

A Preliminary Investigation on Human Tears by Means of Surface Enhanced Raman Spectroscopy [†]

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Abstract: A preliminary investigation on human tears by Surface Enhanced Raman spectroscopy (SERS) is reported. This study is aimed to test the feasibility of SERS for sensing tear composition and potential changes in their composition due to pathologies, eye’s diseases and/or inflammatory processes. Home-made gold nano-particles have been used for preparing glass substrates able to efficiently induce SERS effect in fluid samples excited by HeNe laser ($\lambda = 633$ nm). The system has been preliminary tested on Rhodamine 6G aqueous solutions at different concentrations, proving the possibility to sense substance concentration as low as few μM , i.e. of the order of the main tear organic component levels. Tears have been obtained by informed healthy patients by means of a not-invasive methods based on a direct collection of the tear fluid by means of a suitable micro-capillary tube. A clear SERS response has been also obtained for human tear samples, allowing a preliminary insight on tear composition.

Keywords: SERS; tear; biomedical sensors.

1. Introduction

The human tear represents an exceptionally rich source of information on the health status of eyes, as well as of the whole body functionality, and an increasing attention is presently addressed to the analysis of this human body fluid. This is mainly due to the presence in the tears of a large variety of salts and organic components (including proteins, lipids, metabolites, nucleic acids, and electrolytes) whose concentration can be altered by pathologies, eye’s diseases and/or inflammatory processes [1,2]. The small amount of substance considered and the low concentration usually occurring hampered the access to the direct analysis by biochemistry methods even if the improvement of technologies is overcoming some of the main obstacles and the route to a wider and reliable use of tear diagnostic is in progress. In this framework, micro-Raman spectroscopy is

giving an important contribution. The vibrational spectroscopies can be extremely useful to analyze biological samples, providing valuable insight into the nature of samples and biofluids [3–5] and into the determination of the chemical structure of specific molecules [6]. Raman spectroscopy has been used for investigating tears by different research groups [7–9]. More recently, the use of Surface Enhanced Raman Spectroscopy (SERS) has been proposed with interesting perspectives in terms of sensitivity and specificity of the signal response [10–12]. In this work human tear samples have been investigated by SERS, using home made SERS substrate constituted by gold nano-particles. In order to test the efficiency of the method, preliminary SERS measurements have been performed on Rhodamine 6G.

2. Materials and methods

2.1. Rhodamine 6G solutions

Rhodamine 6G aqueous solutions were prepared diluting Rhodamine in deionized water, with concentrations $c = 0.0025, 0.005, 0.01, 0.1, 0.5, 1$ and 5 mM, respectively. Rhodamine 6G was purchased from Sigma-Aldrich (USA).

2.2. Tears collection

Tear specimens were collected from healthy volunteers. Informed consent was preliminary obtained from them, explaining all the procedures of tear collection. Smooth edge sterile capillary glass tubes, with internal diameter = 0.2 mm, outside diameter = 1 mm and length = 12.7 mm, were used (ACCU-FILL 90 MICROPET by Becton, Dickinson & Co, Clay Adams (CA) USA). During the collection, the volunteer's lower eyelid was gently pulled down and the tip of the open capillary tube was placed in contact with the tear meniscus without irritating the conjunctiva. A minimum of 1.0 ml tears was collected. The samples were stored at 4°C and tested by SERS within 7 days. In this work, 8 patients have been considered, aged between 33 and 85 years (see Table 1).

Table 1. list of volunteers

identifier	birth year	genre
A	1985	f
B	1980	f
C	1972	f
D	1956	f
E	1952	m
F	1948	m
G	1943	f
D	1933	m

2.3. SERS measurements

The SERS measurements were performed by using a Jobin-Yvon system from Horiba Scientific ISA (Osaka, Japan), equipped with a TriAx 180 monochromator, a liquid nitrogen cooled charge-coupled detector and a 1800 grooves/mm grating (final spectral resolution: 4 cm^{-1}). The spectra were recorded in air at room temperature using a 17 mW He-Ne laser source (wavelength 632.8 nm). Accumulation times in the 30–180 sec range were used. The laser beam was focused to a $2\text{ }\mu\text{m}$ spot size on the sample through an Olympus microscope equipped with $100\times$ optical objective.

In order to perform SERS measurements, a small amount of the fluid sample was put on a dried drop of gold nano-particle (GNP) colloid, previously positioned on a conventional microscope glass. The GNP preparations were obtained by conventional citrate reduction method [13] from HAuCl_4 solution reduced by 1% sodium citrate. Final GNP diameter depends on the concentration of sodium citrate, and it is of the order of few tenths of nanometers (about 30 nm, in the case here considered). Details on the fabrication and optical, Dynamic Light Scattering (DLS) and Transmission electron microscopy (TEM) characterization of GNP's are reported in Refs. [14,15].

