Application of surface enhanced Raman scattering spectroscopy in rat serum

Kun Liu (刘 琨)[1], Shifa Wu (吴世法)[1], Yi Zhang (张 毅)[1], and Hui Liu (刘 辉)[2]

[1] *SInstitute of Near-Field Optics and Nanotechnology, Department of Physics, Dalian University of Technology, Dalian 110624*

[2] *College of Medical laboratory, Dalian Medical University, Dalian 116023*

High signal-to-noise surface enhanced Raman scattering (SERS) signal of rat serum was obtained in this article. Two methods were presented to enhance the intensity of rat serum SERS signal. The novel colloid silver was synthesized using heating method by microwave and it was introduced to be the active site of SERS, "hot sites" could be detected in the active site. At same time the high numerical aperture (NA) oil immersed object lens was applied in micro-Raman system so that some new peaks come up in SERS spectrum of rat serum because of the evanescent wave was employed to excite the sample.

OCIS codes: 290.5860, 290.5910, 300.6450, 170.5660, 170.6510.

Raman spectroscopy is a powerful tool to acquire conformational information of biomaromolecule, especially for detecting native conformation of low concentration samples in aqueous solution. The vibration frequency of biomaromolecule is very complicated, which is intimately involved in the information of space geometrical structure and configure of chemical bond[1]. But the cross section of Raman scattering is typically 10^{-29} cm^2, which is less than that of fluorescence about 10^{-13}, most of biological molecules are all belong to intense fluorescence character, so the weak Raman signal generally is buried within a broad, intense fluorescence background. The surface-enhanced Raman scattering (SERS) effect is a good way to increase the cross section of Raman scattering, it was found that some bands in the Raman spectra of molecules adsorbed on specially prepared metal specimens could be strongly enhanced. Among these metal specimens there are electronchemically or lithographically roughed metal surfaces, metal island films and metal colloids. In this article, a novel colloid silver was synthesized using heating method by microwave and it was introduced to be the active substrate of SERS. The rat serum was studied as an example, which is composed of proteins such as albumin, alpha globulin, beta globulin, immune globulin and other product produced in the process of metabolism. The high effective SERS active substrate played an important role in measuring rat serum Raman spectrum.

The beaker which was put in solution of 0.02% $AgNO_3$(150 ml) was placed in the center of micro-wave oven(Glanze WP700, 700 W, 2450 MHz), and was irradiated with 100% power for about 3 minutes,then a solution of 1% 4 ml sodium citrate was added, irradiating was continued for 2 minutes. After irradiation, the colloidal solution was cooled to room temperature, the Ag sols were brownish red. 2 μL Ag colloid solution mixed with 2 μL 0.01% NaCl aqueous solution was coated on to a ZF glass plate then evaporated in room temperature, after about 30 minutes, 2 μL rat serum was dropped on the dry silver aggregates. Another 30 minutes later, the sample was excited by laser beam (wavelength 632.8 nm), the power reached the surface of sample was about 3.5 mW, exposed time was about 10 s, SERS spectra of rat serum were recorded with micro-Raman system which makes up by Raman spectrophotometer (Renishaw Invia, UK) combined with inverse microscope (ZEISS Axiovert25). In this experiment, the high numerical aperture (NA) oil immersed object (NA=1.65) lens was employed in micro-Raman system.

Figure 1 shows the transmission electron micrograph TEM of Ag sols which was added 0.01% NaCl solution in order to make the Ag nanodisks aggregation, the particals have been deposited on a copper grid and allowing the solvent to evaporate. From the micrograph, we have found that the average sizes of the particles were about 40–50 nm, and the shapes of most particles were disk-like. It was observed that most Ag nanodisks were conjunct each other, which accorded with the condition of forming "hot sites". Several papers[2] reported that there existed some "hot sites" which contributed dominantly to SERS in SERS-active substrate, the conclusion of research was that most effective SERS-active regions are the interstices between particles. The work of Brus *et al.*[3] also assigned the SERS "hot sites" at the junctions between individual Ag nanoparticles. The enormous increase in SERS intensity largely comes from the increase in the magnitude of both the incident and the scattered electromagnetic (EM) fields resulting from the excitation of surface plasmons in the "hot sites". In our experiment, the SERS-active substrate with "hot spots" was high effective for detecting SERS of rat serum.

The high NA oil immersed object (NA=1.65) whose magnification factor is 100 was applied in micro-Raman system, which induced that not only the resolution but also the intensity of SERS can be improved. Figure 2

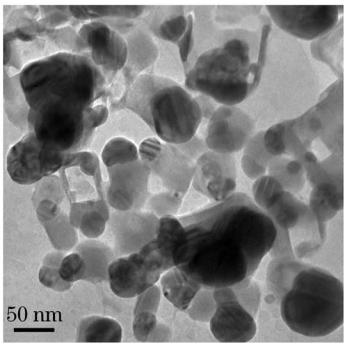

Fig. 1. TEM of silver colloidal nanopaticles dropped 0.01% NaCl aqueous solution.

showed the configuration of high NA objective lens using in micro-Raman system, the exciting light came from evanescent wave in the surface of glass plate when the incident angle of exciting laser was above its critical angle 37.3°. The sample was illuminated by the light focused by the high NA objective lens. The Raman-scattered light by the molecule was collected by the same objective lens. NA of object lens is 1.65, and its critical angle for total reflection is about 37.3°, the evanescent wave formed at the glass surface when the incidence angle of exciting laser was above its critical angle 37.3°. The sample was illuminated both by transmitted light and evanescent light. However, there are fundamental differences between transmitted light and evanescent light. The evanescent wave cannot propagate in space but decay in an exponential way, it is localized in the proximity of the sample. Ayars *et al.*[4]found that there are polarization effect and field gradient effect as the molecule Raman scattering light was excited by evanescent wave, as well as the molecular Raman-scattering light excited by transmitted light, and evanescent light are different. Gradient field Raman (GFR) differs appreciably from Raman spectroscopy in selection rules because there are gradient field components in evanescent wave, and some novel peaks were expected to come up in the Raman spectrum.

