

Figure 2. Heavy atom effect on the fluorescence of *trans*-BPyE in methanol at room temperature; a, by MeI; b, by KI.

creased when the concentration of potassium iodide is very small. This phenomenon is due to the salt effect on the fluorescence by alkaline salt. As the concentration of potassium iodide increases, the fluorescence intensity decreases because of the heavy atom effect. When methyl iodide is added, the intensity of fluorescence decreases showing normal heavy atom effect.

The fluorescence efficiency of *trans*-BPyE at 77K ($\phi_f \cong 0.025$) differs from those of *trans*-styrylpyridines and *trans*-1,2-dipyridylethylenes ($\phi_f \cong 1.0$ at 77 K). The triplet formation in BPyE is also observed by laser flash photolysis at room temperature^{7a} in contrast to styrylpyridines and 1,2-dipyridylethylenes. Azulene quenching studies indicate that the triplet state is the reactive state in photoisomerization.^{7a} From these results, therefore, it is concluded that the intersystem crossing of BPyE is an important process at room temperature and the direct photoisomerization of BPyE proceeds through the triplet manifold in contrast to stilbenes, azastilbenes, and diazastilbenes.

References

1. (a) Y.J. Lee, D.G. Whitten and L. Perderson, *J. Am. Chem.*

- Soc.*, **93**, 6330 (1971); (b) P. Bortolus, G. Favaro and U. Mazzucato, *Mol. Photochem.*, **2**, 311 (1970).
2. (a) U. Mazzucato, *Pure Appl. Chem.*, **54**, 1705 (1982) and references therein; (b) H. Görner and D. Schulte-Frohlinde, *Ber. Bursenges Phys. Chem.*, **81**, 713 (1977).
3. (a) G. Cauzzo, G. Galiazzo, U. Mazzucato, and N. Mongiat, *Tetrahedron*, **22**, 589 (1966); (b) P. Bortolus, G. Cauzzo, U. Mazzucato, and G. Galiazzo, *Z. Phys. Chem. F.M.*, **51**, 264 (1966); **63**, 29 (1969); (c) G. Favaro, F. Masetti and U. Mazzucato, *ibid.*, **66**, 206 (1969).
4. D.G. Whitten and M.T. McCall, *J. Am. Chem. Soc.*, **91**, 5097 (1969).
5. (a) M.A. El-Sayed, *J. Chem. Phys.*, **38**, 2834 (1963); (b) S.K. Lower and M.A. El-Sayed, *Chem. Rev.*, **66**, 199 (1966).
6. (a) M. Kasha, *Discussions Faraday Soc.*, **9**, 14 (1959); (b) L. Goodman, *J. Mol. Spectro.*, **6**, 109 (1961).
7. (a) P.-H. Bong, K.H. Chae, S.C. Shim, N. Nakashima, and K. Yoshihara, *J. Photochem.*, in press (1986); (b) P.-H. Bong, H.J. Kim, K.H. Chae, S.C. Shim, N. Nakashima, and K. Yoshihara, *J. Am. Chem. Soc.*, in press (1986).
8. S.C. Shim, D.S. Lee, J.S. Chae, and P.-S. Song, *J. Korean Chem. Soc.*, **20**, 398 (1976).
9. (a) G. Favaro, U. Mazzucato and F. Masetti, *J. Phys. Chem.*, **77**, 601 (1973); (b) G. Bartocci, P. Bortolus and U. Mazzucato, *ibid.*, **77**, 605 (1973).
10. (a) G. Favaro, F. Masetti, U. Mazzucato, and P. Bortolus, *J. Phys. Chem.*, **79**, 2785 (1975); (b) G. Beggiato, G. Favaro and U. Mazzucato, *J. Heterocyclic Chem.*, **7**, 583 (1970).
11. E.J. Shin, B.S. Ahn and S.C. Shim, *Bull. Chem. Soc. Jpn.*, in press (1986).
12. P.-S. Song, Q. Chae, M. Fujita, and H. Baba, *J. Am. Chem. Soc.*, **98**, 819 (1976).
13. A. Pullman and P. Schuster, *Chem. Phys. Lett.*, **24**, 472 (1974).
14. E.D. Megarity, Ph.D. Thesis, Florida State University (1969).

Surface-enhanced Raman Scattering on Chemically Prepared Silver Film for Trace Analysis

Doo Wan Boo, Kwan Kim*, and Myung Soo Kim*

Molecular Structure Group, Department of Chemistry, Seoul National University,
Seoul 151, Received August 26, 1985

Analytical capability of surface-enhanced Raman scattering has been evaluated. Silver films prepared by homogeneous chemical reduction were used as the substrate. Detection limits for *p*-nitrobenzoic acid, thiophenol and rhodamine 6G were around 10~100 pg.

