

Human blood plasma SERS analysis using silver nanoparticles for cardiovascular diseases detection

Sahar Z. Al-Sammarraie^{1*}, Lyudmila A. Bratchenko¹, Elena N. Tupikova¹, Maria A. Skuratova², Shuang Wang³, Peter A. Lebedev⁴, and Ivan A. Bratchenko¹

¹ Samara National Research University, 34 Moskovskoe Shosse, Samara 443086, Russia

² Samara City Clinical Hospital №1 named after N.I. Pirogov, 80 Polevaya str., Samara 443096, Russia

³ Northwest University, #1 Xuefu Avenue, Xi'an 710127, Shaanxi, China

⁴ Samara State Medical University, 159 Tashkentskaya str., Samara 443095, Russia

*e-mail: saharzead@rocketmail.com

Abstract. In recent years, the use of Raman and surface enhanced Raman spectroscopy 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, and high sensitivity and specificity that they can provide. The aim of this work is the analysis of spectral features of plasma in patients with cardiovascular diseases utilizing surface enhanced Raman spectroscopy to determine the presence or absence of the disease. The investigation revealed spectrum difference between the patient and healthy volunteers' groups at the observed Raman bands. 146 patients and 67 healthy subjects were analyzed. Classification of the patient group with cardiovascular diseases was made based on the projection on latent structures with 99% accuracy. Stability of the classifier was checked with the implementation of cross-validation and separation of analyzed data into training and test sets. The obtained results demonstrate that the proposed SERS technique is stable and has significant potential in clinical diagnostic applications. © 2024 Journal of Biomedical Photonics & Engineering.

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

Paper #9010 received 20 Aug 2023; revised manuscript received 8 Nov 2023; accepted for publication 8 Nov 2023; published online 16 Jan 2024. [doi: 10.18287/JBPE24.10.010301](https://doi.org/10.18287/JBPE24.10.010301).

1 Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide, in 2019, cardiovascular disease, primarily coronary and cerebrovascular atherosclerosis still caused about 18 million deaths per year worldwide (> 30% of all deaths). Coronary heart disease, cerebrovascular disease, rheumatic heart disease, and other illnesses are among the category of heart and blood vessel disorders known as CVDs [1–3]. A range of auxiliary examination methods, both invasive (such as selective coronary angiography) and non-invasive (such as nuclear scans, CT, stress tests, and blood biomarkers), allow assessment of cardiovascular disease risk and treatment targets. However, there is no simple blood biochemical index or biological target for the diagnosis

of atherosclerosis at present; instead, more ultrasonographic screening or angiography are used [4, 5].

In latest years, surface-enhanced Raman scattering (SERS) spectroscopy has been increasingly used with the aim of developing diagnostic applications. SERS is a commonly used sensing technique in which inelastic light scattering by molecules is greatly enhanced when the molecules are absorbed onto corrugated metal surfaces. 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 [6, 7]. The SERS effect is currently being debated

in the literature and two mechanisms have been identified: the electromagnetic mechanism and the chemical mechanism [8]. The first suggests the stimulation of localized surface plasmons on the metallic surface, whereas the second suggests a chemical process for the creation of charge-transfer complexes between the metallic surface and analyte species. Given their sensitivity in the examination of biological material, Raman spectroscopy and SERS have recently been employed as a useful tool in clinical diagnostics and biomedical research [8], including DNA (Pyrak et al., and Zhang et al. [9, 10]), RNA (Lee et al., Han et al. [11, 12]), cancer markers (Choi et al. [13]), bacteria (Andreï et al. [14]), viruses (Chen et al. [15]), genes (Vo-Dinh et al. [16]), drugs (Jaworska et al. [17]), pathological markers on cellular membranes and tissues (Wallace and Masson [18]), other biomolecules, ion concentrations, and redox potential in cells (Jaworska et al. [19]), and even *in vivo* SERS measurements on mice (Wen et al. [20]).

In the current study, a simple approach of SERS substrate implementation for blood plasma/serum analysis is considered. Blood plasma and serum are biofluids that are widely utilized as diagnostic samples because they are rich in biochemical and biological information, are easily accessible, and may be obtained non-invasively. They are routinely preserved in biobanks for research purposes for the same reasons. Regardless of the potential diagnostic capabilities of SERS analysis of blood components, just a few researches have been conducted using it [6, 21].

In order to make SERS trustworthy for the investigation of biofluids, analytical procedures and spectrum interpretation still need to be established. The manufacture and development of SERS substrates that are repeatable, stable, effective, and fairly priced is another significant barrier to the reliable use of SERS in diagnostics [22, 23]. It was found that the cheapest and most efficient substrates were aqueous dispersion of Ag and Au nanoparticles (metal colloids) [24, 25]. Nanoparticle size, shape, and surface properties affect SERS properties in fact, Zeng et al. [26] recently employed aqueous Au and Ag colloids to evaluate the diagnostic potential of SERS spectroscopy of blood serum and plasma in relation to different diseases. Nanoparticle size, shape, and surface properties affect SERS properties for instance Jianqiang et al. [27] prepared starch-capped gold nanoparticles with hexagon and boot shapes through using a nontoxic and biologically benign aqueous-phase synthetic route. They found that different-shaped gold nanoparticles possess different SERS properties. Tian et al. [28] synthesized and characterized gold nanospheres and their aggregates, nanotriangles, and nanostars of similar dimensions according to their average size, zeta potential and UV/visible absorption and explored their relative efficiencies for SERS.

Despite the outstanding signal enhancements accomplished by metal colloids and their simple preparation and use, some substrates frequently struggle

with a lack of consistency due to various preparing protocols, and depending on the preparation methods; the nanoparticles have various physico-chemical surface characteristics, resulting in different stability, SERS efficiency, and repeatability [29].

