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# Vibrational Spectroscopy in Body Fluids Analysis

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**ABSTRACT**

Vibrational spectroscopy offers a unique opportunity to investigate the composition of unknown substances on a molecular basis. The spectroscopy of molecular vibrations using mid-infrared or Raman techniques has been applied to samples of body fluids. This review presents some applications related to body fluids published in the period 2005–2015.

**KEYWORDS**

Body fluids analysis; Raman spectroscopy; vibrational spectroscopy

## Introduction

Vibrational spectroscopy is certainly one of the most important analytical techniques available to today's scientists since is able to investigate simultaneously organic and inorganic compounds.

Two different types of spectroscopic technique are most frequently used to view the fundamental modes of molecular vibrations, namely mid-infrared (MIR) spectroscopy and Raman spectroscopy. The first method measures the absorption, transmission, or reflection of MIR radiation (with wavelengths in the range 2.5 to 25 $\mu$m), which is caused by the interaction of the electric dipole moment of the molecule with the IR radiation. The second method illuminates the sample with radiation of much shorter wavelengths – i.e., far away from the vibrational resonance – and measures that fraction of scattered radiation for which the energy of the photon has changed.

Vibrational spectroscopy includes several different techniques, but the most important techniques are MIR, near-IR (NIR), and Raman spectroscopy. Both MIR and Raman spectroscopy provide characteristic fundamental vibrations that are employed for the elucidation of molecular structure and are the topic of this review.

Every molecule has a unique fingerprint of vibrational frequencies, which makes Raman and Fourier transform infrared (FTIR) spectroscopy highly specific techniques for molecular identification. Both techniques can be employed non-invasively, making them ideal for biomedical applications. Raman and FTIR spectroscopy are sometimes referred to as "sister" techniques and provide complementary information about molecules, but they differ in several fundamental ways.

Almost any compound having covalent bonds, whether organic or inorganic, absorbs various frequencies of electromagnetic radiation in the IR region of the electromagnetic spectrum.

In a medical practice or at a hospital, blood or urine samples are taken from many patients. Analysis of these samples is usually centralized, either in clinics or in specialized laboratories. This leads to a time lapse between taking the sample and the availability of results for the blood or urine constituents, which may delay therapy. Test kits which enable point-of-care-testing are too inaccurate and are also frequently avoided because of a high cost.

Rapid detection of diseases enables the early administration of a therapeutic strategy when the treatment is most effective, thus saving health expenditure and lives. For this purpose, vibrational spectroscopy is a suitable technique as it is non-destructive, label-free, rapid, cost-effective, easy to operate, and requires simple sample preparation. Moreover, the use of serum spectroscopy for diagnostics has the advantage for patients to be relatively non-invasive compared to current diagnostic methodologies such as biopsies.

Vibrational spectroscopy provides information on the composition and structure of matter. The principle of IR spectroscopy is based on the interaction of IR light with matter. Molecular bonds absorb the IR radiation at the resonant frequency of the bond or group, exciting vibrational modes. The resultant spectrum is a biochemical fingerprint of the analyzed sample, each absorption peak/band corresponds to a specific vibration or combination of vibrations of a molecular bond. This absorption phenomenon obeys the Beer–Lambert law, thus allowing to obtain both quantitative and qualitative information (Settle, 1997).

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Vibrational spectroscopy offers an attractive alternative to conventional clinical chemistry analytical methods, with the spectra themselves providing the basis to recognize various components within a mixture and quantify them individually. One obvious advantage is that no reagents are required; and in principle, once a particular analytical method has been established, that analysis may be carried out repetitively with no resources other than the spectrometer itself. The very little sample may be required since a MIR spectrum can be measured using only microliters of the fluid of interest. The most common analysis in the clinical chemistry laboratory is serum, blood, and urine tests. By coincidence, many of the most important analytes lie in concentration ranges that make them suitable for analysis by IR spectroscopy.

Vibrational spectroscopy has emerged in recent years as the analytical method of choice in an enormous variety of applications. What brought about this revolution? The clearest advantage is that no specific reagents are required. Automated, repetitive analyses can, therefore, be carried out at very low cost. They are routine techniques for fingerprinting and identifying chemicals and act as standard methods in analytical chemistry and pharmacy (Wartewig and Neubert, 2005).