Introduction

Raman spectroscopy is a useful technique for the analysis

of organic materials.^{1,2} Like the infrared spectroscopy, various structural information, particularly the presence of certain functional groups, is available from the Raman spectra. Ap-

plication of the Raman spectroscopy to the quantitative analysis is rather limited, however, due to the inherent weakness of the Raman effect. Using the ordinary Raman effect, detection limit of 2 ppm was reported in favorable cases.³ When the exciting light is in resonance with the UV/VIS absorption band of a sample, much stronger ($10^4 - 10^6$) Raman effect is often observed, so-called the resonance Raman effect. With the resonance Raman effect, the detection limit of 10^{-8} M was reported for favorable cases.⁴ However, use of the resonance Raman effect is limited to dye molecules which exhibit absorption in the visible or near ultraviolet spectral region. Moreover, the resonance Raman effect is very frequently masked by the presence of fluorescence.

Recently, it has been found that some molecules adsorbed on the surface of metals such as Ag, Au and Cu show enormously enhanced ($10^4 - 10^6$) Raman effect. This phenomenon, so-called the surface-enhanced Raman scattering (SERS),⁵⁻⁹ is under intensive investigation. It is expected that the technique will be established as a useful tool in surface science for the study of metal/molecule adsorption process. Analytical application^{10,11} of SERS has been limited so far, mainly due to the lack of proper preparation technique for substrate which show strong SERS signal with sufficient reproducibility. We have reported recently¹² a method of substrate preparation which may satisfy such requirements. Silver films prepared by homogenous chemical reduction were found to exhibit strong and reproducible SERS effect. Furthermore, the SERS effect could be controlled with ease by varying the reduction condition.

In this report, we demonstrate the analytical usefulness of SERS on the chemically prepared film. Examples of detection for several organic compounds such as p-nitrobenzoic acid (PNBA), thiophenol and rhodamine 6G (R6G) are given to illustrate the figures of merit of this technique.

Experimental

The silver film preparation technique using homogeneous chemical reduction has been described previously.¹² Briefly, ammonia solution of AgNO_3 was reduced by Rochelle salt ($\text{C}_4\text{H}_4\text{KNaO}_6$). Silver film was deposited on ordinary microscope slides with the size of $18 \text{ mm} \times 18 \text{ mm} \times 0.8 \text{ mm}$. For quantitative analysis, $25 \mu\text{l}$ of methanol solutions with varying sample concentrations was dropped on the film, and solvent was dried. On each film, a grease lining with 1.43 cm inner diameter was printed so that the dropped solution is spread over the same film area in each run.

Details of the apparatus for Raman measurement are the same as those in the previous report.¹² Briefly, Raman spectra were recorded with a JASCO Raman spectrometer (Model R-300) using 514.5 nm line of an Ar^+ laser (Spectra-Physics, Model 164-06). In a typical experiment, 50 mW of laser power was used. Raman scattering was observed at an angle of 90° to the exciting light, and signals were detected using a commercial photon counting system. Sample-coated silver film was spun during the spectral measurement at 3000 rpm using an AC motor in order to insure that there is no laser-induced change in the SERS spectrum.

Results and Discussion

In order that the SERS phenomenon be successfully ap-

plied to the trace organic analysis, the metal substrate should be easily prepared with good SERS reproducibility. In this work, we were able to obtain optimal silver films that exhibited highly intense and reproducible SERS spectra for PNBA. PNBA was selected as the model compound because the SERS spectrum of this compound has been previously investigated in this laboratory. The films selected on the basis of their SERS behavior to PNBA were almost indistinguishable to one another in the SERS efficiency to other adsorbates. Results of multiple measurements conducted on samples prepared under identical condition gave a relative standard deviation (precision) less than 10%. This reproducibility would be quite satisfactory for many analytical applications in trace analysis.

