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Observation of SERS effect in Raman optical activity, a new tool for chiral vibrational spectroscopy

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A new tool for chiral vibrational spectroscopy is reported here. A surface enhanced effect was observed using Raman optical activity (ROA). This observation opens new possibilities for ROA as a tool for vibrational spectroscopy. The combination of surface enhanced effect (SE) and ROA into surface enhanced Raman optical activity (SEROA) takes this tool to another level, where a single molecule may be studied with respect to chirality, secondary structure and fold determination. ROA has been able to provide information about important dynamics in molecular understanding. Until recently, however, ROA measurements required a longer exposure and higher concentration of the sample. With SEROA these obstacles can be overcome because both studies on single molecule, i.e. very low concentration, and faster acquisition of the signal can be carried out. In the present, work silver colloids were mixed with solution, in which a pentapeptide, Met-Enkephalin, was dissolved. SEROA signals were recorded and the results are reported here. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: ROA; SERS; molecular spectroscopy

INTRODUCTION

Surface enhanced Raman scattering (SERS) effect has now been known for three decades,[1–3] and today its applications cover areas in science such as physical chemistry, analytical chemistry and biomedicine. Applications can also be found for bigger systems such as living cells, drug complexes and proteins,[4–8] and for smaller systems such as amino and nucleic acids.[9,10] SERS and the resonant SERS (SERRS) have been developed into powerful Raman Spectroscopy (RS) methods and the weak signal of RS is no longer a disadvantage. SERS can provide typical enhancements of 10^4–10^6 times that provided by RS, and so far an enhancement of up to 10^{14} has been reported.[11] This means that the high concentrations required in RS (normally 10^{-2} M) may be reduced to 10^{-16} M, and hence detection of a single molecule may be achievable.[12,13] Moreover, the advanced optical technology employed in the state-of-the-art Raman apparatus brought the detection time in RS down to milliseconds (Hug W., ICORS2002 Proceedings and Nafie L.A., ICORS2004 Proceedings). Today, SERS as a spectroscopic tool is widely used for molecular detection and structural studies owing to its ultra-high sensitivity and surface selectivity (Refs 14, 15 and references therein).

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Concerning the SERS effect, there are several mechanisms involved in such an enhancement, and generally, it occurs because of two effects: electromagnetic and chemical effects.[16,17] The first is due to the boost in the optical local field due to coupling between photons and electron oscillations at the metal surface. The enhancement factor $\boldsymbol{G}$ to a first approximation can be expressed as[18]

$$\boldsymbol{G}_{em}(\boldsymbol{r}_m, \nu) = |\boldsymbol{E}(\boldsymbol{r}_m, \nu)/\boldsymbol{E}_i(\nu)| \quad (1)$$

where $\boldsymbol{r}_m$ is the molecular location, $\boldsymbol{\nu}$ the laser frequency, $\boldsymbol{E}(\boldsymbol{r}_m, \nu)$ the total electrical field and $\boldsymbol{E}_i(\nu)$ the incident excitation field.

The chemical effect due to charge transfer complexes occurs between adsorbed molecules and a roughened metal surface. This brings the orbital energy levels of the adsorbed molecule closer to the Fermi level of the metal, and when the energy difference happens to be close to those of the incident photons, an enhancement similar to resonance Raman takes place.[19] The cross-section of adsorption that exists between the nano-sized particles, e.g. Au or Ag colloids, and the molecules dissolved in the solvent, plays a big role in this enhancement. This is due to the 10^{14}–10^{16} times bigger cross-section in SERS than in RS, thus a corresponding enhancement of the signal can be expected following the signal power and the cross-section relation. This can be expressed in the power spectrum as

$$\boldsymbol{P}_{\mathrm{SERS}}(\nu) = \boldsymbol{NI}(\nu)|\boldsymbol{A}(\nu_{\mathrm{L}})|^2|\boldsymbol{A}(\nu_{\mathrm{s}})|^2\sigma_{\mathrm{ads}} \quad (2)$$