The main function of the clinical chemistry laboratory is to perform quantitative and qualitative analyses of body fluids such as serum, blood, urine, and spinal fluid, as well as other materials such as tissue, calculi, and faeces. The aim of the present review is to present recent advancements in the potential use of vibrational spectroscopy for discriminating between normal and malignant body fluids, with varying degrees of dysplasia. Biological and medical applications, in particular, have progressed significantly in recent years, many reviews covering this field being published (Lawson et al., 1997; Shaw and Mantsch, 2008; Barths and Harris, 2009; Khaustova et al., 2009; Wetzel, 2012; Perez-Guaita et al., 2013; Alana et al., 2014; Perez-Guaita et al., 2014; Garcia-Garcia et al., 2014; Bunaciu et al., 2015; Pleitez et al., 2012; Ryzhikova et al., 2015; Sevinc et al., 2015).

The objective of this article is to review new developments in applications of vibrational spectroscopy in biomedical investigations, covering the period between 2005 and 2015. Prior to a review on this subject, it is useful to give a short introduction to the theoretical aspects related to body fluids followed by discussion of the quantitative and qualitative biomedical investigations of the technique.

## Theoretical aspects related to the body fluids

Biofluids, such as serum and plasma, represent an ideal medium for the diagnosis of disease due to their ease of collection, that can be performed worldwide, and their fundamental involvement in human function. The ability to diagnose disease rapidly with high sensitivity and specificity is essential to exploit advances in new treatments, in addition, the ability to rapidly profile disease without the need for large-scale medical equipment (e.g., MRI, CT). Vibrational spectroscopy has been investigated as a diagnostic tool and has shown great promise for serum spectroscopic diagnostics.

However, the optimum sample preparation, optimum sampling mode, and the effect of sample preparation on the serum spectrum are not very well known (Lovergne et al., 2015).

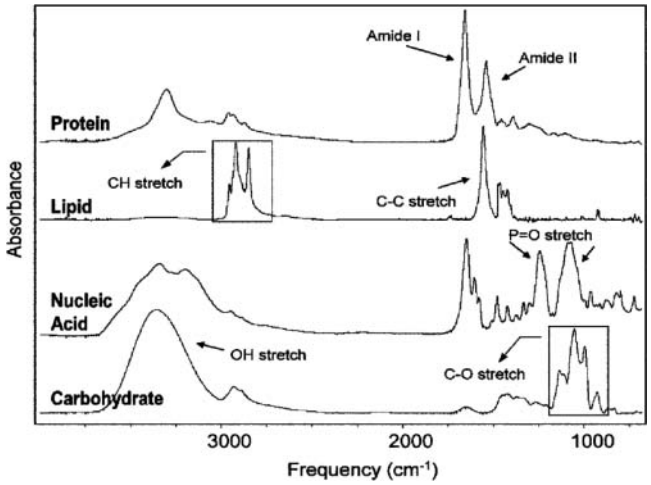


**Figure 1.** Infrared spectra for different cellular components (Shin and Markey, 2006).

Biological samples are composed mainly of proteins, lipids, sugars, and deoxyribonucleic acid (DNA). All those compounds are active in the IR range, as can be seen in Figure 1, and any change produced in the composition or the structure can be evaluated by IR measurements (Shin and Markey, 2006).

Serum is composed of water, organic substances, and inorganic salts; it can reflect human beings' physiological and pathological conditions (Zhang et al., 2008) and is easier to collect and more suitable for rapid diagnosis.

The potential of MIR spectroscopy of body fluids for a quantitative analysis using IR spectra of blood plasma analyzed by partial-least-square (PLS) methods was realized as early as 1989 (Janatsch et al., 1989). Since then, many improvements have been reported.

Water is the major molecular component within biological matrices, and strongly affects the utility of selected electromagnetic spectral regimes due to strong O–H absorptions, especially in the MIR region. However, water has a relatively broad transmission window in the NIR, thereby enabling direct measurement of the biological specimen. However, despite the merits of operating in the NIR region, the information content and data interpretation of biological NIR spectra are frequently affected by relatively weak and highly convoluted absorption features (Shaw and Mantsch, 2008). It is no surprise that the IR spectra of biological fluids are dominated by water absorptions, with features from the dissolved species superimposed on the water absorption profile. Despite the overwhelming strength of the water absorptions, the absorption pattern for the dissolved species can be recovered, as illustrated by the MIR spectrum (Figure 2A) and by the NIR spectrum (Figure 2B) of a typical serum specimen. A typical measurement scheme is to sandwich a small volume of the sample between demountable calcium fluoride or barium fluoride windows that are separated using a Teflon® ring spacer to provide an optical pathlength of 6–10 $\mu$m. This pathlength is short enough to bring the strong water absorption at 1645 $cm^{-1}$ into an absorbance range of 1–1.5, which is low enough that solute absorptions in the same wavenumber range may be recovered by subtracting the spectrum of pure water (see e.g., Figure 2A). Attenuated total reflectance (ATR) spectroscopy provides an alternative means

