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Semi-quantitative Surface Enhanced Raman Scattering Spectroscopic Creatinine Measurement in Human Urine Samples

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Abstract. This paper presents the development of a semi-quantitative method of measuring the creatinine biomolecule in human urine by the surface enhanced Raman scattering (SERS) technique. Creatinine is one of the major components of urine and can be used to represent the metabolic and renal function of the human body. The Raman signal of creatinine is enhanced by 50 nm Au nanoparticles. Raman spectra between 1400 and 1500 cm^{-1} were analyzed to obtain the relationship between the SERS band area and creatinine concentration. The square of the correlation coefficient is 0.99 in artificial urine over the creatinine range 38.4–153.6 mg/dl. In a human urine experiment, a good linear correlation is observed over the creatinine concentration range 2.56–6.4 mg/dl. The square of correlation coefficient is 0.96.

Key words: Au colloid, creatinine, Raman, SERS, urine

1. Introduction

Raman and surface enhanced Raman scattering (SERS) spectroscopic methods have been actively studied recently for applications in cell dynamics monitoring and biomolecule detection, including both qualitative and quantitative measurements (Kneipp *et al*. 1995; Dou *et al*. 1997; McCreery 2000). Raman and SERS methods have many advantages over traditional biochemical methods in body fluid analysis, including non-destructiveness to the specimen, no need for fluorescent labeling, less sample preparation, the provision of molecular structure information, and qualitative analytical abilities (Dou *et al*. 1996).

Raman signals are inelastic scattering signals that represent identification information and molecular vibrational energy. However, the Raman cross section of most molecules is inherently small; therefore the signal from traditional Raman spectroscopy is too weak to measure most biomolecules at a physiological level, because they are mostly at very low concentrations. By

using the SERS technique, the Raman signal of molecules adsorbed onto the surface of nano-metal structures can be greatly enhanced and makes it feasible to detect biomolecules at physiological concentrations (Kneipp *et al*. 1998; Vo-Dinh 1998).

Body fluids contain vital physical information is often used in clinical diagnosis. Urine is a major type of body fluids and one that can be obtained most readily and most easily. Urine analysis provides metabolic information about the body and the condition of renal function. Creatinine is one of the major components of human urine and is an end product of muscle metabolism. The creatinine excretion rate into urine is nearly constant, and it is used as an internal standard for normalizing water variation in urine analysis. Therefore, creatinine is the most commonly used urine component for the renal clearance test, which measures the kidneys' filtration function and health.

The implementation of Raman spectroscopy for the creatinine of urine analysis has been investigated in previous studies. Dou *et al.* quantitatively analyzed the creatinine component in urine at high concentrations by adding urine components into human urine (Dou *et al*. 1996). Premasiri *et al.* utilized gold colloid as SERS active substrate and successfully quantified creatinine concentration in artificial urine (Premasiri *et al*. 2001). McMurdy *et al*. have demonstrated the quantitative measurement of creatinine concentration in multipatient urine by Raman spectroscopy using a long integral time of 900s (McCreery *et al*. 2000). These studies have showed that Raman spectroscopy is feasible for human urine creatinine analysis; however, due to the small cross section of the creatinine molecule, the Raman signal's is too weak to be measured over a short time interval. Therefore, it would be very useful to investigate the feasibility of using SERS method to enhance the signal from the creatinine molecule to allow a sensitive, convenient and quantitative measurement of the molecule for clinical diagnosis purposes. However, SERS intensity is not always proportional to the concentration. Several reports have indicated that SERS intensity depends on the metal surface coverage by adsorbed molecule. Monolayer coverage can achieve the maximum SERS intensity. However, multi-layer coverage may reduce the SERS intensity (Futamata *et al*. 2002; Shadi *et al*. 2003; Joydeep Chowdhury *et al*. 2004).