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where N is the number of molecules, $I(\nu)$ the intensity of the excitation laser, $A(\nu_L)$ and $A(\nu_s)$) the field confinement of the optical coefficients related to the incident and the scattered light frequency, respectively, and σ_{ads} is the adsorption cross-section. The field enhancement factor depends also on the distribution of the colloids in the measured sample. The enhanced factor can be seen to be as big as 10^6 for isolated particles, whereas it is up to 10^{14} for clustered nano-particles.[11] A theory of first-layer and single-molecule SERS is discussed by Otto and can be found in Ref. 20 (and references therein). In brief, SERS can provide an ultra-sensitive detection limit and high structural information without any bleaching owing to the employment of non-resonant Raman process, no labeling is needed because of the short exposure time, and owing to the control and small concentration SERS is suitable for applications in biomolecular studies related to biology.

Another tool that makes use of RS and is used for molecular spectroscopy is Raman optical activity (ROA). The fundamental mechanism responsible for ROA was discovered[21] in 1969 and a more elaborate theory was developed[22] in 1971. Circularly polarized light is utilized in ROA and the information about chirality can be achieved accordingly. The ROA signal is even weaker than ordinary RS, usually by a factor of 10^{-3}, and spectrum detection, consequently, requires longer exposure time. This is because the ROA spectrum represents the difference in intensities between Raman scattered light using right- and left-circularly polarized incident light. The dimensionless circular intensity difference (CID) is defined[23] as follows:

$$\Delta = (I^R - I^L)/(I^R + I^L) \quad (3)$$

where I^R and I^L are the scattered intensities in the right- and left-circularly polarized incident light, respectively. The theory has been further developed and can be found in Refs 22, 24, 25. In spite of the weak ROA signal and the complexity of the optical setup, ROA studies have provided insight into biochemical problems,[26] e.g. fold determination, and structured non-native states studies[27,28] are useful in understanding the conformational plasticity that underlies many of the conformational diseases such as Alzheimer's and prion encephalopathies. In general, ROA is sensitive to folding, secondary and tertiary structural changes, and mostly to chirality, so more accurate information about the chiral molecule can be determined. In spite of these advantages, ROA is made use of today only in a few laboratories and mostly in research applications. In the near future with the use of SERS enhancement, surface enhanced Raman optical activity (SEROA) can find more applications in biochemical, biophysical and biomedical laboratories, where information about chirality can be of significance.

In the following text, we present a novel combination of the surface enhanced effect used normally in SERS with ROA, and give it the name SEROA. To our knowledge no reliable SEROA data has been reported as yet. However, there was a previous report in which the term SEROA was first proposed. It appeared in a Ph.D. thesis [Gu-Sheng Yu *Development and Application of Raman Optical Activity* (Ph.D. Dissertation, Syracuse University) (1994), 134–141], but the work has not been published elsewhere.

In the present experiment we have used Ag colloids in a solution, in which a pentapeptide, Met-Enkephalin (Met-Enke), has been dissolved. The colloids were prepared following the method described elsewhere,[29] and the size was controlled to approximately 40 nm, measured by UV-vis. Met-Enke is a chiral molecule, and we recently have performed vibrational studies of this molecule using Infrared (IR) and vibrational circular diochroism (VCD),[30] RS,[31] and SERS.[32] Therefore, this molecule was chosen as a good candidate to be examined by SEROA. We present the experimental setup and the results from Met-Enke, which were repeated on different instruments. However, other molecules are presently being tried out in order to reach a better understanding of the effects and limits of SEROA as a new and powerful chiral vibrational tool.