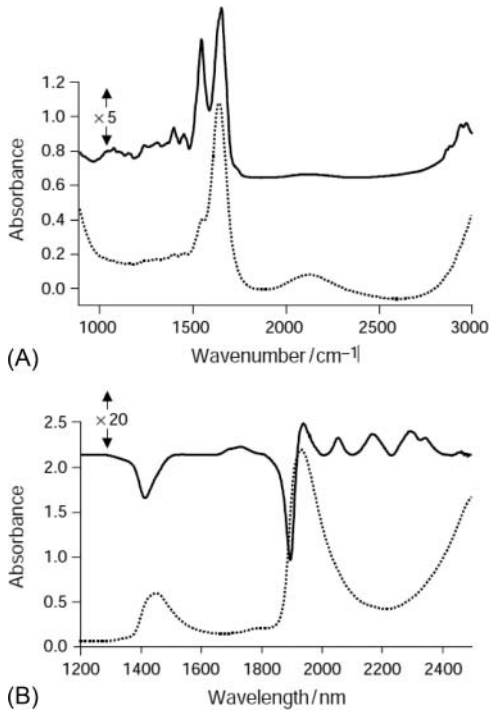


**Figure 2.** Mid-infrared (A) and near-infrared (B) absorption spectra of serum (dashed lines) and the residual spectra with the spectrum of water subtracted from each (solid lines) (Shaw and Mantsch, 2008).

to measure absorption spectra by using the experimental arrangement illustrated in Figure 3.

Although some of the stronger solute absorptions do emerge in the MIR spectra, water clearly dominates the overall appearance. MIR and NIR spectroscopies in fact offer quite different, but complementary, approaches to analysis. The richness of the MIR spectrum makes it instinctively appealing as the method of choice for analytical work, however NIR has practical benefits such as convenience in sample handling and the fact that the sample cells do not require specialized materials. Whereas MIR spectroscopy of aqueous specimens typically requires optical pathlengths of the order of microns, NIR transmission spectra are generally collected using pathlengths of 0.5 mm or greater. The question of whether to use NIR or MIR spectroscopy for analytical purposes then translates to the question of whether the additional effort generally required to acquire MIR spectra is compensated for by other possible benefits such as greater analytical accuracy or smaller sample volume.

Water plays an important role in protein folding/misfolding, protein binding to specific DNA, and many other fundamental biological processes, where the balance between the flexibility of a given protein and DNA sequences and the amount of water released from the interface is essential. The internal molecular flexibility in the proteins necessary for biological activity depends on the level of hydration (Pacaroni et al., 2005). For elucidation of the processes responsible for vibrational IR spectral properties of O–H stretching modes of water involved in H-bonding with the biomolecules of human tissue, the vibrational properties of the interfacial water at the surface of non-cancerous and cancerous tissues were compared.

One obvious impediment to using transmission MIR spectroscopy of aqueous solutions as the basis for routine analysis is a practical one, namely the difficulty in repeatedly draining and refilling cells with such a short pathlength. This impediment has been finessed in two ways. The best solution is to use ATR spectroscopy rather than transmission measurements. The clearest advantage of this method is that it provides a means to measure MIR spectra for strongly absorbing aqueous solutions, without the inconvenience and imprecision involved in working at very short pathlengths that are required for transmission spectroscopy.

The difficulties associated with strong water absorptions can be eliminated by simply eliminating water from the sample. Typically 5–50 $\mu$L of liquid is spread on a suitable substrate and allowed to dry, and a transmission spectrum is acquired for the resulting film. In addition to eliminate the spectral interference of water, this approach can provide inherently better spectral resolution by virtue of eliminating the water/solute interactions. A representative spectrum of a dry serum film is illustrated in Figure 4. The specimen was first diluted two-fold in aqueous 4 g $L^{-1}$ potassium thiocyanate (KSCN) solution. The absorption of $SCN^-$ at 2060 $cm^{-1}$ was used for subsequent normalization of the spectra as part of the development of quantitation models (Shaw et al., 1998).