Surface-enhanced Raman measurements were performed for three organic samples—PNBA, thiophenol and R6G. SERS spectra for PNBA and thiophenol have been reported previously¹² and will not be reproduced here. Typical calibration curves for PNBA are illustrated in Figure 1. The

Table 1. Analysis of PNBA, Thiophenol and R6G in CH_3OH

	Added	Found ^a	% error
PNBA	1.00×10^{-4}	1.06×10^{-4}	6.0
	1.00×10^{-5}	9.47×10^{-6}	-5.3
	1.00×10^{-6}	1.13×10^{-6}	13
	1.00×10^{-7}	1.01×10^{-7}	1.0
Thiophenol	1.00×10^{-4}	1.05×10^{-4}	5.0
	1.00×10^{-5}	9.31×10^{-6}	-6.9
	1.00×10^{-6}	1.01×10^{-6}	1.0
	1.00×10^{-7}	1.02×10^{-7}	2.0
R6G	1.00×10^{-6}	9.73×10^{-7}	-2.7
	1.00×10^{-7}	1.03×10^{-7}	3.0
	1.00×10^{-8}	1.02×10^{-8}	2.0
	1.00×10^{-9}	9.77×10^{-10}	-2.3

^aConcentrations in moles \cdot liter⁻¹. ^bData were obtained from the calibration curves corresponding to the bands centered at 1354, 1575 and 1361 cm^{-1} for PNBA, thiophenol, and R6G, respectively.

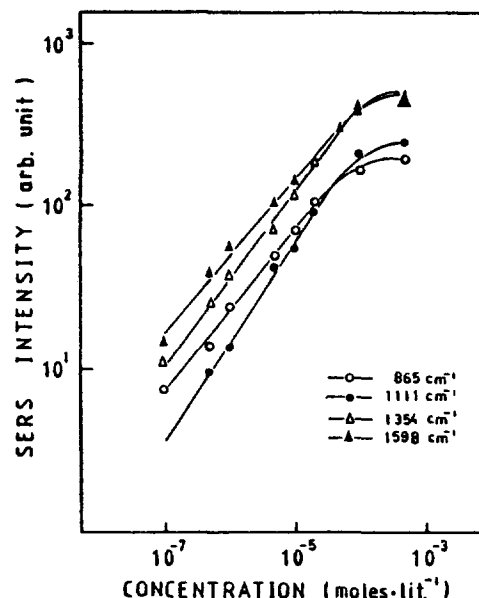


Figure 1. Calibration curves of PNBA on silver films.

data were obtained with four Raman bands centered at 865, 1111, 1354, and 1598 cm^{-1} . The log-log curves are linear from $\sim 10^{-4}$ M to 10^{-7} M and below, for all of four Raman bands. Correlation coefficients for linearity were larger than 0.995. Nonlinearity of the calibration curves occurs above $\sim 10^{-4}$ M. One possible reason for this saturation effect is that above a certain concentration there are not sufficient number of SERS-active sites available for every analyte compound.

Table 1 shows the values of concentration calculated from the calibration curve. Using the present technique, analysis of PNBA in pure methanol would be accurate within $\sim 15\%$ down to 10^{-7} M concentration range. It was also observed that four Raman bands are equally applicable to the analysis of PNBA. Detection limit for PNBA (in methanol) was estimated based on the present experimental results. The detection limit was defined as the amount of sample showing the signal-to-noise (S/N) ratio of 3 when 1 sec time constant was used. For three molecules investigated, the noise levels near the detection limit were mostly due to the broad fluorescence background from the film. In case of PNBA, the detection limit was 74 μg .

It should be noted, however, that the limit of detection is given per sample dose and does not account for the laser/sample illumination ratio. The spot area of the exciting laser at the focused point on the mirror surface was estimated¹³ to be $\sim 7 \times 10^{-5}$ cm^2 . Among the total sample coated on the mirror surface with an area 1.61 cm^2 , only $\sim 0.004\%$ was thus illuminated with the laser. Such a consideration leads to the actual detection limit of ~ 10 fg for PNBA. Hence, the SERS is a promising new technique for trace analysis in picogram range with the improvement in sample delivery method.

Calibration curves for thiophenol are shown in Figure 2. They were drawn for the bands ν_{12} (1001 cm^{-1}), ν_{18a} (1024 cm^{-1}), ν_{18b} (1075 cm^{-1}) and ν_{8a} (1575 cm^{-1}). Details on the spectroscopic assignments and the adsorption mechanism of thiophenol on silver surface are reported elsewhere.¹⁴ Here again, all of four calibration curves show linear region between 10^{-4} M and 10^{-7}

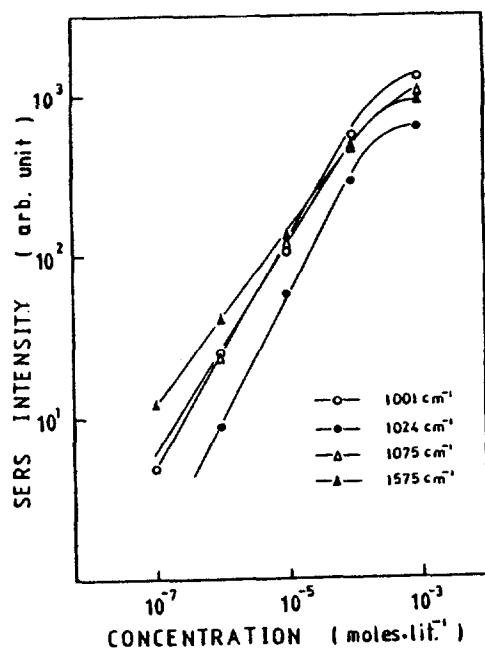


Figure 2. Calibration curves of thiophenol on silver films.