Thus, the main objective of our work is to develop a useful tool for early, stable and accurate diagnosis of CVDs patients based on SERS analysis of blood plasma. The implemented method utilizes a classification model based on projection on latent structures (PLS) to identify CVD patients, and determining the most informative SERS bands associated with differences between the CVD group and control group by variable importance in projection (VIP) distribution.

2 Materials and Methods

2.1 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 1.8% AgNO₃ of and 6 ml of 1% trisodium citrate (Na₃C₆H₅O₇) were 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. The UV-Vis spectrum of the AgNPs solution was obtained by utilizing the Spectrophotometer (UNICO 1201, United Products & Instruments, USA) to track NPs characteristics. Detailed analysis of acquired AgNPs properties may be found elsewhere [30].

2.2 Serum/Plasma Samples Preparation

A standardized sampling was carried out from patients of the Samara Regional Clinical Hospital named after V. D. Seredavin. The study included patients with different cardiovascular diseases. Diagnosis of CVD for each patient was set by doctors in Samara Regional Clinical Hospital named after V. D. Seredavin after examination of patient based on physical test and results of a blood tests according to the guidelines of Russian Ministry of Health. 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 serum/plasma samples were defrosted at room temperature. Each blood plasma samples were dropped in a volume of 1.5 µl and dried for 30 min on aluminum foil with the layer of dried silver colloid for SERS analysis. we measured 1 to 2 specters for each healthy control subjects and 5 to 6 for each patient. The characteristics of the sample of subjects are presented in Table 1.

Table 1 Characteristics of analyzed samples.

Group	Number of patients	Mean age	Number of spectra
Control group	67	33–80 (62)	85
Patients with Cardiovascular Disease	146	40–75 (62)	778

2.3 Experimental Setup and Spectra Collection

The experimental setup for Raman analysis of human serum includes a spectrometric system (EnSpectr R785, Spektr-M, Chernogolovka, Moscow Region, Russia) and a microscope (ADF U300, ADF, China). Focusing the exciting radiation and collecting the scattered radiation were implemented using 50× 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. Exposure time was 4 s. The resulting raw spectrum is an automatic sequential recording of four spectra with subsequent averaging. Thus, the recording time of the SERS raw spectrum is 16 s.

2.4 Spectra Processing

Preprocessing of raw SERS spectra of biofluids consisted of several successive stages: noise smoothing, removal of autofluorescent background, and normalization. Smoothing of the raw spectra was performed with a Savitzky-Golay filter with a filter window width of 15, the first order of the polynomial used for smoothing, and a zero-order derivative (no derivative). Then the smoothed spectra were subjected to the removal of the autofluorescent background by the polynomial method (15th degree polynomial) [31]. Spectral characteristics are normalized using the standard deviation of the method of normal variation (SNV) that consists in subtracting each spectrum by its own mean and dividing it by its own standard deviation. After SNV, each spectrum will have a mean of 0 and a standard deviation of 1.

Each sample of a biofluid corresponds to *a priori* information about belonging to a certain group. Therefore, the data was analyzed through supervised learning. To avoid overfitting, the analysis of the stability of the constructed models and the choice of optimal parameters were implemented using k-fold cross-validation ($k = 7$) [32]. After cross-validation and determination of the optimal parameters of the model, the complete data set was randomly divided: 80% of the subjects for training the model (training set) and 20% of the subjects for testing the model (testing set). It should be noted that when splitting the initial dataset into a training set and a verification set (both in cross-validation and in the final model construction), the split was performed by subjects in order to avoid allocating the spectral characteristics corresponding to one subject in

different sets, which could lead to incorrect overestimation of the model characteristics. Thus, the proposed way of data splitting provides more stable classification models. When building models, the importance of predictors in solving the classification problem was estimated using the distribution of the importance of variables in the constructed model (VIP distribution). An analysis of the VIP distribution makes it possible to determine which spectral bands and related components of the biofluid are characterized by differences in the classification task performed.

Multivariate analysis was implemented using PLS-discriminant analysis (PLS-DA) based on the SIMPLS algorithm in the MDAtools package available in the R studio software.

3 Results and Discussion

Fig. 1 presents the mean plasma SERS spectra with standard deviation (SD) for the control group (healthy) and group of patients with CVD. Fig. 1 demonstrates that the differences between the mean serum spectra for the discriminated groups are visually observed in the intensity of individual spectral bands. Without utilizing multivariate analysis, we can see an increase in the intensity of the peaks at 715 cm^{-1} (C-H) Hypoxanthine, Phosphatidylserine, 856 cm^{-1} (C-O-H) Glutathione, 1003 (C-C) Phenylalanine, 1244 (amide III (proteins)carbohydrates C-O-C, N-H), 1390 cm^{-1} (C-N, C-H group, CH_3 , CH_2 wagging (lipids)), can be identified as distinctive features in the spectral characteristics of the plasma of the patient's group against the control group (healthy), in addition it shows a decrease intensity at 494 and 640 cm^{-1} ((S-S) Phosphatidylserine, (C-S) L- tyrosine, lactose) for the patients' group [33, 34].

Spectral bands and SERS characteristics of the plasma obtained in the current study are similar to those reported in the studies. Huang et al. indicated (856, 1005, and 1244 cm^{-1}) among the informative spectral bands associated with the degree of Coronary artery disease (CAD) spectra showed Raman peaks appeared to originate from lipids and proteins which are the major contributors to Small Extracellular vesicles (sEV) surface. In another work Yang et al. indicated (1244 and 1389 cm^{-1}) as the informative spectral bands from patients with Coronary Heart Disease (CHD). However, it should be noted that Huang et al. and Yang et al. [35, 36] reported a SERS band at 1450 cm^{-1} which was very slightly observed in our study; this band was assigned to the CH_2 bending vibration of proteins and lipids.