EXPERIMENTAL

Met-Enke powder was purchased from Bachem, and the sample specifications provided by the manufacturer were: purity (HPLC) more than 99% and peptide and water contents of 97.5% and 2.4%, respectively. By dissolving 15 mg of the sample powder in 0.5 ml 0.1 M KCl aqueous solution (24 mg KCl in water), a concentration of 10^{-2} M was achieved. Such high concentration is necessary to achieve a reliable intensity of the characteristic Raman bands because the intensity is proportional to the fourth power of the laser frequency. Raman spectra were recorded using a state-of-the-art ChiralRaman instrument (BioTools Inc., USA), which simultaneously provides Raman and ROA spectra. The instrument utilizes a 532-nm laser source (Excel, LaserQuantum, UK), and the CCD camera mounted on the system allows detection of wavenumbers in the range 100–2000 cm^{-1}. In the present work, the sample was contained in a quartz cell of 120 μl volume, and the spectra were recorded for 20 min. The resolution of the ChiralRaman is approximately 7 cm^{-1}, determined by the width of the individual fibers of the fiber optic cable at the entrance to the spectrograph. The fibers were arrayed in a curved line to compensate exactly for the curved image at the detector in the opposite direction, which would result from a straight-line image at the input of the spectrograph.[33]

In Fig. 1 a schematic diagram of the experimental setup is shown. The light, generated by a laser (A), is aligned by passing through double precision slits (B), each of 3 mm in diameter, and reflected off a prism plate (C) on which two 45° mirrors are placed on top of each other. The light is reflected off the first mirror towards the second mirror, off which it is reflected further towards the incident rail, and the beam is

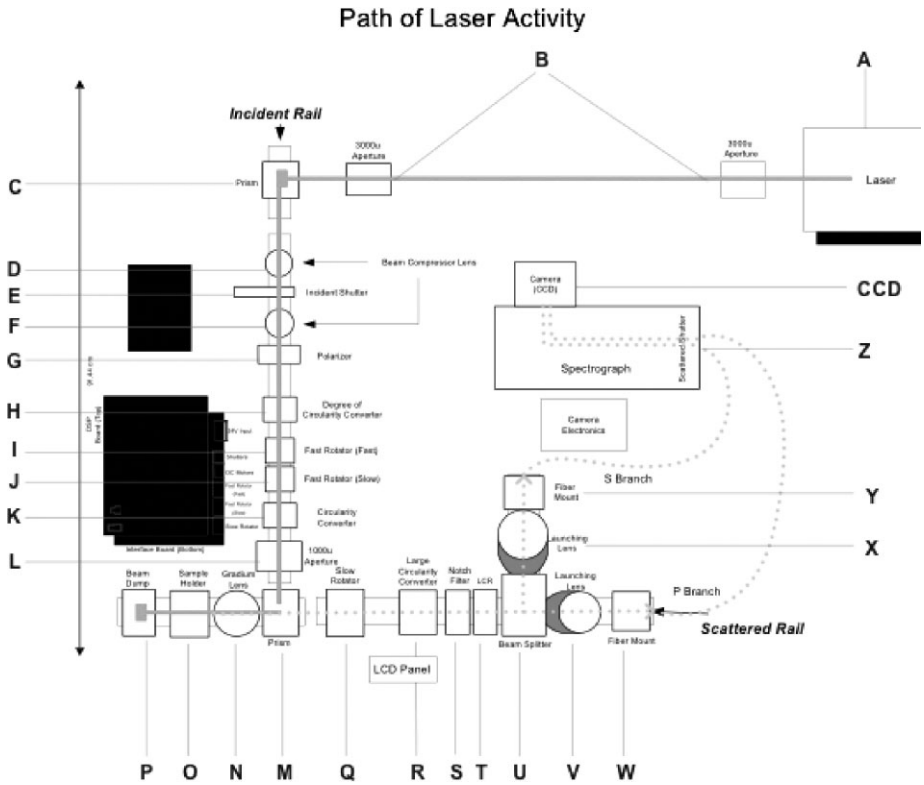

Figure 1. Schematic diagram of the instrument. The solid line represents the incident beam, and the dotted line the scattered.

thus reflected 90° anti-clockwise with respect to the incident beam.

