Applied Chemical Engineering (2023) Volume 6 Issue 2
doi: 10.24294/ace.v6i2.2097

REVIEW ARTICLE

Early diseases diagnosis in body fluids (serum and saliva) using infrared spectroscopy

Andrei A. Bunaciu[1,*], Hassan Y. Aboul-Enein[2]

[1] *S.C. AAB_IR Research S.R.L., 077025 Bragadiru, Romania*

[2] *Pharmaceutical and Medicinal Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Center, Cairo 12622, Egypt*

*** Corresponding author:** Andrei A. Bunaciu, aabunaciu@gmail.com

ABSTRACT

Infrared spectroscopy it is becoming more and more useful in the field of biomedical research. Infrared spectroscopy has been used more and more to characterize biological matrixes, providing a simple way to obtain diagnostic and observational information from easily acquired samples. These tests are performed in order to monitor the changes, and in this way to characterize the biological matrix, with the aim of detecting the first signs that can diagnose a disease. Vibrational spectroscopy analysis of biological fluids has become more and more popular recently. Notably, the development of infrared spectroscopic screening of blood products, particularly blood serum and saliva, for illness diagnosis has attracted economic attention. This review examines some applications of the infrared spectroscopy method that was employed to examine human serum in order to detect disease at an early stage, published between 2017 and 2022.

Keywords: FT-IR analysis; biomedical analysis; human; serum; saliva; early disease diagnosis applications

ARTICLE INFO

Received: 5 May 2023
Accepted: 6 July 2023
Available online: 17 August 2023

1. Introduction

Although theories for vibrational spectroscopy in the biological applications have been discussed for more than three decades and several studies have already shown their suitability in, for instance, histopathologic and biological studies, clinical translation has tends to lag, requiring the consideration of realistic, strategic goals[1,2].

Body fluids (such as plasma, serum, saliva, or urine) are becoming an important source of samples for diseases diagnosis and therapy monitoring in a general clinical setting since their collection is straightforward, mostly non-invasive, and cost-effective. In the present, all over the world there are a lot of tests used for identifying and diagnosing certain diseases using body fluids[3–9]. It is known that any modification of a physiological or pathological condition will determine a change in the concentration of a specific constituent (i.e., biomarker) in body fluids[10]. The number and diversity of diseases have increased rapidly in the last period of time due to changes in genetic factors or lifestyle, as well as different types of rapidly evolving infectious agents[11,12]. Now it is possible to perform a number of analyses for early disease diagnosis in a clinical laboratory using body fluids other than blood, such as cerebrospinal fluid, drainage fluid, urine, amniotic fluid, esoteric fluids (such as sweat,

tears, and saliva), gastric juice, and others, also known as extracellular fluids (ECF)[13]. All these tests will provide doctors with valuable information for effective therapeutic treatment and progress monitoring.

Human serum is one of the most complex biological materials, containing proteins, sugars, lipids, and metabolites[14]. All these components have a unique chemical structure and are characterized by different infrared spectra. In this way, vibrational spectroscopy techniques represent a useful tool in the fields of biology and medicine because of their fast and non-invasive nature.

Taking into account the complex composition of body fluids, it is a challenging aim to obtain methods for the quantitative analysis of all these constituents by IR spectroscopy and there are several reviews, published in this period of time, presenting these results[15–20]. The first step in medical practice is diagnosis, and it is critical for a best clinical decision. Medical diagnosis is considered a very complex process, even for the most experienced clinicians, because it involves taking into account several factors and scenarios in relation to the medical evidence[4,21]. In health care, an early diagnosis is very important because it can slow down or prevent disease progression.

Although aspects of the application of infrared spectroscopy in biomedical analysis have been theoretically discussed many years ago and the feasibility has been demonstrated in various histological and cytological studies, their application has been slow, so lower steps have been considered[2,22]. The basic principle of infrared spectroscopy technique is the oscillations of atoms in molecules, thus generating useful information about molecular conformation, structure, intermolecular interaction(s), and chemical bonding[23]. Since this technique results in a biochemical signature of all the components presented in the sample, it follows that the resultant data set is influenced by all the samples' constituent molecules[24]. Due to the large amount of data obtained from spectra, this technique must be combined with chemometric approaches in order to extract relevant information. Of the most chemometric methods often used for different reasons, Principal Component Analysis (PCA)—the most basic method for analyzing chemometrics data, which involves paring down the data into noise and structural components the most basic method for analyzing chemometrics data, which involves paring down the data into noise and structural components, Random Forest (RF) and Support Vector Machine (SVM)—is a linear model for classification and regression issues that can resolve both linear and non-linear issues and is effective for a variety of real-world issues. SVM's basic premise is as follows: In order to categorize the input, the algorithm generates a line or a hyperplane. Partial Least Squares Regression analysis (PLSR)—is a statistical technique that appears to be principal component regression in some ways; however, it finds a linear regression model by projecting the predicted variables and the observable variables to a new space rather than searching for hyperplanes of maximum variance between the response and independent variables, is commonly used to construct quantitative predictive models[25–28]. For spectroscopy to be more effective at accessing spectrum data, chemometrics must be used. Chemometrics, by definition, is the application of mathematical and statistical techniques to derive pertinent chemical information and relate quality indicators or physical characteristics to analytical data. It means that a chemometrician would use their understanding of chemical and instrumental factors to show data in ways that allowed them to interpret the system they were studying chemically[29]. Biancolillo and Marini briefly reviewed the various chemometric approaches applicable in the context of spectroscopy-based pharmaceutical analysis, discussing the unsupervised exploration of the collected data as well as the potential for developing predictive models for both quantitative (calibration) and qualitative (classification) responses. This is the most straightforward explanation of chemometrics[30].