The analyzed spectral data is characterized by multicollinearity property; thus, application of projection analysis methods can provide a statistically reliable result. The most common approach to solve such problems is the method of discriminant analysis with regression on latent structures – PLS-DA [37]. Discriminant analysis is a technique used to identify the unique features of groups or individuals. The technique assumes that there are common underlying structures in groups or individuals. These common underlying structures are represented by a set of latent variables.

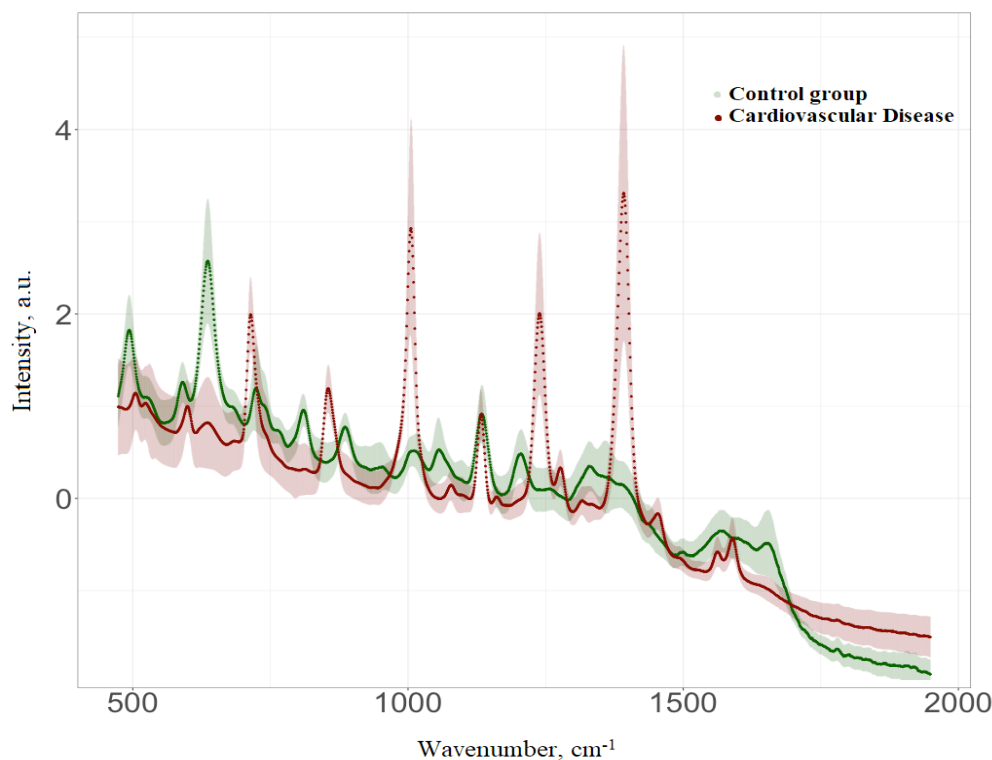


Fig. 1 Mean with standard deviation (SD) for SERS spectra of plasma for control group (healthy) and the patients with (cardiovascular diseases).

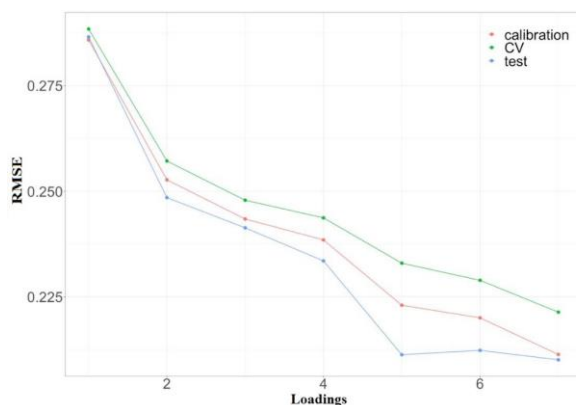


Fig. 2 The relationship between the number of LVs and the root mean square error (RMSE) for PLS-DA model.

To use the SIMPLS algorithm to implement the PLS and PLS portions of the PLS-DA approach [38, 39]. The number of loading vectors (LVs) in the PLS-DA model was determined using training data set through 7 split CV. According to Fig. 2, the RMSE value for the calibration set and CV algorithm is decreasing to 7 LVs. However, in test set RMSE is increasing after LV5, thus, only first 5 LVs were utilized in the analysis.

In order to assess the contribution of each LV we examined their structure. As shown in Fig. 3 LVs peaks show a close correlation with plasma Raman spectra bands at 727, 1003, 1240 and 1390 cm^{-1} , however in the shape of LV4 we observed a negative peak at 1240 and 1375 cm^{-1} but with lower intensity in comparison with

the rest LVs. Table 2 demonstrates characteristics of the constructed classification models. Classifying plasma samples of CVD patients and healthy group is possible with the sensitivity, specificity, and accuracy of 100%, 95% and 0.99% respectively. Stability of the model was verified by CV.

Fig. 4 demonstrates the VIP distribution of the SERS matrix of plasma spectra when constructing discrimination models of control group (healthy donors), with patients with (cardiovascular diseases). The VIP distribution is represented by a gradient fill, where the purple color corresponds to the minimum information content, and the yellow – to the maximum information content. It shows the most informative spectral bands when constructing models coincide with the visible differences in the mean spectra of the discriminated groups. The model identified the following spectral bands as the most informative: 727, 1003, 1244, and 1390 cm^{-1} .