A convex lens (D) compresses the beam before it enters the software-controlled shutter (E) that determines the illumination time (0.07–10 s). The beam is thereafter focused by another lens (F) into the polarizer (G), which in turn eliminates any circularly polarized light. The degree of circularity converter (DCC) is achieved by a quarter wave plate (H) mounted on a motorized arm, which places it in and out of the beam when needed. Furthermore, a double half-wave plate (I and J) synchronized counter-rotating rotor system is mounted in the beam path. This uniformly distributes the polarization direction over the illumination period and thus eliminates any biases due to linear polarization in the incident beam. The beam is then passed through a movable half-wave plate (K) that systematically flips the direction of any residual circularly polarized light so that it can be cancelled by the software. Through a 1-mm aperture (L) the beam is further reflected off the second prism (M), which is similar to the primary prism but mounted so that the beam is now reflected 90° clockwise with respect to the incident beam. The gradium lens (N) utilizes a unique optical glass, in which the refractive index varies along the optical axis. Unlike conventional lenses, light actually bends as it passes through this lens and the beam is focused on the sample. The sample holder (O) is a cradle where a cell of quartz windows is placed.

The transmitted beam through the sample is absorbed in a dumper (P), whereas the scattered beam (180°) travels along the scattered rail through the gradium, the secondary prism and the slow rotor (Q). The degree of circular conversion is repeated for the scattered beam similar to that for the incident beam by utilizing a slow rotor (Q) and a large circularity converter (R). To discriminate the elastically scattered Rayleigh light, a holographic notch filter (S) is mounted on the scattered rail. The beam is thereafter split by a beam splitter (U) into s and p branches and guided by fiber optics to the spectrograph (Z) where a CCD is mounted at its exit.

RESULTS AND DISCUSSION

Using a laser power of 430 mW and a wavelength of 532 nm, both RS and ROA spectra of the 10^{-2} M Met-Enke were simultaneously recorded for 60 min. In Fig. 2 a typical RS spectrum (a) is shown together with the ROA spectrum (b). Considering the RS spectrum (Fig. 2(a)), the peak doublet, known as the Fermi doublet, in RS at 850 and 830 cm^{-1} is characteristic for Tyr, and is due to the Fermi resonance between a ring vibration in tyrosine and an overtone of out-of-plane ring bending vibrations. The peaks at 1032 cm^{-1} and at 1004 cm^{-1} are ring breathing vibrations, which are due to the mono-substituted benzene ring in phenylalanine. The peak at 1208 cm^{-1} is due to the CCO out-of-plane stretching vibrations, which originates from tyrosine.

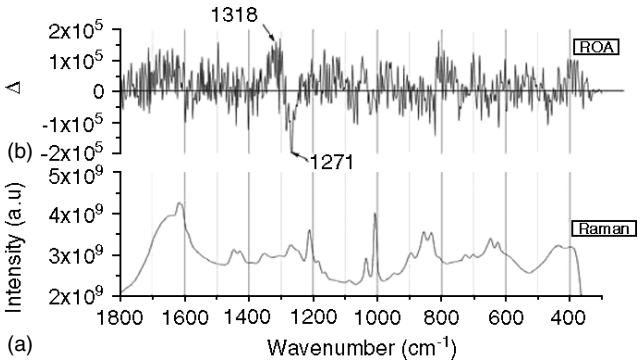

Figure 2. Raman (a) and ROA (b) spectra of 10^{-2} M Met-Enke, obtained in 60 min.

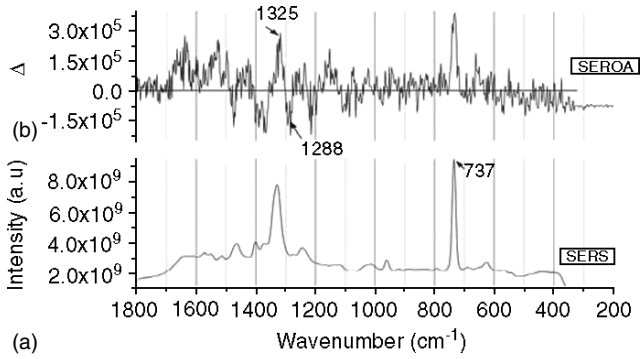

Figure 3. SERS (a) and SEROA (b) spectra of 50 μM Met-Enke, obtained in 20 min.