ATR technique has several limitations since it can only be applied to bands with low absorption. Furthermore, a guessed profile is necessary for the calculation. Subsequently, two new ATR depth profiling techniques based on matrix formalism have been developed: the multiple-angle approach (Ekgasit and Ishida, 1996; Ekgasit and Ishida, 1997) and the multiple-frequency approach (Huang and Urban, 1993). These two techniques overcome several major difficulties with the inverse Laplace transformation technique, which have limited the

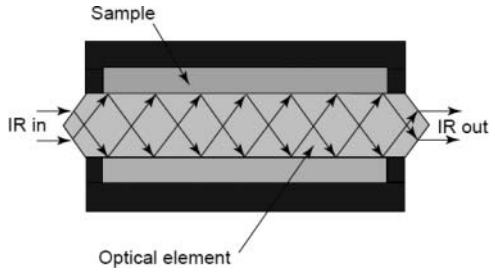


**Figure 3.** Apparatus to measure the ATR spectrum for a liquid specimen. The ATR spectrum is derived by rationing the single-beam spectrum measured with the specimen in place against a single-beam spectrum for the clean optical element.

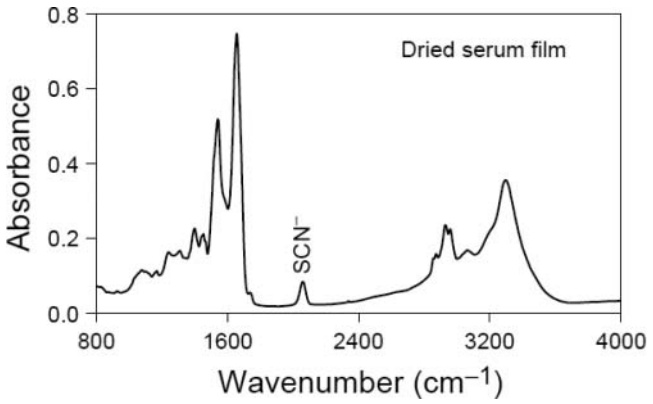


**Figure 4.** Absorption (transmission) spectrum for a serum film dried onto a barium fluoride window (Shaw et al., 1998).

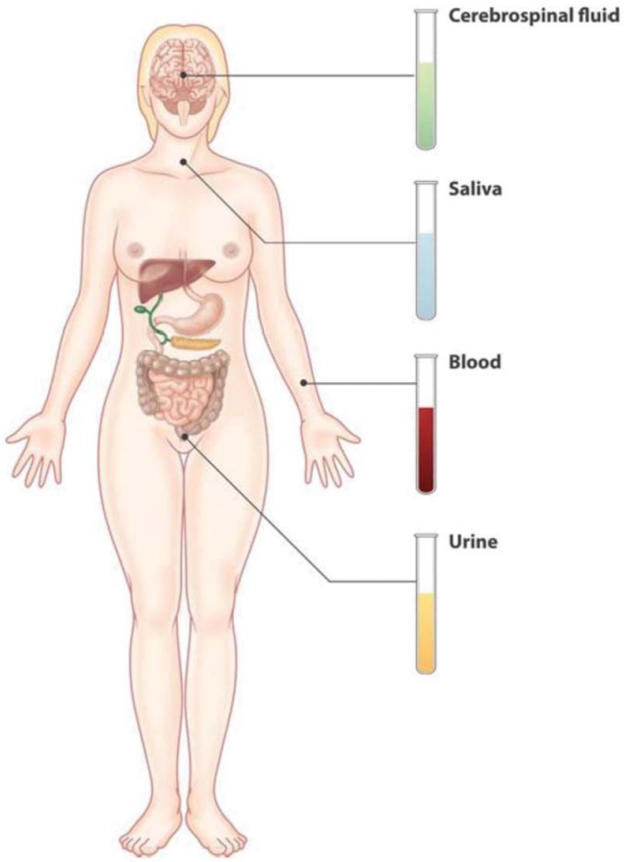


**Figure 5.** A variety of biofluids including blood, saliva, urine, and cerebrospinal fluids are obtainable and applicable in a clinical setting.

applicability of ATR as a quantitative depth profiling technique (Ekgasit and Ishida, 2002).