The aim of this review is to highlight some important applications of infrared spectroscopy for identifying healthy and unhealthy individuals using their body fluids (serum and/or saliva), covering the period between 2017 and 2022, after our previous papers[3,31,32]. The research was performed using "early

disease diagnosis in human serum/saliva using infrared analysis" as keywords in ScienceDirect, Wiley, Taylor & Francis online and SpringerLink sites, and were obtained more than 9000 papers.

2. Ftir applications in early disease diagnosis

Viral infections. One of the major factors affecting public health is the large number and variety of microbial pathogens in the environment. Currently, there are no analytical techniques, that are in the same time specific, fast and cheap and ensures, in a single analysis, a differentiation of viruses. This is the reason for the efforts made in order to develop techniques that can realise the diagnosis that differentiates the viruses[33–35].

SARS-CoV-2, (Severe Acute Respiratory Syndrome Coronavirus 2), more commonly known as COVID-19, is the latest biological hazard to pose threat to healthcare worldwide. A latest world statistic, 3 May 2023, shows the existence of 765,222,932 confirmed cases, of which 6,921,614 death[36]. Some authors, supposed that FTIR spectroscopy, especially on attenuated total reflectance (ATR) crystals can be used for sensing this virus after mass-separating screening while others show that saliva can be used for analysis[37–39].

In **Figure 1**, spectra for control saliva, inactivated-irradiated COVID-19 virus particles, and healthy patients (RT-qPCR negative) are compared[40].

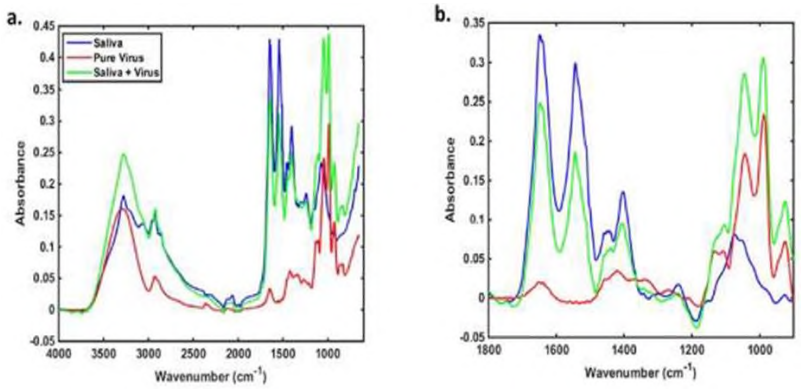

Figure 1. FTIR-ATR spectra for control saliva, inactivated-irradiated COVID-19 virus particles, and healthy patients (RT-qPCR negative)[40].

Control saliva was spiked with varying concentrations of inactivated-irradiated COVID-19 virus particles, as shown in this Figure of a human participant (male, 42 years old, and RT-qPCR negative). The virus particle is obviously capable of altering the IR spectral signature of saliva at low copy counts. When saliva is spiked with a virus at extremely low concentrations (781 copies/mL and lower), the spectral points cocluster with those of control saliva, indicating no changes. At 12,500 copies/mL, there is segregation from the control, though. Exploratory principal component analysis (PCA) is used to clearly separate spectral data points after nucleic acid (RNA/DNA) extraction from saliva samples taken from persons who tested positive (n = 5) or negative (n = 5) for COVID-19.

Diagnosis of HCV (hepatitis C virus from the Flaviviridae family) and HBV (hepatitis B virus from the family Hepadnaviridae) infection are currently based on the detection of antibodies or antigens using serological assays[41].

A similar study was focused on obtaining new biomarkers for the differentiation of chronic infections with HBV and HCV in serum[42]. The ATR-FTIR method proposed initially presents promising results, but after a thorough study it was concluded that it is possible to obtain similar results to infection or compounds synthesized by the virus rather than the presence of the virus itself, so more work is required, before a viral

diagnostic based on ATR-FTIR spectroscopy can be implemented it is necessary to know how specific the reaction is to various viruses and other infectious agents.

Infections with *Helicobacter pylori*, a Gram-negative bacterium, were studied with a highthroughput microplate FTIR spectroscopy system after gastric adenocarcinoma (AGS) cell lines were incubated with H. pylori strains presenting two different genotypes CagA+/Vac-s2[43].

The FTIR spectra obtained using comparatively dry and washed AGS cells, are presented in **Figure 2**.

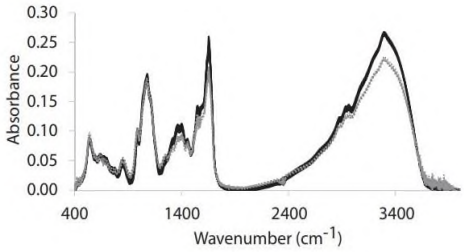

Figure 2. Examples of FTIR spectra of AGS cells incubated (black solid line) and not incubated (gray dashed line) with H. pylori, pre-processed by baseline correction and MSC (multiplicative scatter correction)[43].

The Amide I peak (1650 cm^{-1}) was used to standardize the spectra after baseline correction and multiplicative scatter correction (MSC). A Savitzky-Golay technique was used to construct second derivative spectra from raw spectra while taking into account a third order polynomial across 15-point windows. With Matlab R2012b (Matworks, Natick, MA, USA), the remaining pre-processing work was completed after doing the baseline correction with OPUS software (Bruker, Germany).

Taking into account the results obtained, it can be concluded that the method proposed is useful for the determination of H. pylori strain genotype as well as for the diagnosis of bacterial infections, antibiotic resistence, and gastric disease risk.

Immunoglobulin A (IgA) is an important immunoglobulin (Ig) in serum after immunoglobulin G[44] that acts as a part of the first line of defence against microorganisms invading mucosal surfaces[45].