In order to evaluate the performance of a binary diagnostic classification method, a ROC curve was employed. It is, represented as a graph, that is used to evaluate the performance of a binary diagnostic classification method. The diagnostic test results need to be classified into one of the clearly defined dichotomous categories, such as the presence or absence of a disease. Higher the score, higher the distinction and lower the crossover of the predictions of the two classes [40]. Fig. 5 shows ROC AUC of CVD classification equaled to 0.99. This result suggests the model is successful to perform the correct classification of CVD.

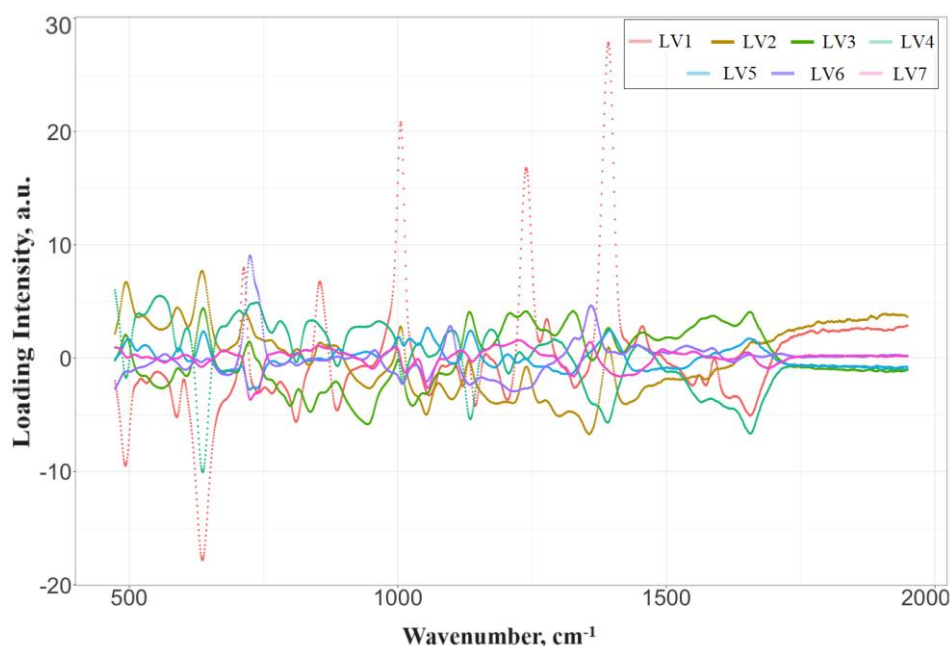


Fig. 3 Shape of the LV1–LV7 for the constructed PLS-DA model.

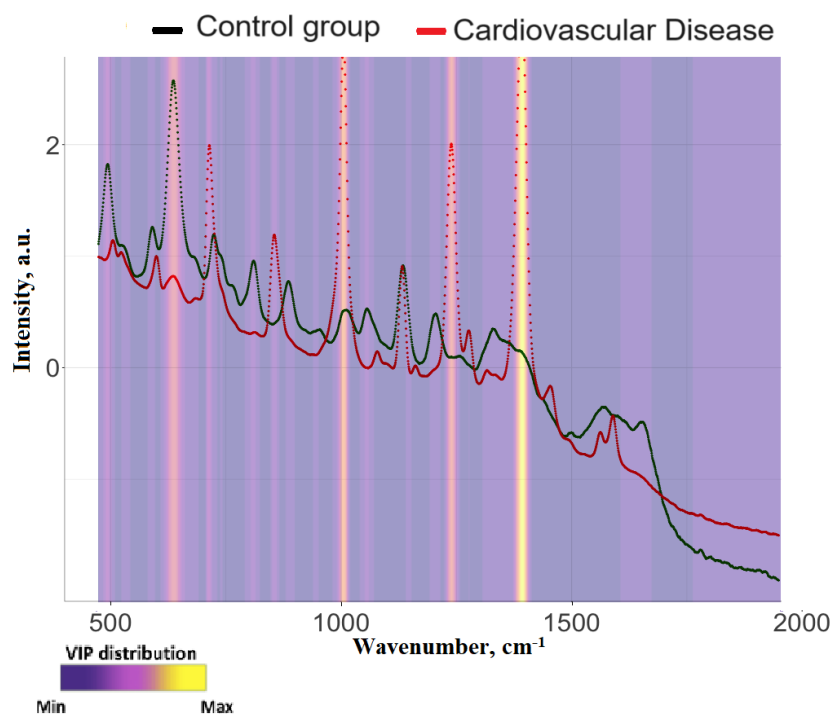


Fig. 4 The mean plasma spectra for the discriminated groups with the overlapping VIP distribution.

Table 3 represent the main informative Raman bands in the discrimination of plasma samples of the control group and the group of patients. One may see that the main contribution to the registered Raman bands with the proposed approach is associated with wide range of chemical groups which can hardly be assumed as markers of CVD. At the same time, it is quite challenging to find an exact biomarker of CVD and instead combinations of biomarkers may be utilized for the efficient detection of CVD [41]. In this regard proposed SERS analysis offers a simple and reliable way to analyze a “spectral

landscape” of chemical components of blood (including biomarkers), and further evaluation of such spectral data gives an opportunity to create a classification model of CVD that involves data from multiple biomarkers together at the same time. However, further investigations are required to estimate possible contribution of exact components to classification of CVD. Such analysis may be performed with utilization of deep-learning and artificial intelligence models [42, 43].

Table 2 Characteristics of the PLS-DA Discrimination Model for the Control Group and the Cardiovascular Disease Group presented as “Mean (95% CI)”.