For the protein molecule, the circumstance of peptide bond is the reflection of conformation of main chain backbone, the vibration band of peptide bond could be used to deduce the conformation of main chain of protein. That is to say, Raman spectra can be used to estimate the secondary structure of proteins. Nine normal modes are allowed for the amide band of proteins. These are called A, B, and I-VII in order of decreasing frequency. The amide bands I (80% C=O stretch, near 1650 cm^{-1}), II (60% N–H bend and 40% C–N stretch, near 1550 cm^{-1}), and III (40% C–N stretch, 30% N-H bend, near 1300 cm^{-1}) are generally employed to study protein structure. Usually the vibration band of amide II can only be detected through surface enhanced resonant Raman scattering (SERRS) or infrared spectra, and the amide I and amide III bands have appreciable Raman intensities. SERS spectrum of Rat serum was shown in Fig. 3. The most discussed issue was that a very strong intensity peak located at 1560 cm^{-1} which is the characteristic vibration band of amide II. The reasonable cause was that the novel silver colloid has high effective in increasing the intensity of SERS in rat serum, as well as that evanescent wave was employed to exciting the sample, the new selection Raman rule of gradient field Raman (GFR) induced the new peak occurring.

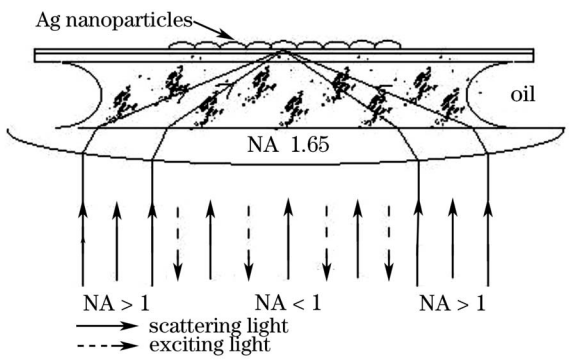

Fig. 2. Configuration of the high numerical aperture objective lens.

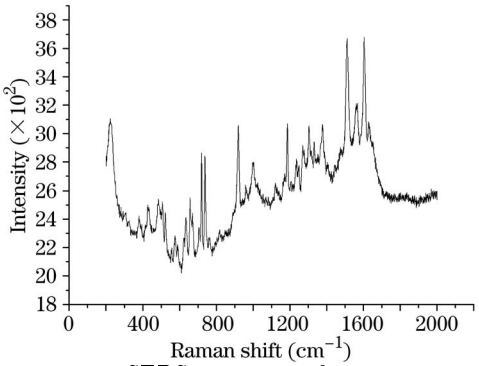

Fig. 3. SERS spectrum of rat serum.

Table 1. Raman Band Assignments for SERS Spectrum of Rat Serum

SERS Spectrum Bands (cm^{-1})	Assignment
1651 (Weak)	Amide I
1626 (Medium)	Parallel β-Sheet
1560 (Medium)	Amide II
1511 (Strong)	COO^- Asymmetric Stretching
1340–1370 (Medium)	Trp
1004 (Weak)	Phe
522 (Medium)	S-S

Assignments for SERS spectrum bands are shown in Table 1. The peak located at 1626 cm^{-1} which was belong to amide I vibration band proved that there was some protein whose structure consisted of parallel β-sheet; the vibration band of S-S bond was at 522 cm^{-1}. COO^- asymmetric stretching vibration contributed to the SER spectrum at 1511 cm^{-1}, according to surface selection rules for Raman scattering, the vibration of adsorbed state molecule, which has one polarizability tensor component perpendicular to the silver surface, will be preferentially enhanced. Stretching vibrations was assumed to have large component of the polarizability along the bond axis. The high intensity of asymmetric stretching bands of COO^- indicates a tilted close to perpendicular orientation of this group to the silver surface, and the excited light comprises evanescent wave component which the gradient electronic field is normal to the surface of the silver so that it polarizes the COO^- normal to the surface of silver, too. 1340–1370 cm^{-1} range is the microenvironment-sensitive region of Trp vibrations[5,6].

The component of rat serum is very complicate, the peaks of spectra were very abundance, and plentiful conformational information of proteins was presented in rat serum. In fact, it is very difficult to explain all the SERS spectra of rat serum entirely until now. It is very useful to do more research work deeply. SERS spectroscopy is expected to be a potential tool to diagnose some disease in early stage in the future.

K. Liu's e-mail address is Liukun_dlut@yahoo.com.cn.

References

1. Z. Q. Wen, L. Hecht, and L. D. Barron, J. Am. Chem. Soc. **116,** 443 (1994).
2. E. V. Albano, S. Daiser, G. Ertl, R. Mirandra, K. Wandelt, and N. Garcia, Phys. Rev. Lett. **51,** 2314 (1983).
3. K. A. Bosnick, J. Jiang, and L. E. Brus, J. Phys. Chem. B **106,** 8096 (2002).
4. E. J. Ayars, C. L. Jahncke, M. A. Paesler, and H. D. Hallen, J. Microscopy **202,** 142 (2001).
5. F. Fleury, A. Lanoul, M. Berjot, A. Feofanov, A. J. Alix, and I. Naviev, FEBS Lett. **411,** 215 (1997).
6. S. K. Freeman, *Applications of Laser Raman Spectroscopy* (John wiley & Sons, New York, 1974) chap.10.