An ATR method coupled with PLS approaches was proposed for the determination of IgA using both whole human serum and ultrafiltrated one[46]. The ATR-IR spectra presents some peaks of interest, which can be easily associated with protein absorption, such as 3300 cm^{-1} (Amide A), 1650 cm^{-1} (Amide I) and 1550 cm^{-1} (Amide II). For whole serum analysis there was necessary a model based on 5 PLS factors and the minimum value detected was 100mg/dL, while for ultrafiltrated serum the model is based on 9 factors with a minimum value of 92 mg/dL.

Figure 3 presents the raw spectra for whole and ultrafiltrated serum samples. The 6–7 spectra with higher than average absorbance in the X-H stretching region 2900–3500 cm^{-1} correspond to samples for which the drying process was not quite complete.

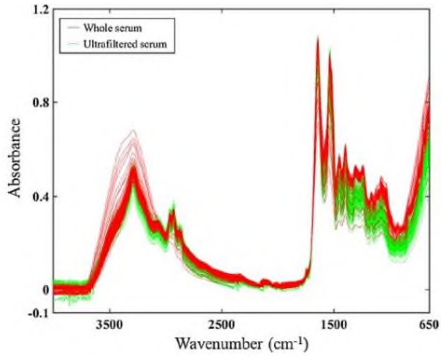

Figure 3. ATR-FTIR spectra of whole and ultrafiltrated human serum samples[46].

This study demonstrates that using centrifugal ultrafiltration as a preprocessing step is a small but important step in achieving an analytical method for the quantitative determination of serum IgA based on infrared spectroscopy. If the procedure is further improved, it might be possible to measure a variety of serum HMW (high molecular weight) proteins, such as IgA and others, that are now just below the concentration threshold needed to use IR-based analytical methods.

Alzheimer's disease (AD), one of the most known forms of dementia, is the result of a neurodegenerative disorder caused by aging of the body for reasons that are not fully known[47].

For an early diagnosis, it was necessary to find some basic criteria for diagnosis of this disease by the American National Institute of Neurological and Communicative Disorders (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA)[48,49]. Taking into account these criteria, the diagnostics is based on the determination of some AD biomarkers as well as a neuropsychological appreciation as presented in a special report[50]. If some of them can be used for the moment, for others additional studies are necessary to be performed.

Near-infrared (NIR) and mid-infrared (MIR) spectra from 265 serum samples from healthy people and Alzheimer's disease patients were collected to examine the viability of applying spectral feature fusion technology to detect Alzheimer's disease (AD)[51] and **Figure 4** presents the average NIR (a) and MIR (b) FTIR spectra for control and patients with Alzheimer's disease.

Water absorption at 1370–1600 nm and 1850–2120 nm dominate the full-spectrum range of 400–2500 nm for the NIR region[52,53], however the spectral characteristics of other components are severely affected by water absorption and are almost completely masked by water absorption bands.

The water molecule absorption peaks in the MIR region were at 3302 and 1639 cm^{-1}, while other blood constituents (such lipids and proteins) were largely absorbed in the fingerprint region of the MIR spectra (1800–800 cm^{-1})[54]. The stretching vibration of the lipid C–O was primarily responsible for the absorption between 1800 and 1700 cm^{-1}; the stretching vibration of the amide carbonyl C–O, also known as the amide I band; the bending vibration of NH_2 in the R $CONH_2$ molecules; and the stretching vibration of the P–O bond and PO– double bond of nucleic acids were both primarily responsible for the absorption between 1640 and 1600 cm^{-1}[55,56]. The spectrum features of other components were incredibly weak because of water absorption, which was clearly visible in the MIR spectra.

Bands that distinguish between the healthy and AD groups can be found by differentiating the spectra and then averaging the derivative spectra.

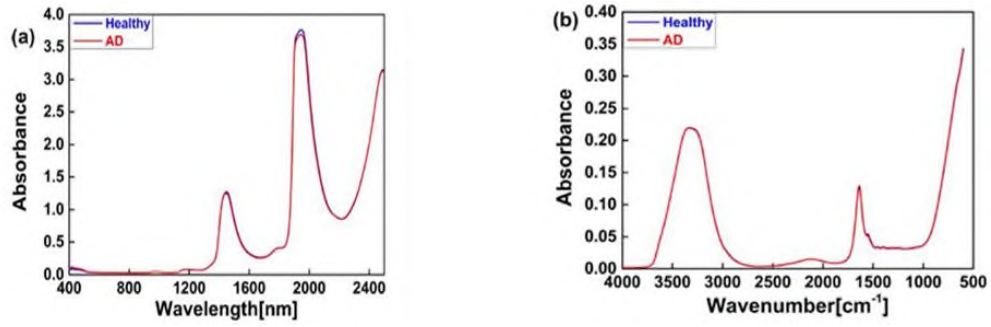

Figure 4. Average FTIR spectra of patients with Alzheimer's disease (red) and controls (blue), **(a)** in the NIR region and respectively **(b)** MIR region[51].

As can be seen, these spectra are very similar. The best pretreatment method chosen for the diagnosis of Alzheimer's disease in order to minimize errors, was accomplished by using a variety of data processing techniques, including the first derivative, second derivative, multiple scattering correction (MSC), and

standard normal variable transform (SNV). The optimization results of the NIR model indicate that the optimal number of PCs (principal components) is four, and the model accuracy is 80.6%, while for the MIR model indicate that the optimal number of PCs is 11, and the model accuracy is 98.51%.

Parkinson disease (PD) is the second most common neurodegenerative neurologic illness, after Alzheimer disease (AD).

A recent study, FTIR spectroscopy was used to discriminate serum samples obtained from PD patients and healthy persons. Studying the average IR spectra, presented in **Figure 5**, it was concluded that there are significant differences in peak-area ratios (A3294/A3067, A1399/A1451 and A1313/A1243), and protein secondary structure[57].