M with the correlation coefficients larger than 0.999. From these results, it was estimated that the analysis of thiophenol would be accurate within $\sim 10\%$ down to 10^{-7} M concentration range using the present technique. The fact that thiophenol exhibits better reproducibility, compared to PNBA, is rather surprising. It seems that thiophenol interacts with the silver surface improving its SERS performance. Possibility for such a phenomenon was extensively investigated in our previous report.^{15, 16} Detection limit for thiophenol was comparable to that of PNBA.

R6G is a strongly fluorescent xanthine derivative which shows absorption and emission maxima at 415 nm and 590 nm, respectively, in methanol solution. Resonance Raman scattering is known to occur but is not detectable by ordinary means due to the presence of much stronger fluorescence band. However, SERS has provided new avenues for dealing with fluorescent materials which can not be easily dealt with in ordinary or resonance Raman spectroscopy. When a dye molecule is adsorbed on silver surface, its excited electronic state is coupled to surface plasmon modes. Weitz et al.¹⁷ noted that such a coupling results in extra decay channel for dyes with high quantum yield of fluorescence. Accordingly, fluorescence quantum yield for R6G adsorbed on silver film is drastically reduced. Hence, the resonance Raman signals which were hidden underneath the fluorescence band in solution spectrum are now exposed as shown in Figure 3. To be sure, Raman signals thus obtained are influenced by the combined effects of resonance Raman and SERS. Calibration curves for R6G based on the major SERS peaks at 610, 772, 1361, 1507, and 1645 cm^{-1} are shown in Figure 4.

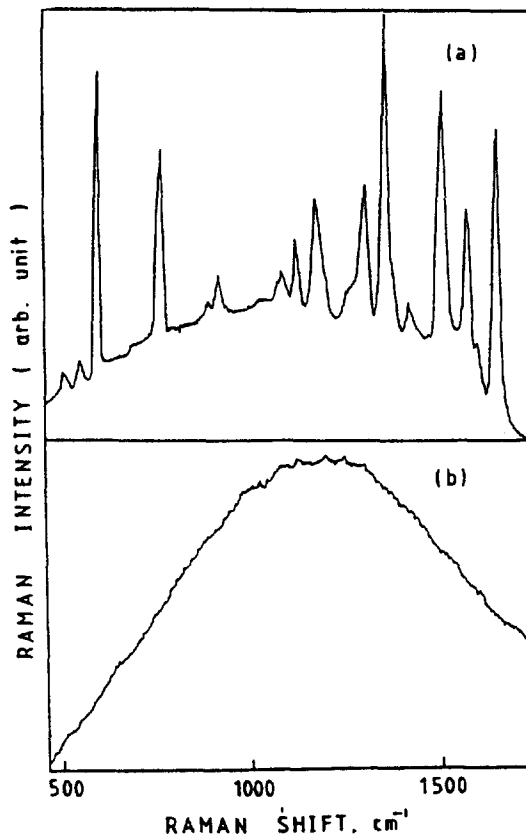


Figure 3. (a) SERS spectrum of R6G on silver film; (b) Raman spectrum of R6G(10^{-7} M) in CH_3OH .

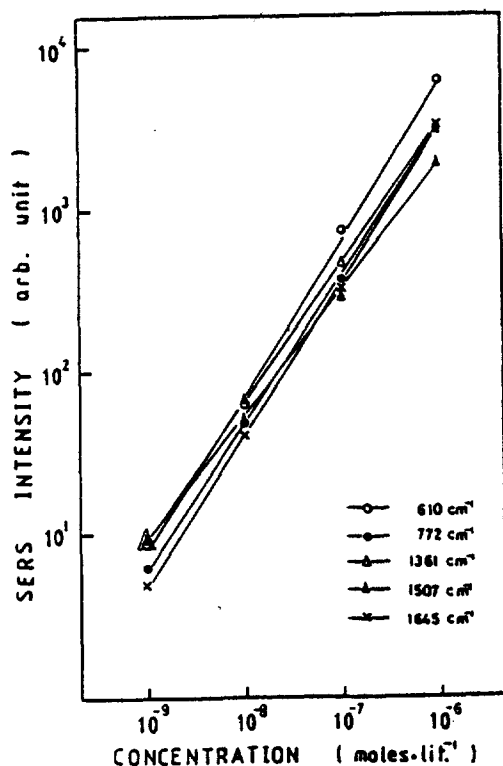


Figure 4. Calibration curves of R6G on silver films.