The reagent-free analysis of biofluids such as whole blood, plasma, or serum takes advantage of the fact that a multitude of analytes can be quantified simultaneously. In addition, the use of Raman spectrometry to analyze biomolecules has the advantages of requiring a small amount of sample, being fast and resistant to water interference, not causing damage to the tissue, and allowing for *in situ* detection. Thus, Raman spectrometry is widely used in medical fields. Its uses include the determination of the secondary structure of proteins and of the interactions between DNA and anticancer drugs, the diagnosis of damaged cells and tissue, and the analysis of patient bodily fluids, such as serum as shown in Figure 5.

In order to realize the full potential of IR spectroscopy as a leading healthcare tool, some issues need to be understood prior to clinical translation (Byrne et al., 2015). One issue, in particular, related to biofluid spectroscopy is due to the strong IR activity of water. As such, the most common protocol for analyzing liquids such as bio-fluids is the drying of drop deposits. However, it has been shown by the optical and spectroscopic assessment that this deposition is not homogenous (Bonier et al., 2014). Fingerprint spectra may diagnose the origin and grade of pathology based on a classification algorithm.

The potential of FTIR and Raman spectroscopy has been widely investigated for diagnostic purposes for cell and tissue analysis and the feasibility to use them for serum sensing has been suggested and applied to a wide range of body fluids (Shaw and Mantsch, 2008), ranging from serum (Rohleder et al., 2004; Bonnier et al., 2014), tears (Lin et al., 2010), urine or saliva (Perez-Guaita et al., 2014).

## Field applications

Diabetes, a disorder in the control of the blood glucose level is considered to be one of the most important metabolic diseases worldwide (Auses et al., 1975). Nowadays, self-monitoring of blood glucose based on painful finger pricking is typically used. Therefore, many researchers have aimed at the development of a non-invasive sensor to monitor the blood glucose level continuously. Although several *in vivo* blood glucose measurement studies have been performed by different research groups NIR absorption and Raman spectroscopic techniques, the prospective prediction has proven to be a challenging problem. An important issue, in this case, is the demonstration of causality of glucose concentration to the spectral information, especially as the intrinsic glucose signal is smaller compared with that of the other analytes in the blood–tissue matrix.

Thus, NIR Spectroscopy coupled with chemometrics is one of the most used method (Haese, 2006). NIR radiation is essential for assays using diffuse reflectance spectroscopy of skin for non-invasive glucose quantification and diabetes screening based on glycation effects. It was found that the first overtone band, 1500–1800 nm, is most informative for aqueous solutions while for glucose measurement of serum samples the combination band was found to be the better choice. Also, Raman spectroscopy was used for quantitative, non-invasive (transcutaneous) measurement of blood analytes, using glucose as an example. Finally, the results suggest that the incorporation of chance correlations for *in vivo* cases can be largely attributed to the uncontrolled physiological sources of variations. Such uncontrolled physiological variations could either be intrinsic to the subject or stem from changes in the measurement conditions. Raman spectrum peaks, using 532 nm laser system, for diabetic blood serum are observed and were attributed to carbohydrates, proteins, lipids, collagen, and skeletal C–C stretch of lipids acyl chains (Firdous et al., 2012). This *in vitro* glucose monitoring methodology will lead *in vivo* non-invasive on-line monitoring having painless and at the same time, the data will be displayed on-line and in real time. The measured Raman peaks provide a detailed biochemical fingerprint of the sample and could confer diagnostic benefit in a clinical setting.

Recent research suggests that specific salivary biomarkers such as glucose, $\alpha$-amylase, and ghrelin appetite hormone exhibit strong diagnostic potential for diabetes (Belazi et al., 1998; Aydin, 2007). IR spectroscopy can be employed to monitor all molecules present in saliva rapidly and simultaneously. Briefly, the attenuation of the intensity of a beam of IR light upon passing through a sample is measured. The intensities of IR spectra provide quantitative information while the frequencies reveal qualitative characteristics about the nature of the chemical bonds, their structure, and their molecular environment (Scott et al., 2010), as can be seen in Figure 6.