Hydrogen tetrachloroaurate (HAuCl_4), and trisodium citrate were purchased from Sigma-Aldrich (Sigma-Aldrich Co., St. Louis, MO, USA). All chemicals were used as received.

2.4. spectral data analysis

The SERS data were preventively elaborated by using a “wavelet” based algorithm in order to reduce background signal and noise level [16]. In order to allow a direct comparison, the obtained spectra were normalized with respect to their total area, and analyzed in terms of convolution of Lorentzian components by using a best-fitting routine of GRAMS/AI (2001, Thermo Electron) program, which is based on the Levenberg-Marquardt nonlinear least-square methods.

3. Results and discussions

Preliminary Raman measurements on Rhodamine 6G solutions at different concentrations were performed with an acquisition time of 30 s in order to obtain a reference signal. SERS measurements on the same Rhodamine 6G solutions in deionized water were performed in order to quantify the signal response enhancement with respect to conventional Raman spectroscopy. SERS spectra have been collected on the Rhodamine-water solutions in similar conditions, by using an acquisition time of 30 sec. The obtained spectra are reported in Fig. 1, arbitrarily shifted along the y-axis in order to improve the readability. They refer to Rhodamine 6G solutions with concentrations $c = 0.0025, 0.005, 0.01, 0.1, 0.5, 1$ and 5 mM, respectively. The intensities of the spectra referring to lower concentrations (red lines) are reported 10 times magnified with respect to the remaining spectra (blue lines). The spectra observed are consistent with data reported in literature [17] and proven the high sensitivity of the method. A SERS efficiency of about 4×10^3 is estimated by comparing the SERS data with signal obtained by conventional Raman spectroscopy.

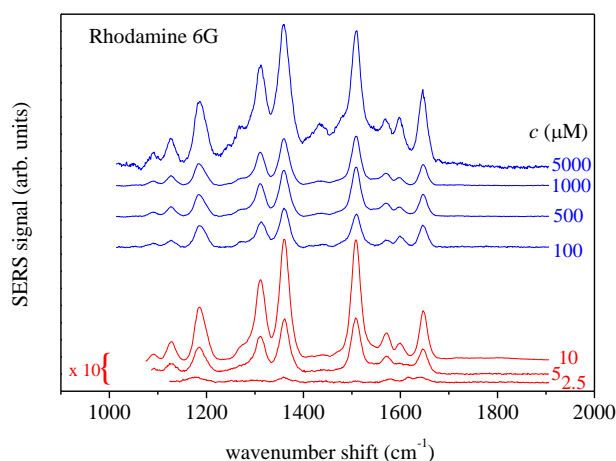


Figure 1. SERS of Rhodamine 6G aqueous solutions at different concentrations in the range of 2.5 – 5000 μM . The intensity of the spectra reported in red is amplified by a factor 10. The spectra are reported arbitrarily shifted along the y-axis.

SERS signals from tears were collected in conditions similar to those used for the preliminary measurements on Rhodamine 6G. In this case, the acquisition time was typically of 180 s. The SERS spectra reported in Fig. 2(a) are obtained by averaging the SERS responses measured in different places of the sample (at less 3 positions) of tear fluids collected from the eight considered healthy volunteers, here identified by letters from A to H (see Table I). Amide I and Amide III Raman bands are clearly evinced in the spectra of Fig. 2(a) at the spectral positions of ~ 1600 cm^{-1} and ~ 1250 cm^{-1} ,

respectively. Relevant similarities occur in the reported spectra. The average spectrum obtained by the sum of the spectra of Fig. 2(a) is reported in Fig. 2(b). By means of a fit numerical procedure, the signal was deconvoluted in terms of Lorentzian components and is reported in Fig. 2(b) as blue curves. The spectrum is rich in components, as expected in an organic fluid. In Fig. 2(b) are indicated some of the most prominent modes, at 855, 1000, 1243, 1342, 1567 and 1624 cm^{-1} . On the basis of SERS investigations on proteins reported in literature [18-19], a tentative assignment of the observed modes indicates that they are mainly due to single amino acid residues [20]. The mode at 855 cm^{-1} is assigned to C-C stretching and is present in many substances as Uracil and Cytosine [19]. Similarly, the modes at 1243 cm^{-1} and 1342 cm^{-1} related to CH_2 and CH_3 vibrations, respectively, are typically observed in amino acids as, for instance, Asparagine and Leucine [19]. The sharp peak at 1000 cm^{-1} is related to Phenylalanine or Thymine [19] and is generally present in the Raman spectra of organic substances. The prominent broad mode at 1567 cm^{-1} is assigned to NH_2 and $\text{C}_2\text{-N}_{10}$ bonds and it is found, for instance, in Guanine spectrum [18]. Finally, the strong peak at 1624 cm^{-1} is related to the Amide I band of protein and vibration of $\text{C}=\text{O}$ bonds. It can be the indication of the presence of Tryptophan or Asparagine, as well of other amino acids [18]. The main protein components revealed by the biochemical analysis in the tear are: Lactoferrin, Lysozyme, Lipocalin, Immunoglobulin (IgA) and Albumine [10]. All these substances have a complex structures where many different amino acids concur to the composition. However, pathological status can alter the fluid composition equilibrium, changing the amino acid relative concentrations and the concerned SERS response. For instance, an increase of the branched-chain amino acids Isoleucine, Leucine and Valine levels, potentially detectable by SERS, can be the mark of neuronal diseases [21].

