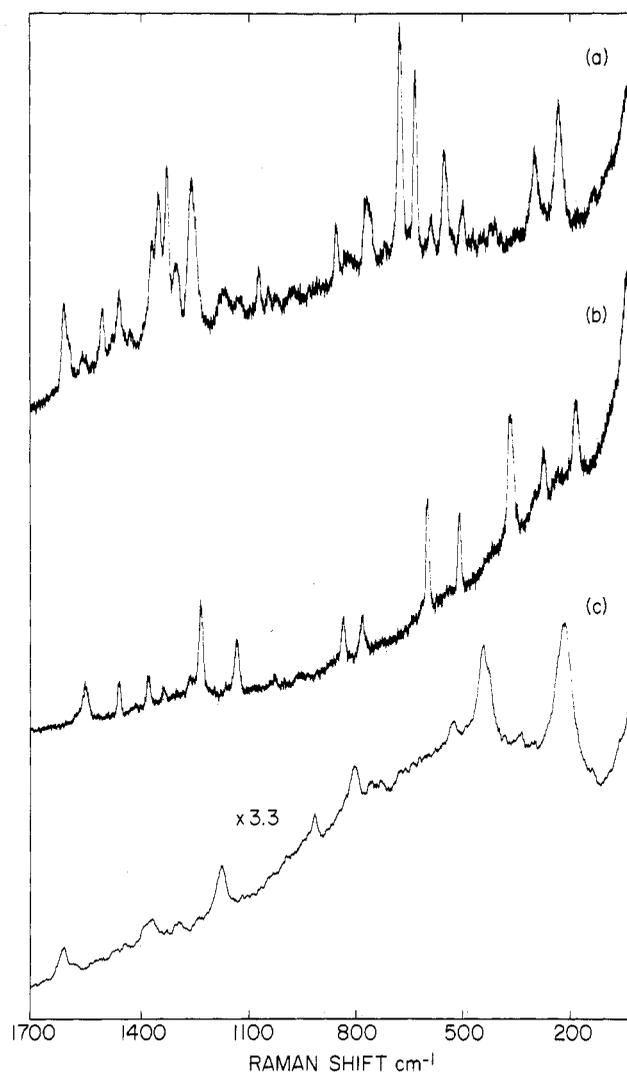
Crystal violet (CV) was chosen because its absorption at the He-Ne laser wavelength, 6328 Å, is relatively high ( $\epsilon = 10333 M^{-1} cm^{-1}$ , Table I) and its resonance Raman spectrum is well documented (10, 11). No resonance Raman scattering was observed from various CV concentrations on filter paper in the absence of the silver colloid hydrosols when they were excited with a 3-mW He-Ne laser. This is probably due to the low Raman cross section of the dye, its self-absorption of any Raman scattering at high concentrations, and the low intensity of the He-Ne laser. Strong Raman signals were measured on a 10K scale when 10 ng (2.5  $\mu$ L) of CV dye was applied in solution with 22.5  $\mu$ L of silver hydrosols onto various filter papers (Figure 1). The enhancement of resonance Raman scattering by silver particles is estimated to be about  $10^3$ , which is comparable to the SERS induced by vacuum deposited silver islands film (12, 13). To this investigator's knowledge this is the first observation of Raman spectra measured on samples at nanogram levels excited by a He-Ne laser. Furthermore, the observed spectra are relatively free of any background scattering from the filter paper (the base line of silver colloids on filter paper is shown in Figure 1a). This is because these silver colloid enhanced resonance Raman spectra were obtained by the exclusive excitation at the 632.8 nm absorption line of the dye.