PD group consisted of 61 PD patients (stage 1: 18 cases; stage 1.5: 12 cases; stage 2: 18 cases; stage 3: 9 cases; stage 4: 4 cases) who came from The First Affiliated Hospital of Anhui Medical University. The control group consisted of 22 healthy volunteers. PD group and the control group had no significant difference in gender and age.

Peak-area ratios (A3294/A3067, A1399/A1451, and A1313/A1243) revealed substantial variations between the serum of PD patients and healthy individuals, illustrating the changes in the serum's proteins, lipids, and nucleic acids that occur with the progression of PD. Discriminant analysis was carried out on the basis of A3294/A3067, A1399/A1451 and A1313/A1243, which obtained the sensitivity of 93.44%, the specificity of 77.27%, the positive predictive value of 91.94% and the accuracy of 89.16%.

These results prove that FTIR spectroscopy can be used with good success for early diagnosis of PD.

Cirrhosis is a disease associated with liver-related complications and mortality. Prognosis of cirrhosis assessment is thus critical to the management of patients, especially regarding prioritization for liver transplantation.

Figure 6 presents the representative spectra, for patients with cirrhosis, after a 6 months study.

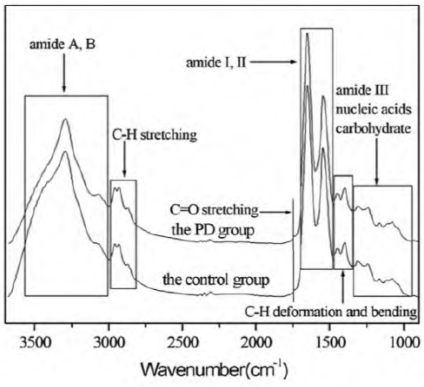

Figure 5. The average FTIR spectra of serum from PD patients and healthy persons[57].

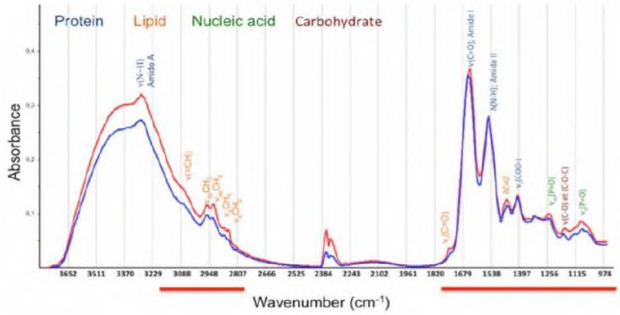

Figure 6. Mid-infrared absorbance representative spectrum for a patient that was alive (blue) and a patient that was deceased (red) at 6 months[58].

The results show that this method appears to be more efficient at identifying patients at risk for short-term mortality compared with the MELD (model for end-stage liver disease) and Child-Pugh scores[59,60].

This pilot study demonstrates that MIR-FEWS has a very high prognostic value for metabolic profile of individuals with cirrhosis and ascites. Area under the receiver operating characteristic curve (AUROC) was 0.90 (CI95: 0.88–0.91) for mortality over six months. With a 0.30 cut-off, the mean sensitivity was 0.79 (CI95: 0.75–0.83) and the mean specificity was 0.85 (CI95: 0.84–0.87). The AUROC of the MIR model was much higher compared to the MELD (model for end-stage liver disease) and Child-Pugh scores.

Usually the changes from oral cavity can be monitored by a set of rapid test performed on saliva samples[61], gingival crevicular fluid[62], or inflammatory factors according to serum analysis[63,64].

Similarly, FTIR investigations of blood, dentine and gingival fluids have been performed in order to test their potential in diagnostic for preventive screening of oral cavity pathologies[65].

Figure 7 depicts the IR spectra of the dentine and gingival fluids and blood.

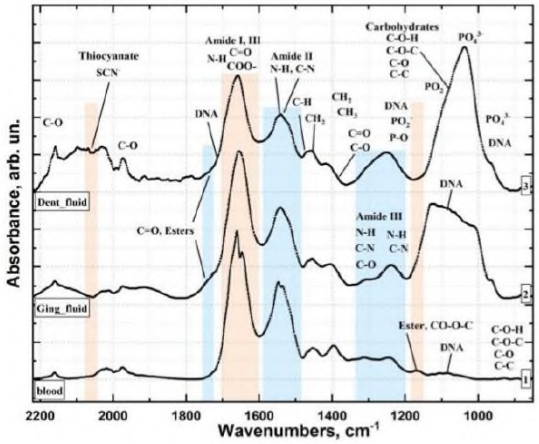

Figure 7. Comparison of IR spectra in the 2200–850 cm^{-1} range of the gingival and dentine fluids and blood averaged over the participant groups[64].

It was concluded that both dentine and gingival fluids have high diagnostic potential for the study of pathological processes in the human oral cavity. Additionally, it was found that patients with the development of pathological carious processes in the dental tissues had higher levels of thiocyanates and complex esters in their dentine fluid and gingival crevicular fluid. The use of gingival fluid for screening would support a transition to personalized treatment as well as the growth of high-tech public health and health care technology in general because its sampling for analysis is less challenging than that of dentine fluid.

In another research, FTIR spectroscopy was used for studying all the changes that appear in gingival crevicular fluid (GCF) during the orthodontic treatment with fixed appliances[65]. The most important changes take place at proteins, lipids, carbohydrates and nucleic acids levels, and thus provide useful information about the processes which occur in the alveolar bone and can be a useful tool for monitoring orthodontic treatment.

Cancer is the leading cause of mortality in many countries, according to the estimates of premature deaths from the World Health Organization (WHO) in 2015[66].

There are several reviews published in this period, related to cancer diagnosis using FTIR spectroscopy[7,67–69].