Classification	Sensitivity			Specificity			Accuracy		
	train	cross-validation	test	train	cross-validation	test	train	cross-validation	test
Control group (Healthy)	1 (0.99–1)	1 (0.99–1)	1 (0.99–1)	0.95 (0.89–99)	0.96 (0.92–1)	0.95 (0.91–1)	0.95 (0.92–0.97)	0.97 (0.94–0.99)	0.99 (0.96–1)
Cardiovascular Disease group	0.89 (0.86–0.94)	0.89 (0.88–0.92)	0.95 (0.91–1)	1 (0.99–1)	1 (0.99–1)	1 (0.99–1)	0.96 (0.90–98)	0.96 (0.91–0.99)	0.99 (0.97 1)

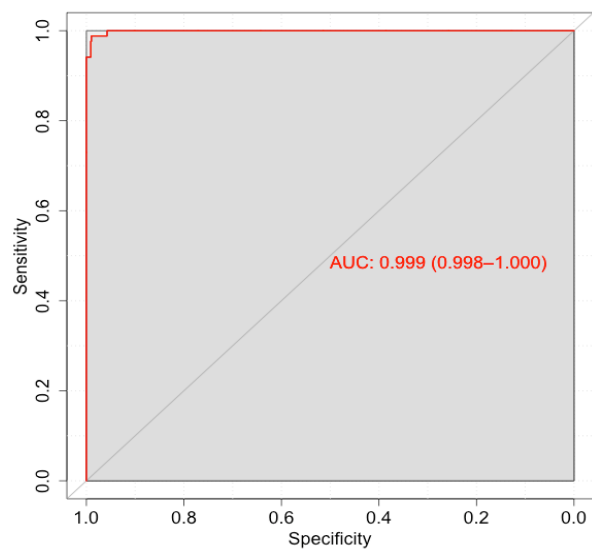


Fig. 5 ROC curves of the constructed models for the classification of “healthy” control group vs “patients” group on the verification dataset.

In contrary to the proposed approach it is possible to analyze exact biomarkers of CVD. As example, Cheng et al. [44] created a SERS-based platform for the ultrasensitive detection of cardiac troponin and creatine kinase-MB by combining a novel SERS nanoprobe with a monoclonal antibody immobilized gold-patterned chip. This SERS platform offers a limit of detection of 8.9 pg/mL and 9.7 pg/mL. Another rapid, cost-effective SERS immunoassay approach developed by Chon et al. [45]. This method was effective in the detection of 42.5 pg/mL and 33.7 pg/mL of creatinine kinase and troponin respectively and the fitted regression line was within the 95% CI. In similar approaches Hu et al. [46] used a magnetic immunoassay technique based on SERS to create a SERS immunoprobe to detect heart-type fatty acid-binding protein and cardiac troponin. The concentration of the biomarkers was determined by observing the distinctive Raman peak intensities of the two Raman reporter molecules. The minimal detection limits of H-FABP and cTnI at the optimal conditions were 0.6396 and 0.0044 ng/mL, respectively. Fu et al. [47] studied the usage of graphene oxide-gold nanoparticle combination

for effective signal amplification for troponin detection in another promising SERS lateral immunoassay. As compared to immunoassays lacking graphene oxide, this test was able to detect troponin quantitatively across a much wider range (5 pg/mL to 1000 ng/mL). Application of discussed SERS platforms is effective for exact biomarkers detection (as troponin), but further investigations are required to estimate exact accuracy of CVD detection in clinical settings. In addition to, the discussed approaches are more complex, expensive, and complicated to manufacture in comparison to the proposed technique.

Moreover, we achieved a high diagnostic classification result in comparison to other studies. For instance, in our previous work we analyzed the spectral features of the serum in hemodialysis patients with end-stage chronic kidney disease using a combination of SERS and machine learning methods. The specificity was found to be 0.95, 0.92 sensitivity, and 0.94 accuracy [48]. Huang et al. [35] demonstrated a study for early diagnosis of coronary artery disease detection by isolated small extracellular vesicles analysis from the human plasma samples. The achieved sensitivity, specificity, and accuracy of 97%, 95%, and 92.3% respectively. Yanga et al. [36] measured and analyzed urine samples from congenital heart disease patients, including those who had and had not undergone percutaneous coronary intervention. The authors showed that the two patient groups had classification sensitivity and specificity of 90% and 78.9%, respectively.

Lin et al. [49] studied the variability of different tumor stages in nasopharyngeal cancer in blood plasma using SERS. High diagnostic sensitivities of 84% and 92%, specificities of 83.3% and 95% and accuracies of 83.5% and 93.3%, respectively, were achieved for classification of cancer and normal blood groups. Feng et al. [50] analyzed blood plasma samples from healthy control subjects and patients diagnosed with adenomas and colorectal cancer. The corresponding diagnostic sensitivity, specificity and accuracy were 0.938, 0.869 and 0.945 respectively. Tahir et al. [51] used SERS for analysis of serum samples of late acute stage of typhoid in comparison with healthy samples, accuracy, sensitivity and specificity were 91%, 89%, and 97 %, respectively.

Table 3 Major Raman peak positions and their characteristic assignments [33–36].

Raman shift (cm ⁻¹)	Peak assignment
641	(C–S) L-tyrosine, lactose
727	(C–H) Hypoxanthine, Phosphatidylserine, amide III
1003	(C–C) Phenylalanine
1244	amide III (proteins)carbohydrates C–O–C, N–H
1390	C–N, C–H group, CH ₃ , CH ₂ wagging (lipids), Tyrosine (proteins), and nucleic acid, lipoproteins

In general, the presented data shows that the proposed approach of human blood plasma analysis and spectra classification is stable for the detection of patients with CVD. This method offers an opportunity to achieve 99% accuracy and 0.99 ROC AUC of CVD detection, however, further clinical trials are required to ensure that the proposed approach may provide high specificity of CVD detection among other diseases.

