Silver nanoparticles-based substrate for blood plasma analysis under 785 nm laser excitation

S.Z. Al-Sammarraie
Samara National Research University
Samara, Russia
saharzead@rocketmail.com

L.A. Bratchenko
Samara National Research University
Samara, Russia
shamina94@inbox.ru

E.N. Typikova
Samara National Research University
Samara, Russia
nil-6ssau@mail.ru

P.A. Lebedev
Samara State Medical University
Samara, Russia
palebedev@yahoo.com

V.P. Zakharov
Samara National Research University
Samara, Russia
zakharov@ssau.ru

I.A. Bratchenko
Samara National Research University
Samara, Russia
bratchenko@ssau.ru

***Abstract*—The implementation of Raman and surface enhanced Raman spectroscopy (SERS) for the detection of disease has increased in recent years. The reasons for their increased implementation have often been attributed to their well-known advantages, including the production of narrow spectral bands, which are characteristic of the molecular components present, their non-destructive method of analysis and the sensitivity and specificity which they can confer. In this study, plasma samples were examined using conventional Raman (CR) and SERS. The obtained results demonstrate that the proposed SERS technique is stable and has significant potential in clinical diagnosis applications.**

Keywords— surface-enhanced Raman spectroscopy, Enhancement Factor, blood plasma, silver nanoparticles, Raman band shift.

1. INTRODUCTION

In latest years, Surface-enhanced Raman Scattering (SERS) spectroscopy has been increasingly used with the aim of developing diagnostic applications. The high sensitivity, the ease of use and the increasing availability of relatively inexpensive portable Raman instruments, make SERS particularly attractive for the development of point-of-care and screening tests of biological samples, such as blood derivatives or tissues [1–3]. Blood plasma are biofluids universally used as samples in diagnostics, since they are rich in biochemical and biological information, they are easily available and non-invasively collected. For the same reasons, they are commonly stored for research purposes in biobanks. In the current work to implement a simple analysis of human plasma using SERS, a silver SERS substrate was prepared [4]. The goal of this work was to develop a SERS technique based on sliver nanoparticles (Ag NPs) application for simple, reliable and rapid analysis of human plasma. To assess the prospects of the proposed SERS technique and a comparative analysis of the conventional Raman (CR) spectra and SERS characteristics of plasma samples.

2. MATERIALS AND METHODS

A. Colloidal silver nanoparticles solution

Silver nitrate and trisodium citrate were used as starting materials for the preparation of (AgNPs). The silver colloid was prepared by using chemical reduction method. All solutions of reacting materials were prepared in distilled water. In typical experiment 20 ml of distilled water heated to boil. To this solution 3 mL of AgNO3 of and 6 mL of trisodium citrate (Na3C6H5O7) was added. The resulting solution was heated at 95°C for 20 min until a yellow-green solution is formed. Then the solution was removed from the heating device and stirred until cooled to room temperature.

B. Plasma samples preparation

A standardized sampling was carried out from patients of the Samara Regional Clinical Hospital named after V.D. Seredavina. The study included patients with stages 1-3a of chronic kidney disease. The study protocols were approved by the ethical committee of Samara State Medical University. All the subjects who participated in this study gave their written informed consent at the beginning of the study. The blood plasma samples were collected from patients in fasting condition and placed in sealed containers, followed by freezing at a temperature of -16 °C. Immediately before the start of the analysis, the blood plasma samples were defrosted at room temperature. Each blood plasma samples were dropped in a volume of 1.5 μl and dried for 30 minutes: on aluminum foil for CR analysis; on aluminum foil with the layer of dried silver colloid for SERS analysis.

C. Experimental setup and spectra collection

The experimental setup for Raman analysis of human plasma includes a spectrometric system (EnSpectr R785, Spektr-M, Chernogolovka, Russia) and a microscope (ADF U300, ADF, China). Focusing the exciting radiation and collecting the scattered radiation were implemented using 50x Objective LMPlan. The stimulation of collected spectra was performed by the laser module with central wavelength 785 nm. The diameter of the laser spot at the focus on the sample surface was 5 μm. The laser power was 10 mW for the SERS technique and 60 mW for the CR technique. Exposure time was 4 seconds for SERS and 20 seconds for CR.