The enhancements were found to be dependent on the (1) preparation of the silver colloidal hydrosols, (2) sampling



**Figure 1.** SERS of 10 ng of crystal violet adsorbed on 22.5  $\mu\text{L}$  of silver hydrosols on (b) quartz tissue, (c) 3MM cellulose paper, (d) glass fiber GF/F, (e) glass fiber GF/B, (f) glass fiber GF/C, and (g) glass fiber GF/D. (a) is Raman spectrum of 22.5  $\mu\text{L}$  of silver hydrosols on GF/F glass fiber.

method, and (3) filter paper type. It has been known that surface-enhanced Raman scattering depends on the size of metal particles (3, 14). Any impurities present in the process of silver colloid preparation will introduce irreproducible SERS since they are known to induce the coagulation of silver particles. By use of freshly cleaned glassware, freshly made solution, and identical conditions for the preparation of silver colloidal hydrosols, the SERS can be reproduced within 80%. The colloid solutions used in this study had an absorption peak at 400 nm which is a characteristic of the well-known dipolar surface plasmon for silver spheres with radii small compared to the wavelength of light (6). Transmission electron microscopic examination revealed that the size of the particles was in the range of 100–300 Å (not shown).



**Figure 2.** SERS of (a) 150 ng of TMCP, (b) 300 ng of DECC, and (c) 1  $\mu\text{g}$  of MR adsorbed on 22.5  $\mu\text{L}$  of silver hydrosols on GF/F glass fibers. Spectrum (c) was taken with a 10-s integration time.

Higher Raman signals and better reproducibility were found when dye and silver hydrosols were introduced onto filter paper as a premixed mixture rather than separately. This effect can be explained by the interactions between filter fibers and silver particles which result in the latter being buried inside filter fibers, thereby making fewer silver particles available for adsorption of the dye.

SERS intensities of CV varied with different filter papers. Highest intensities were obtained on glass fibers GF/F (Figure 1d); chromatographic cellulose 3MM papers gave the lowest SERS (Figure 1c), with quartz fiber filters, between the two (Figure 1b). A variety of reasons might account for this observation, including the ability of the incident laser light to reach the dye as it adsorbed onto the filter fibers, but the most likely one is the difference in the average distance between the dye and silver particle. As SERS is a short range interaction process (12, 13), the closer the dye is to the silver particles, the higher are the SERS intensities. Glass and quartz fibers have crystalline structures that are much denser than the polymeric cellulose paper so that silver particles and dyes can be adsorbed more deeply into the fibers of the latter compared to the glass and quartz. In addition, competition between the silver particles and the filter fibers for dye adsorption may lead to less dye adsorption on those silver particles imbedded further into the filter. This effect would be most significant on the lower density cellulose filters. Consequently, SERS is lower on cellulose and higher on glass and quartz. This observation is further confirmed by com-

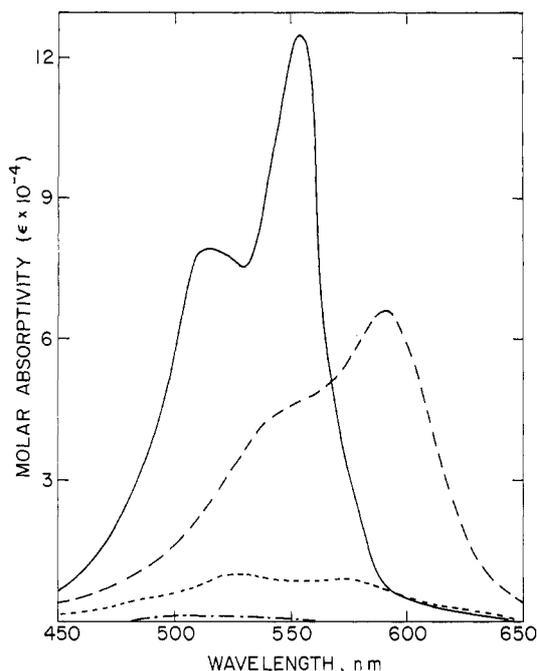


Figure 3. Absorption spectra of CV (---), TMCP (---), DECC (—), and MR (-.-).

paring SERS intensities of CV on glass fiber filters with different effective pore sizes (Figure 1). The densest sub-micrometer glass fiber filter, GF/F, provides the best support for CV as it gave the highest SERS (Figure 1d); FG/B, a lower density filter gave the next highest intensities (Figure 1e), while no additional effect was seen for filter type GF/C or GF/D which have even lower densities (Figure 1f,g). The ability of the incident laser light to excite the dye buried inside filter fibers does not seem to be a major reason for the difference in the Raman intensities. This stems from the study of the silver particle enhanced fluorescence of dyes and will be published in detail elsewhere (15).