4 Conclusions

This study shows that plasma SERS spectra can be a useful resource of information for the noninvasive diagnosis of CVD. In this investigation, 67 plasma samples from healthy volunteers and 146 plasma samples from patients with CVD were analyzed using the 785 nm laser excitation. The investigation revealed spectrum difference between the patient and healthy volunteer groups at the characteristic Raman bands, which can be linked to biochemical changes in blood during CVD progression. The achieved classification accuracy was 99%. SERS substrate based on AgNPs is a useful platform for the intensification of Raman signals of blood

plasma, allowing to observe significant differences between analyzed groups.

In comparison to standard diagnostic procedures, the results imply that the proposed approach of blood plasma analysis with SERS has the benefit of being less invasive, fast, and simple to use. Thus, the proposed SERS platform has the potential to be a useful diagnostic tool suitable for clinical detection and monitoring of CVD severity. However, further investigations with the proposed SERS platform are required for better understanding of spectral contributions of chemical components of plasma in patients with CVD. Moreover, combination of SERS serum analysis and skin optical biopsy [52] may be tested in future.

Acknowledgments

This work was funded by the Russian Science Foundation Project No. 21-75-10097, <https://rscf.ru/project/21-75-10097/>.

Disclosures

The authors declare that they have no conflict of interest.

References

1. R. V. John, T. Devasia, N. Mithun, J. Lukose, and S. Chidangil, “[Micro-Raman spectroscopy study of blood samples from myocardial infarction patients](#),” *Lasers in Medical Science* 37(9), 3451–3460 (2022).
2. R. Ross, “[The pathogenesis of atherosclerosis](#),” *Nature* 362(6423), 801–809 (1993).
3. R. A. Hegele, “[The genetic basis of atherosclerosis](#),” *International Journal of Clinical and Laboratory Research* 27, 2–13 (1997).
4. D. Bennett, R. V. Krishnamurthi, S. Barker-Collo, M. H. Forouzanfar, M. Naghavi, M. Connor, C. M.M. Lawes, A. E. Moran, L. M. Anderson, G. A. Roth, G. A. Mensah, M. Ezzati, C. J. L. Murray, and V. L. Feigin, “[The global burden of ischemic stroke: findings of the GBD 2010 study](#),” *Global Heart* 9(1), 107–112 (2014).
5. A. E. Moran, M. H. Forouzanfar, Gregory A. Roth, George A. Mensah, M. Ezzati, Christopher, J. L. Murray and M. Naghavi, “[Temporal trends in ischemic heart disease mortality in 21 world regions, 1980 to 2010: the Global Burden of Disease 2010 study](#),” *Circulation* 129(14), 1483–1492 (2014).
6. Y. Khristoforova, L. Bratchenko, and I. Bratchenko, “[Raman-Based Techniques in Medical Applications for Diagnostic Tasks: A Review](#),” *International Journal of Molecular Sciences* 24(21), 15605 (2023).
7. Y. Zhang, X. Mi, X. Tan, and R. Xiang, “[Recent progress on liquid biopsy analysis using surface-enhanced Raman spectroscopy](#),” *Theranostics* 9(2), 491(2019).
8. N. Guillot, M. L. de la Chapelle, “[The electromagnetic effect in surface enhanced Raman scattering: Enhancement optimization using precisely controlled nanostructures](#),” *Journal of Quantitative Spectroscopy and Radiative Transfer* 113(18), 2321–2333 (2012).
9. E. Pyrak, J. Krajczewski, A. Kowalik, A. Kudelski, and A. Jaworska, “[Surface enhanced Raman spectroscopy for DNA biosensors—How far are we?](#)” *Molecules* 24(24), 4423 (2019).