3. RESULTS AND DISCUSSION

At the first stage, the characteristic spectral features of a human plasma sample using the SERS technology, using the CR technology, and the spectral characteristics of the prepared silver SERS substrate were considered. Fig. 1 shows the recorded raw SERS and CR spectra of plasma. As can be seen from Fig. 1, the spectral contribution of the silver substrate to the SERS spectrum of the plasma sample is insignificant. Only a few Raman peaks could be observed in the regular Raman spectra of the plasma sample. The SERS spectra of the plasma sample, obtained with the proposed technique with AgNPs shows multiple distinctive peaks, and the intensity of these peaks is enhanced compared with that of CR spectra.

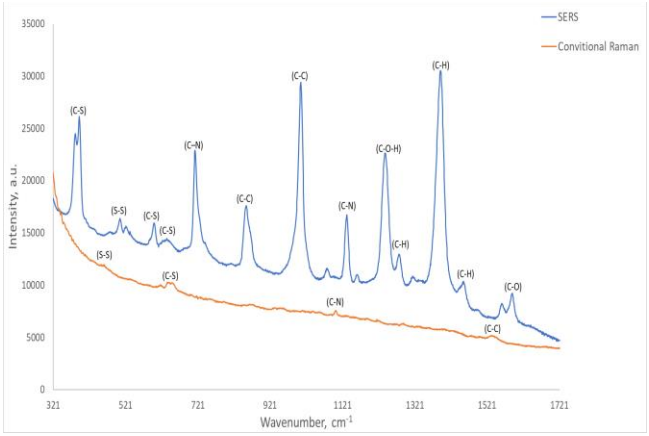

Fig. 1. Comparison of the raw spectrum of a blood plasma sample for CR and SERS

The major CR peaks are observed at 480, 641, 1100 and 1540 cm^{-1} and may be attributed to biochemical components of nucleic acids, fatty acids and Amide I group. For SERS measurements we observe strongly enhanced bands which may be attributed to such biochemical components as nucleic acids (607, 641, 714, 856, 1132, 1240 and 1400 cm^{-1}), carbohydrates (641 and 1094 cm^{-1}), lipids (1280 and 1338 cm^{-1}) etc. The observed SERS peaks are indicators of the corresponding plasma components [5-7].

Several of the observed bands clearly stand out by the impact of SERS technique at 400, 607, 714, 856, 1003,1240 and 1400 cm^{-1}. These bands were undetectable by CR spectroscopy, as the intensities of these bands are weaker than the intensity of the autofluorescence and noise signals. The SERS spectrum of plasma with AgNPs showed many dominant vibration bands, indicating a strong interaction between the silver colloids and the plasma substances. [5-7]. In SERS spectrum, bands of several molecules could be shifted compared with the bands of these molecules in the CR spectrum. Such shift may be observed from 480, 1100 and 1540 cm^{-1} bands to 505, 1132 and 1560 cm^{-1} bands respectively. This might be due to the strong interaction between prepared substrate and analyte [8]. Which is demonstrates the blueshift of the SERS spectrum relative to the CR spectrum that observed shift is related to the change of the chemical bond's energy. Moreover, the reason for the blueshift or the redshift of the Raman peak is the change of the corresponding chemical bonds, leading to the migration of electron clouds. Specifically, the change involves the transformation of interatomic bond force and distance [9].