For all of five Raman bands, the calibration curves are linear from 10^{-6} M to 10^{-9} M. Linearities are excellent here again with correlation coefficients larger than 0.999. Nonlinearity above 10^{-6} M is not due to the saturation of active sites as was proposed for the PNBA and thiophenol cases. For R6G, background fluorescence signals from molecules remaining unadsorbed at high concentration interfered with Raman measurement. Based on the present data, the detection limit for R6G was ~ 6 pg, while the actual limit is expected to lie around 1 fg. Using absorption or fluorescence spectroscopy, detection sensitivity for R6G can be better than the present result. However, the present technique is much superior to those techniques in selectivity.

We have demonstrated the utility of SERS in trace analysis. Use of very small amount of sample is one obvious advantage of the present technique. Another advantage of SERS technique is its spectral selectivity. Especially, such a selectivity is achievable at high sensitivity. To summarize, SERS makes avail the selectivity of vibrational spectroscopy at the sensitivity of electronic spectroscopy. Hence, SERS would be proven useful not only for quantitation, as demonstrated here, but also

for identification of trace amount of sample. Similar analytical performance is also achieved in resonance Raman spectroscopy. However, it is to be noted that samples showing such effect are limited. Even for those molecules, SERS effect may be used advantageously to improve the spectral quality.¹⁸

Finally, we would like to compare the present results with those reported by Vo-Dinh *et al.*¹⁰ where an elaborate vacuum deposition technique was utilized for substrate preparation. The present method is simpler and easier. Also, the precision of analysis is better at least by a factor of 2. Based on the actual detection limit, the present film was more sensitive by a factor of 100.

Acknowledgement. This work was financially supported by the Ministry of Education, Republic of Korea [ED 85-303].

References

1. D.A. Long, "Raman spectroscopy", McGraw-Hill, New York, 1977.
2. J.G. Grasselli, M.K. Snavely and B.J. Bulkin, *Phys. Reports*, **65**, 231 (1980).
3. N. Furuya, A. Matsuyuki, S. Higuchi and S. Tanaka, *Water Res.* **13**, 371 (1979).
4. L.C. Hoskins and U. Alexander, *Anal. Chem.* **49**, 695 (1977).
5. R.K. Chang, T.E. Furtak Eds., "Surface Enhanced Raman Scattering" Plenum Press, New York, 1982.
6. J. Gersten, and A. Nitzan, *J. Chem. Phys.* **76**, 3023 (1980).
7. C.A. Murray, and D.L. Allara, *J. Chem. Phys.* **76**, 1290 (1980).
8. J.A. Creighton, *Surface Sci.* **124**, 209 (1983).
9. M.E. Lippitsch, *Phys. Rev.* **B29**, 3101 (1984).
10. T.Vo-Dinh, M.Y.K. Hiromoto, G.M. Begun and R.L. Moody, *Anal. Chem.* **56**, 1667 (1984).
11. C. Jennings, R. Aroca, A. Hor and R.O. Loutfy, *Anal. Chem.* **56**, 2033 (1984).
12. D.W. Boo, W.S. Oh, M.S. Kim, K. Kim and H.C. Lee, *Chem. Phys. Lett.* **120**, 301 (1985).
13. A.E. Siegman, "An introduction to lasers and masers", Chap. 8, p 317, McGraw-Hill, New York, NY, 1971.
14. T.H. Joo, K. Kim and M.S. Kim, *J. Raman Spectrosc.* in press.
15. T.H. Joo, K. Kim and M.S. Kim, *Chem. Phys. Lett.* **112**, 65 (1984).
16. H.S. Kim, M.S. Kim and K. Kim, *Bull. Kor. Chem. Soc.* **6**, 61 (1985).
17. D.A. Weitz, S. Garoff, J.I. Gersten and A. Nitzan, *J. Chem. Phys.* **78**, 5324 (1983).
18. T.M. Cotton, S.G. Schultz and R.P. Van Duyne, *J. Am. Chem. Soc.* **104**, 6528 (1982).