Nowadays the most useful method for most cancer diagnosis is the microscopic test, but presents the great disavantage that can be performed only when the cancerous or pre-cancerous lesions are observable

and already contain significant genetic changes. More than that, this procedure is invasive, time consuming and has a limited sensitivity that depends on the pathologists' experience.

Breast cancer (BC) is the most common invasive cancer worldwide[70]. FTIR spectroscopy has been used for easier discrimination of the changes that best reflects protein conformational changes in the serum samples of BC patients[71]. These alterations were associated with the amide I (1600–1700 cm^{-1}) band, as well as by comparing the ratio of the absorbance values at the amide II and amide III bands. Thus, infrared spectroscopy can serve as a powerful tool to understand the protein structural alterations besides distinguishing breast cancer and healthy serum samples, as can be seen in **Figure 8**.

It is challenging to distinguish between the absorbance of the functional components of the two groups alone by glancing at the FTIR spectra. However, when the absorbance spectra of the two groups were compared using a student's *t*-test (with two-tailed unequal variance), it was discovered that the amide regions (1541–1656 cm^{-1}) and mixed regions of carbohydrates and nucleic acids (1018–1076 cm^{-1}) had the greatest potential for discrimination. RNA/DNA nucleotides, C=O/C–N stretching, N–H bends in amides, and C–O vibrations of carbohydrates are some of the key discriminating areas[72,73].

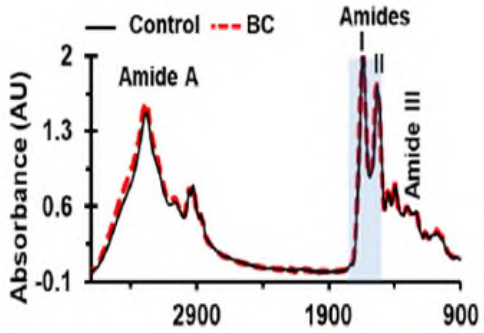

Figure 8. Identification of discriminatory bands. Ensemble averages of normalized serum spectra derived from control, n = 10, and BC, n = 10. This wider range of spectra is presented to show the quality of spectra, which overcomes the noise and atmospheric contamination, while measuring them at a resolution of 4 cm^{-1}[71].

In another research, a home-made hollow optical fiber attenuated total reflection (HOF-ATR) probe was tested to perform breast cancer research at molecular level[74]. **Figure 9** presents comparatively, the mean FTIR spectra of healthy and cancerous breast tissues, which clearly make differences between the healthy and cancerous tissues without spectral overlaps.

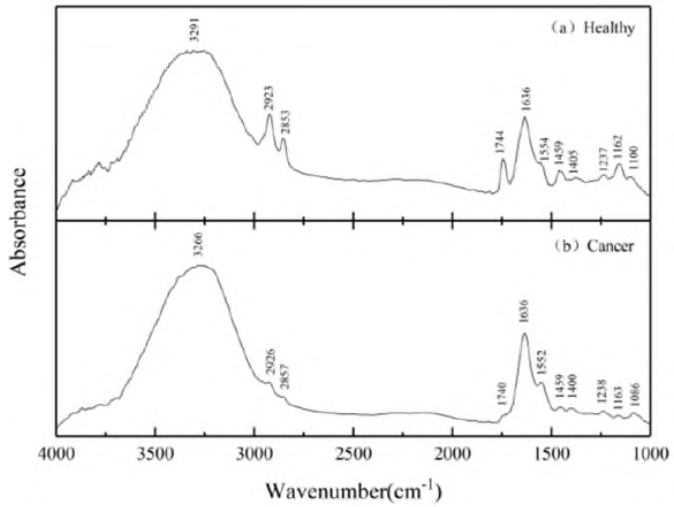

Figure 9. HOF-ATR-FTIR spectra of **(a)** healthy and **(b)** cancerous breast tissues[74].

Urine collection is quick and non-invasive, making it a wonderful choice as a vehicle for repeated measurements. This makes it easier to track the evolution of the disease, its remission or return, or even its reaction to treatment.

3. Future perspectives and conclusion

This review summarizes the spectral signatures recorded from saliva, serum and urine collected from the published studies to unleash the aptitude of ATR-FTIR to be a possible future diagnostic tool. Further trials with larger sample size are required to accept these sets of signatures as ATR-FTIR based diagnostic tool for clinical diagnosis. As screening of body fluid using spectroscopy is still emerging and many questions need to be addressed in order to conclude that this tool can be in accordance with the standard diagnostic tools being used at present. Unification of the sample processing methodology and data processing techniques will be beneficial and can lead to radical improvements in their clinical implementation in the form of next generation diagnostic tools. FTIR spectroscopy allows the advancement of next-generation clinical systems that could revolutionize disease diagnostics.

As with all diseases, early detection is critical for the best patient outcome and there is a need for cheaper, more accurate and ideally automated methods for diagnosis and for identification of progressing/regressing during the treatment.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Conceptualization, AAB and HYAE; methodology, AAB and HYAE; writing—original draft preparation, AAB; data curation, HYAE; writing—reviewing and editing, HYAE.

Conflict of interest

The authors declare no conflict of interest.