In this paper, we developed Au colloidal nanoparticles SERS semi-quantitative analysis technique for measuring creatinine concentration in the human urine. We have developed 50 nm sized colloidal gold particles as the SERS active material. Urine samples were diluted to 13 different concentrations. The SERS spectra showed the presence of a creatinine characteristic band at 1400–1500 cm^{-1}. In human urine experiment, a good linear correlation is observed over the creatinine concentration range: 2.56–6.4 mg/dl in the diluted human urine sample. The square of correlation coefficient is 0.96 for this concentration range.

2. Experimental system

A Helium-Neon laser, 632.8 nm, was used to provide the Raman excitation. The laser spectral line width was further spectrally purified with an interference type laser line band-pass filter. A notch filter (HSPF-632.8-1.0, Kaiser, Ann Arbor, MI) was used to reject the excitation light while allow the Raman signal to enter the spectrometer system. An 80 cm focal length spectrometer system (HR800, Jobin Yvon, Longjumeau Cedex, France) was used with an 1800 g/mm holographic grating to provide spectral resolution at 1 cm^{-1}. A liquid nitrogen cooled CCD 2D array detector was used to measure the Raman signal by integration.

We developed an Au colloid, which was used as SERS active substrate to enhance the Raman signal of creatinine in human urine samples. The colloidal gold nanoparticles were made by the chemical reduction method. Hydrogenaurotetrachloride (0.1%) ($AuCl_4$) (Sigma–Aldrich, St. Louis, MO) was prepared and stored in an amber glass bottle. Sodium borohydride solution was prepared by dissolving 0.0066 g of sodium borohydride powder (Sigma–Aldrich, St. Louis, MO) in 400 ml of distilled water. This solution (25 ml) was added to 25 ml of 0.1% $AuCl_4$ solution with vigorous stirring for 30 minutes. Another 75 ml of sodium borohydride was added into this mixture solution and 2 hours allowed to complete the reaction. The final solution was a clear red color with Au nanoparticles of a diameter of 50 nm. This Au colloid has an absorption peak at 520 nm.

In this study, we have conducted two series of SERS creatinine measurements, one on artificial urine samples and the other on human urine samples. In artificial urine measurements, urine control (InstruChemie, Delfzijl, Netherlands). This is a type of artificial urine that contains all the various urine components at normal physiological concentration ranges and this was used in the measurement. The concentration of creatinine in urine control is 96 mg/dl. We added different volume (from 20 to 80 μl) of urine control into 1 ml Au colloid to obtain eight samples with creatinine from 38.4 to 153.6 mg/dl. In human urine measurements, 13 urine samples were obtained from a healthy volunteer's urine specimens. All samples were kept at 4°C and measured within few hours after preparation. Creatinine samples were measured by the Jaffe method to provide the reference concentration. The human creatinine was in the concentrations range from 2.56 to 115.2 mg/dl. For the Raman measurements, the urine samples were taken out from the refrigerator and returned to 22°C. Urine sample (50 μl) was mixed with 1 ml Au colloid and then samples were placed in quartz cuvettes (QG, Hellma, NY, USA) to reduce the interfering fluorescence signals that occur with glass or plastic cuvettes, during the spectrum acquisition. The Raman spectra were acquired for 20 seconds and a relatively good signal-to-noise ratio was obtained from the samples.

For the Raman spectroscopic data analysis, Raman band areas were calculated for quantitative analysis. Before band area calculation, all the SERS spectra were smoothed and baseline-subtracted by 5th degreed average filtering and 5th degreed line-segment baseline subtraction using LabSpec software (Jobin Yvon). Raman band area then was then calculated using the LabSpec software. All samples were measured three times and their band areas were averaged.

3. Results and discussion

Figure 1 compares the Raman spectrum of Au colloid and SERS spectra of creatinine, the urine control and the human urine. The concentration of creatinine is 104 mg/dl; the artificial urine control is in the form of liquid solution, with the creatinine concentration at 96 mg/dl. We can observe that there is no obvious Raman peak in the spectrum of Au colloid; however, there are two creatinine peaks between 1400 and 1500 cm^{-1} in all of the creatinine, urine control and human urine samples. These two-peak spectra were used during this analysis to obtain the relationship between Raman band area and the creatinine concentration.