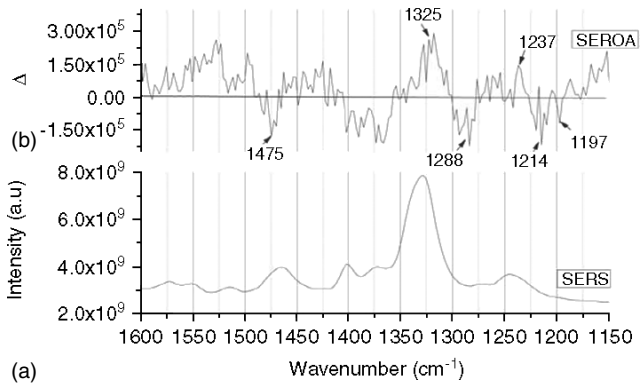

Figure 4. A blow-up of the frequency region 1150–1600 cm^{-1} of the spectra in Fig.3.

The Amide III band can be seen at 1230 cm^{-1}, and at 1443 cm^{-1} there is a peak due to CH2 and CH3 bending vibrations. The strong peak at 1626 cm^{-1} is due to the aromatic C–C stretch vibrations of the aromatic side chains in phenylalanine and tyrosine. The ROA spectrum is shown in Fig. 2(b), and the strong doublet, positive centered around 1318 cm^{-1} and negative around 1271 cm^{-1}, is usually assigned to polyproline II helix or another disordered structure.[34,35] Similarly, the negative ROA band at 1271 cm^{-1} is probably indicative of a β-turn-type local conformation. These assignments make more sense since a short peptide like Met-Enke is expected to have disordered type structure at both ends due to fraying effects. This observation agrees with that shown in earlier studies.[30,31]

In order to carry out SERS and SEROA measurements, the sample was diluted (1 : 100) in water and 60 μl of the new concentration was mixed with 60 μl Ag-colloid solution in the 120 μl cell, and the measurement was carried out for 20 min. In Fig. 3(a) and (b), SERS and SEROA spectra are shown, respectively. The peaks at 737 cm^{-1} and at 1340 cm^{-1} have also been seen in our earlier SERS study[34] on Met-Enke. In the earlier study a pronounced peak at 1004 cm^{-1}, attributed to the phenylalanine, was also seen, while in the present study (Fig. 3(a)) this peak seems to be absent. This may be due to difference in the method of preparation of the sample, whereas in the earlier study the sample was placed on a glass plate under a confocal microscope, and only a smaller, dry area of the sample was exposed to the incoming beam, in this study the sample was an aqueous solution contained in a 120 μl quartz cell. Nevertheless, the ROA signal attributed to the same β-conformation (doublet around 1300 cm^{-1}) is still seen in Fig. 3(b), positive centered around 1325 cm^{-1} and negative around 1288 cm^{-1}. The region 1600–1150 cm^{-1} of the spectrum in Fig. 3 is also shown in Fig. 4, in which the SEROA peaks are assigned.

It is obvious that a ROA enhancement (SEROA) has occurred owing to the low concentration of the sample and the short recording time of the spectra compared to that used in RS and ROA. Moreover, the SEROA signal in Fig. 3 is even stronger than that of the ROA (Fig. 2).

Accordingly, SEROA is now feasible. Within our group, research is in progress on other known molecules and proteins, such as insulin, myoglobin and glucagons, in order to explore the new possibilities of this tool and to prove the efficiency of SEROA in studying biomolecular dynamics. In these studies, the effects of pH-value, temperature and concentration will be taken into consideration. In an attempt to utilize SERS and SEROA, we were able to synthesize our own Ag- and Au-colloids of high stability and controlled sizes in the range of 30–100 nm.

CONCLUSION

SERS and ROA have been combined into SEROA. The effect was shown to be present when a known pentapeptide molecule, Met-Enke, was examined. The SERS signal was confirmed from an earlier study, while the SEROA was seen here for the first time. The study has so far shown that SEROA can be achieved with modern instruments by using Ag colloids and for 10^{-2} concentrations of that used in ROA. Studies on other molecules combined with other colloid solutions, e.g. Au-colloids, are still in progress.