The SERS effect from the interaction of CV and silver colloidal hydrosols is not unique to CV as it was also found for other dyes adsorbed on silver particles on filter paper. Figure 2 shows SERS from 150 ng of 1,1',9-trimethyl-2,2'-cyanine perchlorate (TMCP) (Figure 2a), 300 ng of 3,3'-diethylthiacarbocyanine chloride (DECC) (Figure 2b), and 1  $\mu$ g of Methyl Red (MR) (Figure 2c) adsorbed onto 22.5  $\mu$ L of silver colloidal hydrosols on GF/F glass fibers. The ability of this technique to detect TMCP and DECC dyes at the nanogram level on filter paper is of special interest because those cyanine dyes are used as spectral sensitizers in photographic techniques.

Calibration curves were obtained for those dyes by plotting the intensities of the Raman signals at 210  $\text{cm}^{-1}$  (for CV), 230  $\text{cm}^{-1}$  (TMCP), and 179  $\text{cm}^{-1}$  (DECC) against various concentrations of the dyes at an excess and fixed silver particle concentration. In all cases, the calibration plots exhibited a linear response over a wide ranges of concentrations with their slopes close to unity: slopes were 0.85 for CV, 1.10 for TMCP, 0.78 for DECC, and 1.2 for MR. From the calibration plots, limits of detection (LOD) were estimated for those dyes by determining the dye spotted amount that yielded a signal to noise ratio of two. Those values are shown in Table I. The lowest LOD was found for CV (500 pg) whereas values for other dyes are higher: 7 ng for TMCP, 15 ng for DECC, and 240 ng for MR to give an order of CV > TMCP  $\approx$  DECC > MR. A variety of reasons might account for this observation, including the difference in the binding of the dyes to silver colloidal hydrosols, but the most likely one is the difference in absorptivity of those dyes at the excitation wavelength, 632.8 nm (Figure 3). Since the observed Raman signals are

due to the silver hydrosols enhanced resonance Raman scattering, the greater the absorption of the excitation laser light by the dye, the more pronounced is Raman signal obtained. As can be seen in the Table I and Figure 3, the optical density of CV at 632.8 nm is 10333  $\text{M}^{-1} \text{cm}^{-1}$  which is the highest among the four dyes. The difference between the molar absorptivity of TMCP (1379  $\text{M}^{-1} \text{cm}^{-1}$ ) and DECC (1250  $\text{M}^{-1} \text{cm}^{-1}$ ) is very small whereas the optical density of MR at 632.8 nm is only 12.8  $\text{M}^{-1} \text{cm}^{-1}$ . Therefore, the order of molar absorptivity of dyes at 632.8 nm is CV > TMCP  $\approx$  DECC > MR which is same order as the LOD.

It is expected that the excellent subnanogram range LOD can be still further improved by some minor changes such as using argon ion and dye lasers to tune the excitation wavelengths to the absorption peaks of the dyes, these are 590 nm for CV, 530 nm for TMCP, 553 nm for DECC, and 500 nm for MR (Figure 3) (12, 16). Replacing the PMT by one with a higher gain such as a RCA 31034A-02 or a vidicon would enhance the signal to noise ratio (17). Increasing the power or focusing the laser would enhance the signal but it would also decrease the SERS intensity due to its heating effect. The latter will be ameliorated somewhat by sample rotation. These possibilities are now under investigation.

It has been demonstrated that the Raman spectra of an analyte at subnanogram levels can be measured by a simple and inexpensive technique. Further applications of this excellent technique is that the silver colloidal hydrosols not only enhance Raman cross section of the analytes but also enhance their fluorescences (12, 13) and phosphorescence (18). Experiments are now in progress to explore these potential applications.

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