The urine control was measured first by the SERS technique to verify its sensitivity during creatinine measurement in a semi complicated environment. Urine control is an artificially made standard solution that contains all urine components within the normal physiological concentration range. Figure 2(a) shows the SERS spectra of urine control solution different creatinine concentration created by adding different volume of urine control into Au colloid. We can easily observe the Raman peaks between 1400 and 1500 cm^{-1}, which represents the Raman band of creatinine. Figure 2(b) represents the relationship between the Raman band area and the creatinine concentration the R-square value between them is 0.9945. The error bars denote to two standard deviations. From this result, we have demonstrated that the sensitivity level of SERS technique using Au colloid as the active SERS substrate is suitable for quantification of creatinine in urine at physiological concentrations.

In human urine measurements, a human urine specimen with creatinine concentration of 128 mg/dl was obtained and diluted to 13 different concentrations. Figure 3(a) shows the 13 SERS spectra of human urine samples with different creatinine concentration. It is observed that the SERS intensity is not concentration dependent over the full concentration range. The SERS intensity decreased at higher creatinine concentration and this may be due to the coverage of colloidal Au particles being more than a full monolayer of the adsorbed particle. Figure 3(b) shows the relationship between SERS band area of creatinine and concentration. In the

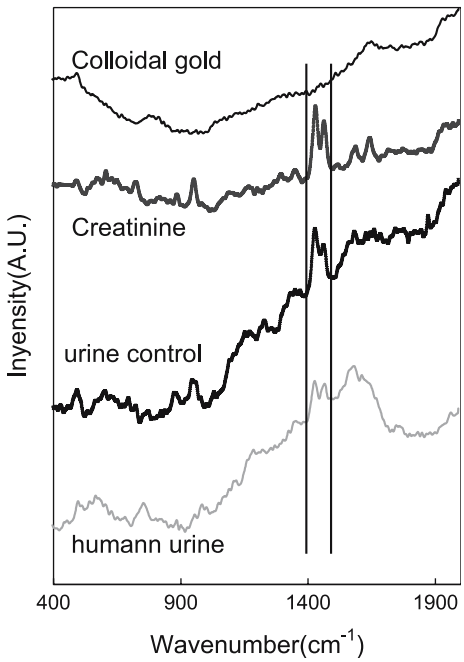

Fig. 1. Raman spectrum of Au colloid and the SERS spectra of creatinine, the urine control, and the human urine. There are two obvious peak of creatinine at 1430 and 1464 cm^{-1}. These spectra were vertically shifted for clarity.

concentration range 2.56 to 6.4 mg/dl, the SERS band area is linearly proportional to creatinine concentration; in the concentration range 7.68–115.2 mg/dl, the SERS band area does not show linearity against concentration.

In the creatinine concentration range from 2.56 to 6.4 mg/dl, the relationship between SERS band area and creatinine concentration is shown in Fig. 4. The square of correlation coefficient is 0.96. The linear regression range of creatinine in human urine is different from that in artificial urine because the constituents and their concentration are different. Other constituents besides creatinine may compete with creatinine to adsorb onto the Au particles. With the variation in concentrations of the different constituents in human urine and artificial urine, the number of creatinine molecule adsorbed on Au particles is different.

The application of Raman for biomolecule measurements has been over looked for many years due to the complicated and expensive system configuration needed for Raman system and the weak Raman signal. Recent developments emerging in optics, sensors and electronics have significantly reduced the cost of the Raman system and therefore, many Raman systems are currently used in many industries for field monitoring purposes. In

