# Human blood analysis based on silver nanoparticles Substrate under 785 nm laser excitation

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***Abstract*— In recent years, the use of Raman and surface enhanced Raman spectroscopy (SERS) for disease detection has grown. The motives for their increased use have commonly been attributed to their well-known benefits, such as the creation of narrow spectral bands that are characteristic of the molecular components present, their non-destructive method of analysis, and the sensitivity and specificity that they can confer. In this study, human blood samples were examined using SERS. The obtained results demonstrate that the proposed SERS technique is stable and has significant potential in clinical diagnosis applications. In this study, human blood samples were examined using 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, blood plasma, silver nanoparticles, Raman scattering**

## I. INTRODUCTION

Surface-enhanced Raman Scattering (SERS) spectroscopy has been widely employed in recent years with the goal of creating diagnostic applications. SERS is particularly appealing for the development of point-of-care and screening tests of biological samples, such as blood derivatives or tissues, due to its high sensitivity, convenience of use, and increasing availability of very affordable portable Raman devices [1–3]. Blood plasma are biofluids that are widely utilized as diagnostic samples as they are rich in biochemical and biological information, are easily accessible, and can be collected non-invasively. They are frequently preserved in biobanks for research purposes for the same reasons. A silver SERS substrate was created in the current study to accomplish a simple analysis of human plasma using SERS [4]. The purpose of this study was to create a SERS approach based on silver nanoparticles (Ag NPs) for easy, reliable, and fast analysis of human plasma. To assess the prospects of the proposed SERS technique.

## II. 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 $AgNO_3$ of and 6 mL of trisodium citrate ($Na_3C_6H_5O_7$) 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. 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 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. Exposure time was 4 seconds for SERS. Raw spectra were processed by carrying baseline correction and smoothing to remove noise and fluorescence background. Prior to analysis, the raw spectral data were smoothed by the Savitsky-Golay filter. The spectral characteristics were normalized by linear normalization (max – min).

## III. RESULTS AND DISCUSSION

The observed SERS bands were analyzed. Several of these bands clearly stand out by the impact of SERS technique as seen in Fig. 1. The SERS spectrum of blood showed many dominant vibration bands, indicating a strong interaction between the silver colloids and the blood substances. This interaction also indicated that biochemical ingredients in the blood were closely adsorbed onto the surfaces of the silver nanoparticles, and Raman scattering took place in the highly localized optical fields of these structures, which resulted in a strong enhancement in the intensity.

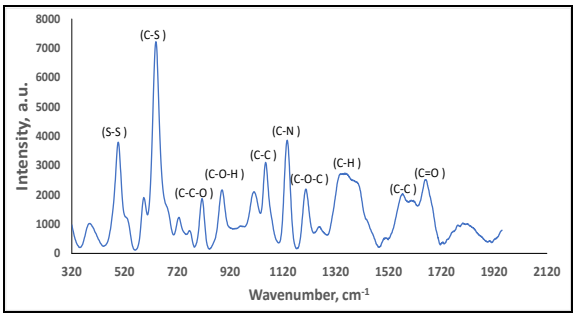


Fig. 1 the preprocessed SERS spectra of the blood sample

Box plots have been used to highlight various regions of the spectrum, as shown in Fig. 2, including specific peaks of Raman spectra from plasma of patients with heart disease.

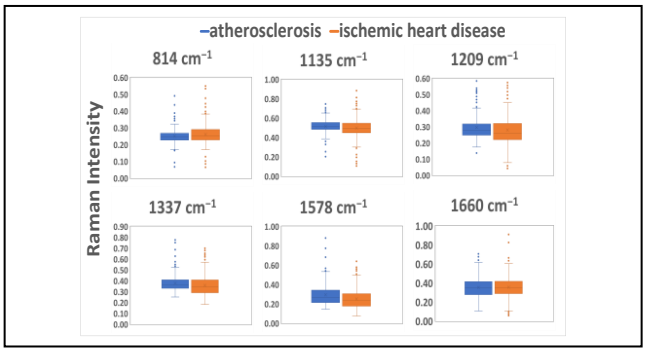


Figure 2 -Box plots of specific peaks of Raman spectra from plasma of patients with heart disease

A few of the small differences between the spectra are shown as the box plots in Fig. 2, including (1209, 1337,1578) $cm^{-1}$.

The disulfide stretching in proteins Phosphatidylserine, glycogen and deformation vibrations of FeO2 group are identified at 498 $cm^{-1}$ referents to (S-S). At 641-727 $cm^{-1}$ (C–S) L- tyrosine, lactose, (C–H): Hypoxanthine, Phosphatidylserine, vibrations of pyrrole, ring breathing mode tryptophan, and amide III. 815: corresponded to DNA, (C–C–O) L-Serine, glutathione, amino acids phenylalanine and tyrosine, (proteins- Tyrosine and Proline)). 1057-1139 $cm^{-1}$: carbohydrates C-C (stretching), (deformation vibrations of C-N group) Ascorbic acid, (C–H) Nucleic acid base, L-serine, aromatic ring of Phenylalanine and tyrosine (proteins) which corresponds to a protein amino acid, (glucose), DNA base [5,6].

AS seen in fig.2, a few of the small differences are seen in the region of 1139-1660 $cm^{-1}$, which have been attributed to O-O, C–C and C–O stretches, as well as varying concentrations of lipid, and proline. 1200-1330: carbohydrates C-O-C , (deformation vibrations of (N-H, C-N, C-H group), amide III (proteins), $CH_3$, $CH_2$ wagging (lipids), Tyrosine (proteins), collagen and nucleic acid, lipoproteins, but their concentrations and even absence can change because the blood from some patients presents individual differences; some include large amounts of proteins and lipids whereas others contain only a large amount of glucose because the metabolites presents in blood fundamentally depend upon the alimentation of each person. The most important components found in healthy human blood and their average concentrations are summarized in [7,8].

The Amide III band from 1220 to 1280 $cm^{-1}$ is based on N-H in-plane bending vibration. the difference is associated with a disorder in the secondary structure of the protein components [7,8].

1577: Amide II, (N-H, C-N, C–C) bending mode of phenylalanine). differences are observed in the region 1577 $cm^{-1}$ due to changes in the Amide II band is derived from N-H bending and C-N stretching vibration in parallel or antiparallel beta sheets from 1500 to 1577 $cm^{-1}$. 1656: proteins, Amide I vibration mode relative to peptide chain (C=O) of lipids, Fatty acid. Notable differences are observed in the region of 1659 $cm^{-1}$ due to Changes in the shoulders of the amide I peak which usually due to changes in the secondary structures of amide I, such as the β pleated sheet proteins [9].

## IV. CONCLUSION

In this study human blood samples were examined using 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 which may be attributed to such biochemical components as nucleic acids, carbohydrates, lipids, etc. 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

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