10. J. Zhang, Y. Dong, W. Zhu, D. Xie, Y. Zhao, D. Yang, and M. Li, “[Ultrasensitive detection of circulating tumor DNA of lung cancer via an enzymatically amplified SERS-based frequency shift assay](#),” *ACS Applied Materials & Interfaces* 11(20), 18145–18152 (2019).
11. J. U. Lee, W. H. Kim, H. S. Lee, K. H. Park, and S. J. Sim, “[Quantitative and specific detection of exosomal miRNAs for accurate diagnosis of breast cancer using a surface-enhanced Raman scattering sensor based on plasmonic head-flocked gold nanopillars](#),” *Small* 15(17), 1804968 (2019).
12. Y. Han, L. Qiang, Y. Gao, J. Gao, Q. He, H. Liu, L. Han, and Y. Zhang, “[Large-area surface-enhanced Raman spectroscopy substrate by hybrid porous GaN with Au/Ag for breast cancer miRNA detection](#),” *Applied Surface Science* 541, 148456 (2021).
13. N. Choi, H. Dang, A. Das, M. S. Sim, I. Y. Chung, and J. Choo, “[SERS biosensors for ultrasensitive detection of multiple biomarkers expressed in cancer cells](#),” *Biosensors and Bioelectronics* 164, 112326 (2020).
14. C. C. Andrei, A. Moraillon, E. Larquet, M. Potara, S. Astilean, E. Jakab, J. Bouckaert, L. Rosselle, N. Skandrani, R. Boukherroub, F. Ozanam, S. Szunerits and A. C. Gouget-Laemmel, “[SERS characterization of aggregated and isolated bacteria deposited on silver-based substrates](#),” *Analytical and Bioanalytical Chemistry* 413, 1417–1428 (2021).
15. H. Chen, A. Das, L. Bi, N. Choi, J. Moon, Y. Wu, S. Park, and J. Choo, “[Recent advances in surface-enhanced Raman scattering-based microdevices for point-of-care diagnosis of viruses and bacteria](#),” *Nanoscale* 12(42), 21560–21570 (2020).
16. M. B. Wabuyele, M. E. Martin, F. Yan, D. L. Stokes, J. Mobley, B. M. Cullum, A. Wintenberg, R. Lenarduzzi, and T. Vo-Dinh, “[Portable Raman device for detection of chemical and biological warfare agents](#),” *Proceedings of SPIE* 5692, 330–336 (2005).
17. A. Jaworska, S. Fornasaro, V. Sergo, and A. Bonifacio, “[Potential of surface enhanced Raman spectroscopy \(SERS\) in therapeutic drug monitoring \(TDM\). A critical review](#),” *Biosensors* 6(3), 47 (2016).
18. G. Q. Wallace, J. F. Masson, “[From single cells to complex tissues in applications of surface-enhanced Raman scattering](#),” *Analyst* 145 (22), 7162–7185 (2020).
19. A. Jaworska, K. Malek, and A. Kudelski, “[Intracellular pH—Advantages and pitfalls of surface-enhanced Raman scattering and fluorescence microscopy—A review](#),” *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 251, 119410 (2021).
20. C. Wen, H. Chen, X. Guo, Z. Lin, S. Zhang, X. C. Shen, and H. Liang, “[Lysosome-targeted gold nanotheranostics for in situ SERS monitoring pH and multimodal imaging-guided phototherapy](#),” *Langmuir* 37(1), 569–577 (2020).
21. E. Critselis, H. L. Heerspink, “[Utility of the CKD273 peptide classifier in predicting chronic kidney disease progression](#),” *Nephrology Dialysis Transplantation* 31(2), 249–254 (2015).
22. A. Bonifacio, S. D. Marta, R. Spizzo, S. Cervo, A. Steffan, A. Colombatti, and V. Sergo, “[Surface-enhanced Raman spectroscopy of blood plasma and serum using Ag and Au nanoparticles: a systematic study](#),” *Analytical and Bioanalytical Chemistry* 406, 2355–2365 (2014).
23. V. V. Volkov, J. McMaster, J. Aizenberg, and C. C. Perry, “[Mapping blood biochemistry by Raman spectroscopy at the cellular level](#),” *Chemical Science* 13(1), 133–140 (2022).
24. E. J. Want, I. D. Wilson, H. Gika, G. Theodoridis, R. S. Plumb, J. Shockcor, E. Holmes, and J. K. Nicholson, “[Global metabolic profiling procedures for urine using UPLC–MS](#),” *Nature Protocols* 5(6), 1005–1018 (2010).
25. F. Selimoğlu, M. E. Ayhan, “[Silver nanoparticle decorated graphene-based SERS electrode towards procalcitonin detection](#),” *Vibrational Spectroscopy* 126, 103539 (2023).
26. S. Feng, R. Chen, J. Lin, J. Pan, G. Chen, Y. Li, M. Cheng, Z. Huang, J. Chen, and H. Zeng, “[Nasopharyngeal cancer detection based on blood plasma surface-enhanced Raman spectroscopy and multivariate analysis](#),” *Biosensors and Bioelectronics* 25(11), 2414–2419 (2010).
27. Z. Hu, B. J. Deibert, and J. Li, “[Luminescent metal–organic frameworks for chemical sensing and explosive detection](#),” *Chemical Society Reviews* 43(16), 5815–5840 (2014).
28. F. Tian, F. Bonnier, A. Casey, A. E. Shanahan, and H. J. Byrne, “[Surface enhanced Raman scattering with gold nanoparticles: effect of particle shape](#),” *Analytical Methods* 6(22), 9116–9123 (2014).
29. V. Moisoiu, S. D. Iancu, A. Stefancu, T. Moisoiu, B. Pardini, M. P. Dragomir, N. Crisan, L. Avram, D. Crisan, I. Andras, and D. Fodor, “[SERS liquid biopsy: An emerging tool for medical diagnosis](#),” *Colloids and Surfaces B: Biointerfaces* 208, 112064 (2021).
30. S. Z. Al-Sammarraie, L. A. Bratchenko, E. N. Typikova, P. A. Lebedev, V. P. Zakharov, and I. A. Bratchenko, “[Silver nanoparticles-based substrate for blood serum analysis under 785 nm laser excitation](#),” *Journal of Biomedical Photonics & Engineering* 8(1), 010301 (2022).
31. J. Zhao, H. Lui, D. I. McLean, and H. Zeng, “[Automated autofluorescence background subtraction algorithm for biomedical Raman spectroscopy](#),” *Applied Spectroscopy* 61(11), 1225–1232 (2007).
32. P. Refaeilzadeh, L. Tang, H. Liu, L. Liu, and M. T. Özsu, “[Encyclopedia of database systems](#),” *Cross-validation* 5, 532–538 (2009).
33. J. Guo, Z. Rong, Y. Li, S. Wang, W. Zhang, and R. Xiao, “[Diagnosis of chronic kidney diseases based on surface-enhanced Raman spectroscopy and multivariate analysis](#),” *Laser Physics* 28(7), 075603 (2018).