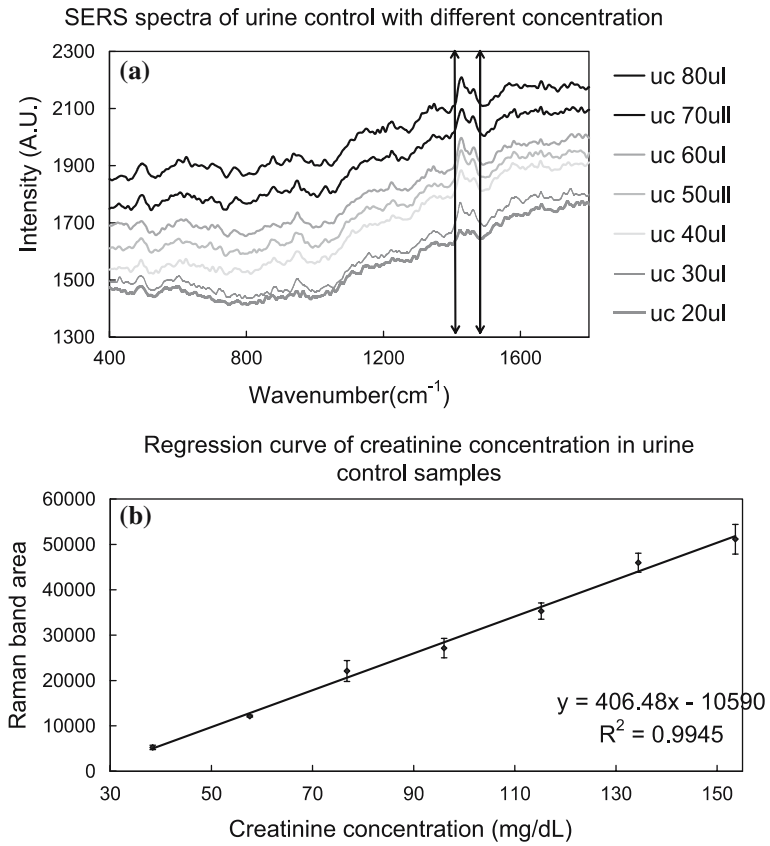

Fig. 2. (a) SERS spectra of the urine control at different concentration. 20–80 μl of urine control was added to gold colloid. The Raman band of creatinine (1400–1500 cm^{-1}) were analyzed. (b) Calibration curve of the urine control. The square of the correlation coefficient is 0.99.

addition, the SERS technique has demonstrated its ability to enhance the Raman signal by as much as 12–14 orders of magnitude over the Raman scattering signal. Therefore, the SERS technique may be feasible for the detection and measurements of biomolecules of body fluids at normal psychological concentrations.

One advantage of SERS technique is that the enhanced Raman signal of targeted biomolecules can be obtained in a shorter time due to a higher S/N ratio. The other advantage is that the sample preparation is much simpler than traditional biochemical methods and other vibrational spectral methods, in which many external chemicals are required to be added for selective binding and labeling purposes. In addition, the Raman band is very narrow, and has weak interference from water; therefore multiple molecules detection in body fluid may be feasible using Raman analysis.