References

1. Byrne HJ, Baranska M, Puppels GJ, et al. Spectropathology for the next generation: quo vadis? *The Analyst* 2015; 140(7): 2066–2073. doi: 10.1039/ c4an02036g
2. Baker MJ, Byrne HJ, Chalmers J, et al. Clinical applications of infrared and Raman spectroscopy: State of play and future challenges. *The Analyst* 2018; 143(8): 1735–1757. doi: 10.1039/c7an01871a
3. Bunaciu AA, Aboul-Enein HY, Hoang VD. Body fluids analysis. In: *Vibrational Spectroscopy Application in Biomedical, Pharmaceutical and Food Sciences*. Elsevier; 2020. pp. 39–70.
4. Shahid AH, Singh MP. Computational intelligence techniques for medical diagnosis and prognosis: Problems and current developments. *Biocybernetic and Biomedicale Engineering* 2019; 39(3): 638–672. doi: 10.1016/j.bbe.2019.05.010
5. Song CL, Kazarian SG. Micro ATR-FTIR spectroscopic imaging of colon biopsies with a large area Ge crystal. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 2019; 228: 117695. doi: 10.1016/j.saa.2019.117695
6. Willetts K, Farr L, Foreman L. From stellar composition to cancer diagnostics. *Contemporary physics* 2019; 60(3): 221–225. doi: 10.1080/00107514.2019.1645492
7. Su KY, Lee WL. Fourier transform infrared spectroscopy as a cancer screening and diagnostic tool: A review and prospects. *Cancers* 2020; 12(1): 115. doi: 10.3390/cancers12010115
8. Khulla N, Salmann A, Javaria Q. ATR-FTIR spectroscopy as the future of diagnostics: A systematic review of the approach using bio-fluids. *Applied Spectroscopy Reviews* 2020; 56(2): 85–97. doi: 10.1080/05704928.2020.1738453

9. Sparrow RL, Greening DW, Simpson RJ. A protocol for the preparation of cryoprecipitate and cryodepleted plasma. In: Simpson RJ, Greening DW (editors). *Serum/Plasma Proteomics—Methods and Protocols*. Springer Science+Business Media; 2011. pp. 259–265.
10. Anderson NL, Anderson NG. The human plasma proteome: History, character, and diagnostic prospects. *Molecular Cellular Proteomics* 2002; 1(11): 845–867. doi: 10.1074/mcp.R200007-MCP200
11. Fu Y, Lin W, Yang Y, et al. Analysis of diverse factors influencing the health status as well as medical and health service utilization in the floating elderly of China. *BMC Health Services Research* 2021; 21(1): 438. doi: 10.1186/s12913-021-06410-7
12. Amos W, Harwood J. Factors affecting levels of genetic diversity in natural populations. *Philosophical Transactions of the Royal Society B* 1998; 353: 177–186. doi: 10.1098/rstb.1998.0200
13. Cameron JM, Bruno C, Parachalil DR, et al. Vibrational spectroscopic analysis and quantification of proteins in human blood plasma and serum. In: Ozaki Y, Baranska M, Lednev I, et al. (editors). *Vibrational Spectroscopy in Protein Research*, 1st ed. Academic Press; 2020. pp. 269–314.
14. Schrader M, Schulz-Knappe P. Peptidomics technologies for human bodyfluids. *Trends in Biotechnology* 2001; 19: S55–S60. doi: 10.1016/S0167-7799(01)01800-5
15. Cameron JM, Butler HJ, Palmer DS, et al. Biofluid spectroscopic disease diagnostics: A review on the processes and spectral impact of drying. *Journal of Biophotonics* 2018; 11(4): e201700299. doi: 10.1002/jbio.201700299
16. De Bruyne S, Speeckaert MM, Delanghe JR. Applications of mid-infrared spectroscopy in the clinical laboratory setting. *Critical reviews in clinical laboratory sciences* 2018; 55(1): 1–20. doi: 10.1080/10408363.2017.1414142
17. Leal LB, Nogueira MS, Canevari RA, et al. Vibration spectroscopy and body biofluids: Literature review for clinical applications. *Photodiagnosis and Photodynamic Therapy* 2018; 24: 237–244. doi: 10.1016/j.pdpdt.2018.09.008
18. Paraskevaidi M, Martin-Hirsch PL, Martin FL. ATR-FTIR spectroscopy tools for medical diagnosis and disease investigation. In: Kumar CSSR (editor). *Nanotechnology Characterization Tools for Biosensing and Medical Diagnosis*. Springer Berlin, Heidelberg; 2018. pp. 163–211. doi: 10.1007/978-3-662-56333-5_4
19. Rohman A, Windarsih A, Lukitaningsih E, et al. The use of FTIR and Raman spectroscopy in combination with chemometrics for analysis of biomolecules in biomedical fluids: A review. *Biomedical Spectroscopy and Imaging* 2019; 8(4): 55–71. doi: 10.3233/BSI-200189
20. Cameron JM, Butler HL, Anderson DJ, et al. Exploring pre-analytical factors for the optimisation of serum diagnostics: Progressing the clinical utility of ATR-FTIR spectroscopy. *Vibrational Spectroscopy* 2020; 109: 103092. doi: 10.1016/j.vibspec.2020.103092
21. Naseer K, Ali S, Qazi J. ATR-FTIR spectroscopy as the future of diagnostics: A systematic review of the approach using bio-fluids. *Applied Spectroscopy Reviews* 2021; 56(2): 85–97. doi: 10.1080/05704928.2020.1738453
22. National Academies of Sciences and Medicine E. *Improving Diagnosis in Health Care*. National Academies Press; 2016.
23. Li YS, Church JS. Raman spectroscopy in the analysis of food and pharmaceutical nanomaterials. *Journal of Food and Drug Analysis* 2014; 22(1): 29–48. doi: 10.1016/j.jfda.2014.01.003
24. Finlayson D, Rinaldi C, Baker MJ. Is infrared spectroscopy ready for the clinic? *Analytical Chemistry* 2019; 91: 12117–12128. doi: 10.1021/acs.analchem.9b02280
25. Shaw RA, Mantsch HH. Infrared spectroscopy in clinical and diagnostic analysis. *Encyclopedia of Analytical Chemistry* 2006; 1–20. doi: 10.1002/9780470027318.a0106
26. Spalding K, Bonnier F, Bruno C, et al. Enabling quantification of protein concentration in human serum biopsies using attenuated total reflectance—Fourier transform infrared (ATR-FTIR) spectroscopy. *Vibrational Spectroscopy* 2018; 99: 50–58. doi: 10.1016/j.vibspec.2018.08.019
27. Xu Y, Muhamadali H, Sayqal A, et al. Partial least squares with structured output for modelling the metabolomics data obtained from complex experimental designs: A study into the Y-block coding. *Metabolites* 2016; 6(4): 38. doi: 10.3390/metabo6040038
28. Walther BA, Moore JL. The concepts of bias, precision and accuracy, and their use in testing the performance of species richness estimators, with a literature review of estimator performance. *Ecography* 2005; 28: 815–829. doi: 10.1111/j.2005.0906-7590.04112.x
29. Davies AMC. What IS and what is NOT chemometrics. *Spectroscopy Europe* 2012; 24(4): 33–36.
30. Bunaciu AA, Aboul-Enein HY, Hoang VD. *Vibrational Spectroscopy Application in Biomedical, Pharmaceutical and Food Sciences*. Elsevier; pp. 227–247.
31. Bunaciu AA, Fleschin Ş, Aboul-Enein HY. Biomedical investigations using fourier transform-infrared microspectroscopy. *Critical Reviews in Analytical Chemistry* 2014; 44(3): 270–276. doi: 10.1080/10408347.2013.829389
32. Bunaciu AA, Fleschin Ş, Hoang VD, et al. Vibrational spectroscopy in body fluids analysis. *Critical Reviews in Analytical Chemistry* 2017; 47(1): 67–75. doi: 10.1080/10408347.2016.1209104
33. Chaudhary I, Jackson N, Denning D, et al. Contributions of vibrational spectroscopy to virology: A review. *Clinical Spectroscopy* 2022; 4: 100022. doi: 10.1016/j.clispe.2022.100022