34. A. Bonifacio, S. D. Marta, R. Spizzo, S. Cervo, A. Steffan, A. Colombatti, and V. Sergo, “[Surface-enhanced Raman spectroscopy of blood plasma and serum using Ag and Au nanoparticles: a systematic study](#),” *Analytical and Bioanalytical Chemistry* 406, 2355–2365 (2014).
35. X. Huang, B. Liu, S. Guo, W. Guo, K. Liao, G. Hu, W. Shi, M. Kuss, M. J. Duryee, D. R. Anderson, Y. Lu, and B. Duan, “[SERS spectroscopy with machine learning to analyze human plasma derived sEVs for coronary artery disease diagnosis and prognosis](#),” *Bioengineering & Translational Medicine* 8(2), e10420 (2023).
36. H. Yang, C. Zhao, R. Li, C. Shen, X. Cai, L. Sun, C. Luo, and Y. Yin, “[Noninvasive and prospective diagnosis of coronary heart disease with urine using surface-enhanced Raman spectroscopy](#),” *Analyst* 143(10), 2235–2242 (2018).
37. S. Kucheryavskiy, “[mdatools — R package for chemometrics](#),” *Chemometrics and Intelligent Laboratory Systems* 198, 103937 (2020).
38. H. J. Koster, A. Guillen-Perez, J. S. Gomez-Diaz, M. Navas-Moreno, A. C. Birkeland, and R. P. Carney, “[Fused Raman spectroscopic analysis of blood and saliva delivers high accuracy for head and neck cancer diagnostics](#),” *Scientific Reports* 12(1), 18464 (2022).
39. F. S. Nahm, “[Receiver operating characteristic curve: overview and practical use for clinicians](#),” *Korean Journal of Anesthesiology* 75(1), 25–36 (2022).
40. Z. Wang, Y. Liu, W. Lu, Y. V. Fu, and Z. Zhou, “[Blood identification at the single-cell level based on a combination of laser tweezers Raman spectroscopy and machine learning](#),” *Biomedical Optics Express* 12(12), 7568–7581 (2021).
41. C. Polonschii, M. Potara, M. Iancu, S. David, R. M. Banciu, A. Vasilescu, and S. Astilean, “[Progress in the Optical Sensing of Cardiac Biomarkers](#),” *Biosensors* 13(6), 632 (2023).
42. L. A. Bratchenko, S. Z. Al-Sammarraie, E. N. Tupikova, D. Y. Konovalova, P. A. Lebedev, V. P. Zakharov, and I. A. Bratchenko, “[Analyzing the serum of hemodialysis patients with end-stage chronic kidney disease by means of the combination of SERS and machine learning](#),” *Biomedical Optics Express* 13(9), 4926–4938 (2022).
43. I. A. Bratchenko, L. A. Bratchenko, Y. A. Khristoforova, A. A. Moryatov, S. V. Kozlov, and V. P. Zakharov, “[Classification of skin cancer using convolutional neural networks analysis of Raman spectra](#),” *Computer Methods and Programs in Biomedicine* 219, 106755 (2022).
44. Z. Cheng, R. Wang, Y. Xing, L. Zhao, J. Choo, and F. Yu, “[SERS-based immunoassay using gold-patterned array chips for rapid and sensitive detection of dual cardiac biomarkers](#),” *Analyst* 144(22), 6533–6540 (2019).
45. H. Chon, S. Lee, S. Y. Yoon, E. K. Lee, S. I. Chang, and J. Choo, “[SERS-based competitive immunoassay of troponin I and CK-MB markers for early diagnosis of acute myocardial infarction](#),” *Chemical Communications* 50(9), 1058–1060 (2014).
46. C. Hu, L. Ma, M. Guan, F. Mi, F. Peng, C. Guo, S. Sun, X. Wang, T. Liu, and J. Li, “[SERS-based magnetic immunoassay for simultaneous detection of cTnI and H-FABP using core-shell nanotags](#),” *Analytical Methods* 12(45), 5442–5449 (2020).
47. X. Fu, Y. Wang, Y. Liu, H. Liu, L. Fu, J. Wen, J. Li, P. Wei, and L. Chen, “[A graphene oxide/gold nanoparticle-based amplification method for SERS immunoassay of cardiac troponin I](#),” *Analyst* 144(5), 1582–1589 (2019).
48. J. H. Lin, R. A. Patil, R. S. Devan, Z. A. Liu, Y. P. Wang, C. H. Ho, Y. Liou, and Y. R. Ma, “[Photoluminescence mechanisms of metallic Zn nanospheres, semiconducting ZnO nanoballoons and metal-semiconductor Zn/ZnO nanospheres](#),” *Scientific Reports* 4(1), 6967(2014).
49. S. Feng, W. Wang, I. T. Tai, G. Chen, R. Chen, and H. Zeng, “[Label-free surface-enhanced Raman spectroscopy for detection of colorectal cancer and precursor lesions using blood plasma](#),” *Biomedical Optics Express* 6(9), 3494–3502 (2015).
50. M. Tahira, H. Nawaz, M. I. Majeed, N. Rashid, S. Tabbasum, M. Abubakar, S. Ahmad, S. Akbar, S. Bashir, M. Kashif, S. Ali, and H. Hyat, “[Surface-enhanced Raman spectroscopy analysis of serum samples of typhoid patients of different stages](#),” *Photodiagnosis and Photodynamic Therapy* 34, 102329 (2021).
51. 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, and V. P. Zakharov, “[Comparative study of multivariate analysis methods of blood Raman spectra classification](#),” *Journal of Raman Spectroscopy* 51(2), 279–292 (2020).
52. Y. A. Khristoforova, L. A. Bratchenko, M. A. Skuratova, E. A. Lebedeva, P. A. Lebedev, and I. A. Bratchenko, “[Raman spectroscopy in chronic heart failure diagnosis based on human skin analysis](#),” *Journal of Biophotonics* 16(7), e202300016 (2023).