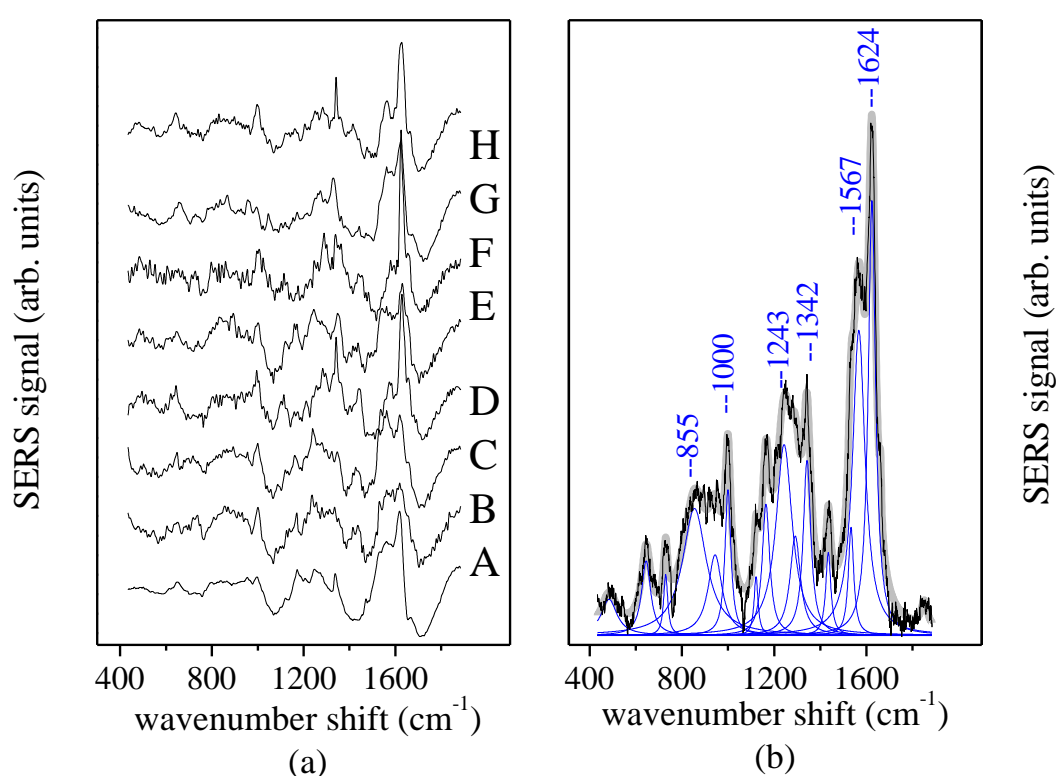


Figure 2. SERS of tear fluid: (a) Spectra obtained from tear fluid collected from the eight considered healthy volunteers, here identified by letters from A to H (see Table I) . (b) Average spectrum obtained by the sum of the spectra reported in (a). The signal have been deconvoluted in terms of Lorentzians (blu lines) and the main Raman mode components are indicated.

Conclusions

A preliminary investigation on tear fluids by SERS is reported. The SERS is implemented by using home-made GNP's that, suitably tuned to the HeNe laser excitation light, allow a considerable enhancement of the Raman signal has proven by the test measurements on Rhodamine 6G water solutions. Concentrations of Rhodamine 6G as low of few μM , comparable of concentrations of organic component of the tear, have been clearly revealed. Tear sample from eight healthy subjects have been tested. From the SERS data, an average spectrum has been obtained that allowed the individuations of the main components, mainly related to amino acids contents. The considered tear test is completely not invasive and represents as appealing potential alternative to biochemical assays for health diagnostics through tear fluid analysis.

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