34. Rumaling MI, Chee FP, Bade A, et al. Methods of optical spectroscopy in detection of virus in infected samples: A review. *Heliyon* 2022; 8: e10472. doi: 10.1016/j.heliyon.2022.e10472
35. Santos MCD, Morais CLM, Lima KMG, et al. Vibrational spectroscopy in protein research toward virus identification: Challenges, new research, and future perspectives. In: Ozaki Y, Baranska M, Lednev I, et al. (editors). *Vibrational Spectroscopy in Protein Research*, 1st ed. Elsevier Academic Press; 2020. pp. 315–335.
36. World Health Organization, Coronavirus (COVID-19), Dashboard. Available online: https://covid19.who.int/.info (accessed on 14 July 2023).
37. Bunaciu AA, Aboul-Enein HY. Determination of COVID-19 viruses in Saliva using fourier transform infrared spectroscopy. *Chinese Journal of Analytical Chemistry* 2022; 50(12): 100178. doi: 10.1016/j.cjac.2022.100178
38. de Carvalho LFCS, Nogueira MS. Optical techniques for fast screening—Towards prevention of the coronavirus COVID-19 outbreak. *Photodiagnosis and Photodynamic Therapy* 2020; 30: 101765. doi: 10.1016/j.pdpdt.2020.101765
39. Khan RS, Rehman HU, Rehman IU. Saliva for the diagnosis of COVID-19. *Applied Spectroscopy Reviews* 2020; 55: 805–809. doi: 10.1080/05704928.2020.1809442
40. Barauna VG, Singh MN, Barbosa LL, et al. Ultrarapid on-site detection of SARS-CoV-2 infection using simple ATR-FTIR spectroscopy and an analysis algorithm: High sensitivity and specificity. *Analytical Chemistry* 2021; 93(5): 2950–2958. doi: 10.1021/acs.analchem.0c04608
41. Stanaway JD, Flaxman AD, Naghavi M, et al. The global burden of viral hepatitis from 1990 to 2013: Findings from the Global Burden of Disease Study 2013. *The Lancet* 2016; 388(10049): 1081–1088. doi: 10.1016/S0140-6736(16)30579-7
42. Roy S, Perez-Guaita D, Bowden S, et al. Spectroscopy goes viral: Diagnosis of hepatitis B and C virus infection from human sera using ATR-FTIR spectroscopy. *Clinical Spectroscopy* 2019; 1: 100001. doi: 10.1016/j.clispe.2020.100001
43. Marques V, Cunha B, Couto A, et al. Characterization of gastric cells infection by diverse helicobacter pylori strains through fourier-transform infrared spectroscopy. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 2019; 210: 193–202. doi: 10.1016/j.saa.2018.11.001
44. Woof JM, Kerr MA. The function of immunoglobulin A in immunity. *Journal of Pathology* 2006; 208(2): 270–282. doi: 10.1002/path.1877
45. Kerr MA. Function of immunoglobulin A in immunity. *Gut* 2000; 47(6): 751–752. doi: 10.1136/gut.47.6.751
46. Elsohaby I, McClure JT, Riley CB, et al. Centrifugal ultrafiltration of human serum for improving immunoglobulin A quantification using attenuated total reflectance infrared spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis* 2018; 150: 413–419. doi: 10.1016/j.jpba.2017.12.031
47. Li Y, Qiang XM, Luo L, et al. Multitarget drug design strategy against Alzheimer's disease: Homoisoflavonoid Mannich base derivatives serve as acetylcholinesterase and monoamine oxidase B dual inhibitors with multifunctional properties. *Bioorganic & Medicinal Chemistry* 2017; 25(2): 714–726. doi: 10.1016/j.bmc.2016.11.048
48. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease. *Neurology* 1984; 34(7): 939–944. doi: 10.1212/WNL.34.7.939
49. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers & Dementia* 2011; 7(3): 263–269. doi: 10.1016/j.jalz.2011.03.005
50. Hrubešova K, Fousková M, Habartová L, et al. Search for biomarkers of Alzheimer's disease: Recent insights, current challenges and future prospects. *Clinical Biochemistry* 2019; 72: 39–51. doi: 10.1016/j.clinbiochem.2019.04.002
51. Yang CM, Guang PW, Li L, et al. Early rapid diagnosis of Alzheimer's disease based on fusion of near- and mid-infrared spectral features combined with PLS-DA. *Optik* 2021; 241: 166485. doi: 10.1016/j.ijleo.2021.166485
52. Huck CW, Ozaki Y, Huck-Pezzei VA. Critical review upon the role and potential of fluorescence and near-infrared imaging and absorption spectroscopy in cancer related cells, serum, saliva, urine and tissue analysis. *Current Medicinal Chemistry* 2016; 23(27): 3052–3077. doi: 10.2174/ 092986732366616060711050 7
53. Sakudo A. Near-infrared spectroscopy for medical applications: Current status and future perspectives. *Clinica Chimica Acta* 2016; 455: 181–188. doi: 10.1016/j.cca.2016.02.009
54. Bruyne SD, Speeckaert MM, Delanghe JR. Applications of mid-infrared spectroscopy in the clinical laboratory setting. *Critical Reviews in Clinical Laboratory Sciences* 2018; 55(1): 1–20. doi: 10.1080/10408363.2017.1414142
55. Lopez-Lorente AI, Mizaikoff B. Mid-infrared spectroscopy for protein analysis: Potential and challenges. *Analytical and Bioanalytical Chemistry* 2016; 408: 2875–2889. doi: 10.1007/s00216-016-9375-5
56. Bunaciu AA, Fleschin S, Hoang VD, et al. Vibrational spectroscopy in body fluids analysis. *Critical Review in Analytical Chemistry* 2017; 47(1): 67–75. doi: 10.1080/10408347.2016.1209104
57. Wang X, Wu Q, Li C, et al. A study of Parkinson's disease patients' serum using FTIR spectroscopy. *Infrared Physics and Technology* 2020; 106: 103279. doi: 10.1016/j.infrared.2020.103279