4. Conclusion

In this study human blood plasma samples were examined using CR and newly proposed SERS technique. The obtained results indicate accuracy and stability of the proposed SERS substrates., the proposed SERS technique provides a capability to detect Raman bands 480, 641, 1100 and 1540 cm^{-1} which may be attributed to such biochemical components as nucleic acids, carbohydrates, lipids, etc. These bands were not presented in the CR spectra of human plasma; thus, SERS analysis increases the possibility to detect disease biomarkers during blood samples analysis. The obtained results demonstrate that the proposed SERS technique is stable, no-invasiveness with no blinking phenomenon and has significant potential in clinical diagnosis applications

Acknowledgment

The study was supported by a grant from the Russian Science Foundation No. 21-75-10097 https://rscf.ru/project/21-75-10097/.

References

[1] Bonifacio, A. Surface-enhanced Raman spectroscopy of blood plasma and serum using Ag and Au nanoparticles: a systematic study / A. Bonifacio, S. Dalla Marta, R. Spizzo, S. Cervo, A. Steffan, A. Colombatti, V. Sergo // Analytical and Bioanalytical Chemistry. – 2014. – P. 2355-2365.

[2] Bratchenko, L.A. Comparative study of multivariative analysis methods of blood Raman spectra classification / L.A. Bratchenko, I.A. Bratchenko, A.A. Lykina, M.V. Komarova, D.N. Artemyev, O.O. Myakinin, A.A. Moryatov, I.L. Davydkin, S.V. Kozlov, V.P. Zakharov // Journal of Raman Spectroscopy. – 2020. –Vol. 51(2). – P. 279-292.

[3] Bratchenko, L.A. Raman spectroscopy of human skin for kidney failure detection / L.A. Bratchenko, I.A. Bratchenko, Y.A. Khristoforova, D.N. Artemyev, D.Y. Konovalova, P.A. Lebedev, V.P. Zakharov // Journal of Biophotonics. – 2021. – Vol. 14(2). – P. e202000360.

[4] Bratchenko, I.A. In vivo diagnosis of skin cancer with a portable Raman spectroscopic device / I.A. Bratchenko, L.A. Bratchenko, A.A. Moryatov, Y.A. Khristoforova, D.N. Artemyev, O.O. Myakinin, A.E. Orlov, S.V. Kozlov, V.P. Zakharov // Experimental Dermatology. – 2021. – Vol. 30(5). – P. 652- 663.

[5] Subaihi, A. Rapid, accurate, and quantitative detection of propranolol in multiple human biofluids via surface-enhanced Raman scattering / A. Subaihi, L. Almanqur, H. Muhamadali, N. AlMasoud, D.I. Ellis, D.K. Trivedi, K.A. Hollywood, Y. Xu, R. Goodacre // Analytical chemistry. – 2016. – Vol. 88(22). – P. 10884-10892.

[6] Witkowska, E. Detection and identification of human fungal pathogens using surface-enhanced Raman spectroscopy and principal component analysis / E. Witkowska, T. Jagielski, A. Kamińska, A. Kowalska, A. Hryncewicz-Gwóźdź, J. Waluk // Analytical Methods. – 2016. – Vol. 8(48). –P. 8427-8434.

[7] Liu, R. Surface-enhanced Raman scattering study of human serum on PVA– Ag nanofilm prepared by using electrostatic self-assembly / R. Liu, X. Zi, Y. Kang, M. Si, Y. Wu // Journal of Raman Spectroscopy. – 2011. –Vol. 42(2). – P.137-144.

[8] Pichardo-Molina, J.L. Raman spectroscopy and multivariate analysis of serum samples from breast cancer patients / J.L. Pichardo-Molina, C. Frausto-Reyes, O. Barbosa-García, R. Huerta-Franco, J.L. González-Trujillo, C.A. Ramírez-Alvarado, G. Gutiérrez-Juárez, C. Medina-Gutiérrez // Lasers in medical science. – 2007. – Vol. 22(4). – P. 229-236.

[9] Liao, Q. Surface-enhanced Raman scattering and DFT computational studies of a cyanuric chloride derivative / Q. Liao, M. Y. Li, R. Hao, X. C. Ai, J. P. Zhang, Y. Wang // Vibrational spectroscopy. – 2007. – Vol. 44(2). – P. 351-356.