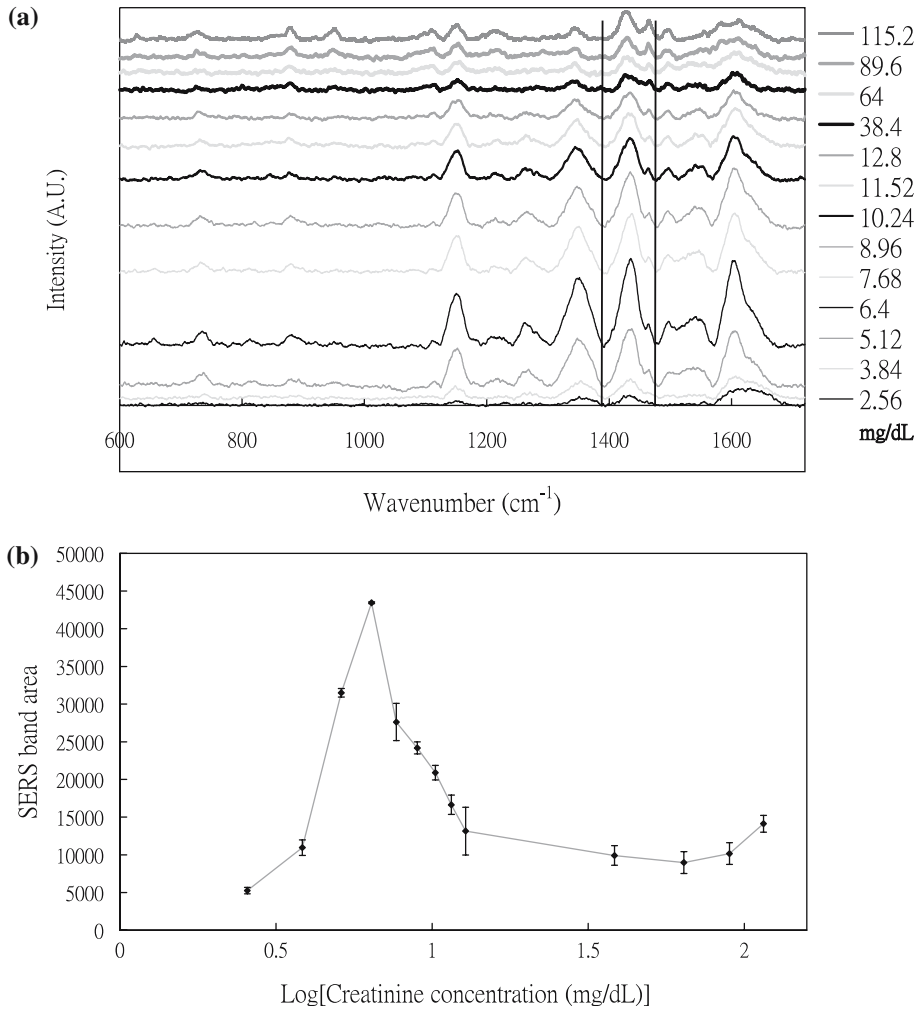

Fig. 3. (a) SERS spectra of 13 human urine samples, diluted from a human specimen, at the creatinine concentration range 2.56–115.2 mg/dl. These spectra were vertically shifted for clarity. (b) Relationship between the SERS band area and the creatinine concentration in urine sample. In the concentration range 2.56–6.4 mg/dL, the SERS band area is linearly proportional to the creatinine concentration; in the concentration range 7.68–115.2 mg/dl, the SERS band area does not show a linear concentration dependence. The error bar denotes one standard deviation.

4. Conclusions

In this paper, we have demonstrated the feasibility of the SERS technique with Au colloid for semi-quantitative creatinine measurements in human urine samples. The SERS based spectroscopic method has the advantage of providing a non-destructive and short-time platform technique for bodily

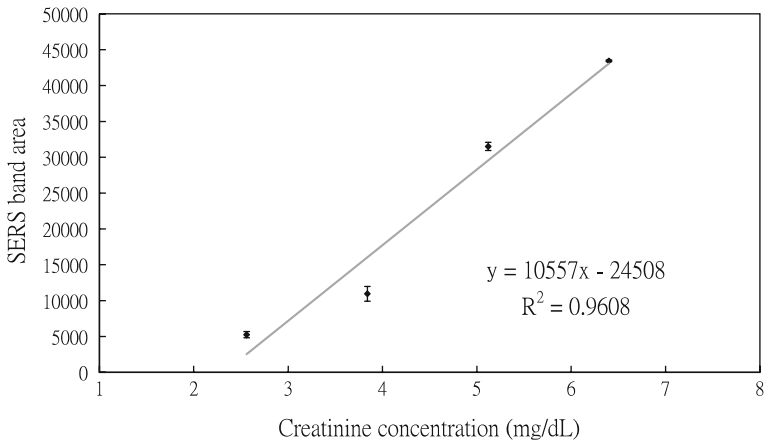

Fig. 4. Regression curve of the human urine samples over the concentration range 2.56–6.4 mg/dl. The square of the correlation coefficient is 0.96.

fluid measurement. The SERS bands between 1400 and 1500 cm^{-1} represents the vibrational features of creatinine and were used for quantitative measurement of creatinine in artificial urine and human urine samples. We have achieved a square of the correlation coefficient as 0.99 in the urine control samples, and 0.96 in human urine samples. This study has demonstrated the feasibility of the SERS technique using Au colloid for detecting and analyzing creatinine biomolecules in a semi-quantitative way.

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