58. Le Corvec M, Jezequel C, Monbet V, et al. Mid-infrared spectroscopy of serum, a promising non-invasive method to assess prognosis in patients with ascites and cirrhosis. *PLoS One* 2017; 12(10): e0185997. doi: 10.1371/journal.pone.0185997
59. Malinchoc M, Kamath M.D PS, Gordon FD, et al. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000; 31(4): 864–871. doi: 10.1053/he.2000.5852
60. Child CG, Turcotte JG. Surgery and portal hypertension. In: Child CG (editor). *The liver and portal hypertension.* Philadelphia: Saunders; 1964. pp. 50–64.
61. Pugh RNH, Murray-Lyon IM, Dawson JL, et al. Transection of the oesophagus for bleeding oesophageal varices. *British Journal of Surgery* 1973; 60(8): 646–649. doi: 10.1002/bjs.1800600817
62. Xiang XM, Liu KZ, Man A, et al. Periodontitis-specific molecular signatures in gingival crevicular fluid. *Journal of Periodontal Research* 2010; 45(3): 345–352. doi: 10.1111/j.1600-0765.2009.01243.x
63. Bonnier F, Brachet G, Duong R, et al. Screening the low molecular weight fraction of human serum using ATR-IR spectroscopy. *Journal of Biophotonics* 2016; 9(10): 1085–1097. doi: 10.1002/jbio.201600015
64. Seredin P, Goloshchapov D, Ippolitov Y, et al. Spectroscopic signature of the pathological processes of carious dentine based on FTIR investigations of the oral biological fluids. *Biomedical Optics Express* 2019; 10(8): 4050–4058. doi: 10.1364/BOE.10.004050
65. Portaccio M, d'Apuzzo F, Perillo L, et al. Infrared microspectroscopy characterization of gingival crevicular fluid during orthodontric treatment. *Journal of Molecular Structure* 2019; 1176: 847–854. doi: 10.1016/j.molstruc.2018.09.013
66. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians* 2021; 71(3): 209–249. doi: 10.3322/caac.21660
67. Bel'skaya LV. Use of IR spectroscopy in cancer diagnosis—A review. *Journal of Applied Spectroscopy* 2019; 86(2): 187–205. doi: 10.1007/s10812-019-00800-w
68. Kumari A, Kaur J, Bhattacharyya S. Application of fourier transform-infrared spectroscopy as a tool for early cancer detection. *American Journal of Biomedical Sciences* 2018; 10(3): 139–148. doi: 10.5099/aj180300139
69. Sala A, Anderson DJ, Brennan PM, et al. Biofluid diagnostics by FTIR spectroscopy: A platform technology for cancer detection. *Cancer Letters* 2020; 477: 122–130. doi: 10.1016/j.canlet.2020.02.020
70. Stewart BW, Wild CP. *World Cancer Report 2014.* WHO Press; 2019.
71. Ghimire H, Garlapati C, Janssen EAM, et al. Protein conformational changes in breast cancer sera using infrared spectroscopic analysis. *Cancers* 2020; 12(7): 1708. doi: 10.3390/cancers12071708
72. Ollesch J, Drees SL, Heise HM, et al. FTIR spectroscopy of biofluids revisited: An automated approach to spectral biomarker identification. *Analyst* 2013; 138(14): 4092–4102. doi: 10.1039/c3an00337j
73. Elmi F, Movaghar AF, Elmi MM, et al. Application of FT-IR spectroscopy on breast cancer serum analysis. *Spectrochim Acta Part A: Molecular and Biomolecular Spectroscopy* 2017; 187: 87–91. doi: 10.1016/j.saa.2017.06.021
74. Lu YF, Zhao Y, Zhu YK, et al. In situ research and diagnosis of breast cancer by using HOF-ATR-FTIR spectroscopy. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 2020; 235: 118178. doi: 10.1016/j.saa.2020.118178