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REFERENCES

1. Fleishmann M, Hendra PJ, McAuillan AJ. *Chem. Phys. Lett.* 1974; **24**: 163.
2. Jeanmaire DL, Van Duyne RP. *J. Electroanal. Chem.* 1977; **84**: 1.
3. Albrecht MG, Greighton JA. *J. Am. Chem. Soc.* 1977; **99**: 5215.
4. Kim SK, Kim MS, Suth SW. *J. Raman Spectrosc.* 1987; **18**: 171.
5. Dou X, Jung YM, Cao Z-Q, Ozaki Y. *Appl. Spectrosc.* 1999; **53**: 1440.
6. Watanabe T, Maeda H. *J. Phys. Chem.* 1989; **93**: 3258.
7. Suh JS, Moskovits M. *J. Am. Chem. Soc.* 1986; **108**: 4711.
8. Chumanov GD, Efromov RG, Nabiev IR. *J. Raman Spectrosc.* 1991; **21**: 43.
9. Tessier PM, Velev OD, Kalambur AT, Rabolt JF, Lenhoff AM, Kaler EW. *J. Am. Chem. Soc.* 2000; **122**: 9554.
10. Olson LG, Lo Y-S, Beebe TP Jr., Harris JM. *Anal. Chem.* 2001; **73**: 4268.
11. Kneipp K, Kneipp H, Itzkan I, Dasari PR, Feld MS. *Chem. Rev.* 1999; **99**: 2957.
12. Kneipp K. *Exp. Techn. Phys.* 1988; **36**: 161.
13. Kneipp K, Wang Y, Dasari PR, Feld MS. *Appl. Spectrosc.* 1995; **49**: 78.
14. Brolo AG, Irish DE, Smith BD. *J. Mol. Struct.* 1997; **405**: 29.
15. Campion A, Khambhampati P. *Chem. Soc. Rev.* 1998; **27**: 241.
16. Otto A. In *Light Scattering in Solid*. Cardona M, Guentherodt G (eds). Springer: Berlin, 1984.
17. Moskovits M. *Rev. Mod. Phys.* 1985; **57**: 783.
18. Garcia-Vidal FJ, Pendry JB. *Phys. Rev. Lett.* 1996; **77**: 1163.
19. Clark RJH, Dines TJ. *Angew. Chem.* 1986; International Edition in English **25**: 131.
20. Otto A. *Phys. Stat. Sol. A* 2001; **188**(4): 1455.
21. Atkins PW, Barron LD. *Mol. Phys.* 1969; **16**: 453.
22. Nafie LA, Che D. *Adv. Chem. Phys.* 1994; **85**: 105.
23. Barron LD, Buckingham AD. *Mol. Phys.* 1971; **20**: 1111.
24. Hecht L, Barron LD. *Appl. Spectrosc.* 1990; **44**: 483.
25. Polavarapu PL. *Vibrational Spectra*. Elsevier: Amsterdam, NY, 1998.
26. Barron LD, Hecht L, Blanch EW, Bell AF. *Prog. Biophys. Mol. Biol.* 2000; **73**: 1.
27. Dill KA. *Nature* 1999; **400**: 309.
28. Carrell RW, Lomas DA. *Lancet* 1997; **350**: 134.
29. Lee PC, Meisel D. *J. Phys. Chem.* 1982; **86**: 3391.
30. Abdali S, Jalkanen K, Cao X, Nafie LA, Bohr H. *Phys. Chem. Chem. Phys.* 2004; **6**: 2434.
31. Abdali S, Refstrup P, Faurskov-Nielsen O, Bohr H. *Biopolymers (Biospectroscopy)* 2003; **72**: 318.
32. Kneipp K, Kneipp H, Abdali S, Berg RW, Bohr H. *Spectroscopy* 2004; **18**: 433.
33. Hug W, Hangartner G. *J. Raman Spectrosc.* 1999; **30**: 841.
34. IMcColl IH, Blanch EW, Gill AC, Rhie Alexandre GO, Ritchie MA, Hecht L, Nielsen K, Barron LD. *J. Am. Chem. Soc.* 2003; **125**: 10 019.
35. McColl IH, Blanch EW, Hecht L, Kallenbach NR, Barron LD. *J. Am. Chem. Soc.* 2004; **126**: 5076.