IR spectroscopy was used as a novel diagnostic tool in the prediction of diabetic status by analyzing the molecular and sub-molecular spectral signatures of saliva collected from subjects with diabetes and healthy controls. Spectral analysis

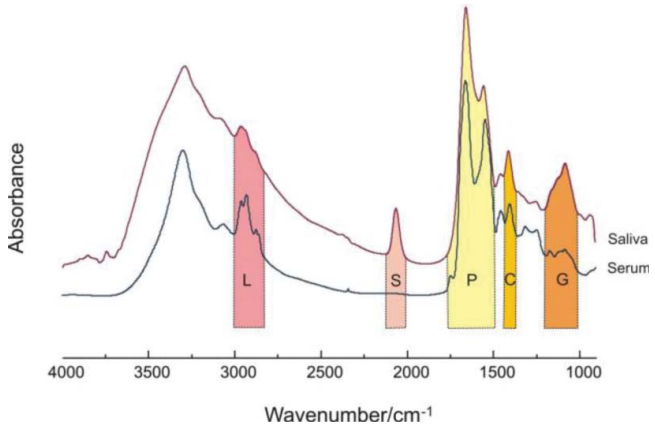


**Figure 6.** Comparison of IR spectra obtained from films of normal human saliva and serum. Areas marked L, S, P, and G represent lipid, thiocyanate, protein, and glucose, respectively (Scott et al., 2010).

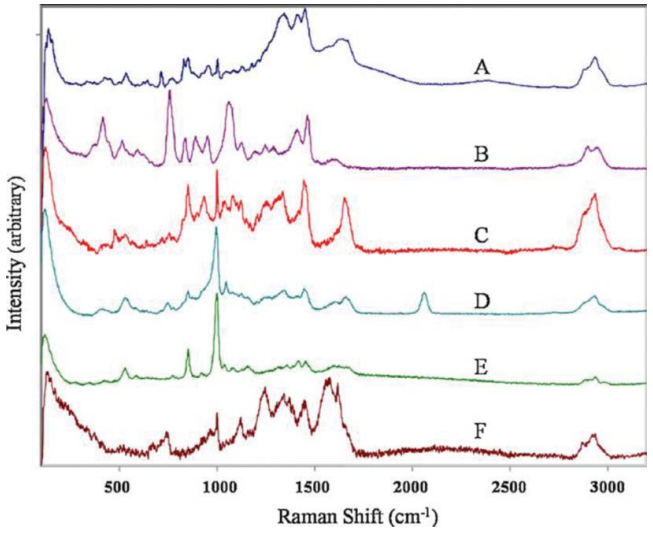


**Figure 7.** Raman spectra of human semen (A), canine semen (B), vaginal fluid (C), saliva(D), sweat (E), and blood (F) (Orphanou, 2015).

revealed differences in several major metabolic components—lipid, proteins, glucose, thiocyanate, and carboxylate—that clearly demarcate healthy and diseased saliva. It was established that IR spectroscopy can be used to generate complex biochemical profiles in saliva and identify several potential diabetes-associated spectral features. IR spectroscopy may represent an appropriate tool with which to identify novel diseases mechanisms, risk factors for diabetic complications, and markers of therapeutic efficacy.

Human blood, saliva, semen, and vaginal secretions could successfully be detected and differentiated from one another when analyzed with ATR FT-IR spectroscopy (Orphanou, 2015). This enabled identification based on the unique spectral pattern, combination of peaks, and peak frequencies corresponding to the macromolecule groups common within the biological material, such as proteins, sugars, and phosphates. This study and other similar studies (Hoşafçı et al., 2007; Virkler and Lednev, 2008; Sikirzhytski et al., 2010; Sikirzhytskaya et al., 2014) proved the discrimination and identification of each body fluid and demonstrate the potential for ATR FT-IR and Raman spectroscopy to be utilized as a confirmatory method for body fluid identification with high confidence, as can be seen in Figure 7.

In the same filed of applications, forensic analysis, it was found that vibrational spectroscopy can be used for determination of drugs of abuse (cocaine, diazepam, methamphetamine, cotinine, and benzoylecgonine) in oral fluids (D'Elia et al., 2015). Comparing IR, Raman, and NMR spectroscopy techniques, was proved that Surface-Enhanced Raman Spectroscopy (SERS) is the most sensitive technique for the detection of illicit drugs in oral fluid. The use of IR spectroscopy for determining drugs of abuse in oral fluid is growing although the LODs obtained until now do not yet satisfy the necessities in the forensic field. Finally, NMR spectroscopy has been seldom used to determine drugs in oral fluid. Furthermore, those techniques that already dispose of good portable instrumentation, in particular Raman and IR spectroscopy seem the most promising spectroscopic tools for determining drugs in oral fluid.

Immunoglobulin G (IgG) is crucial for the protection of the host from invasive pathogens. The concentration of IgG in blood and other biofluids is directly related to the level of humoral immunity where by abnormal IgG concentrations are often indicative of disease or the risk of susceptibility to infection. Due to its importance for human health, tools that enable the monitoring of IgG levels are highly desired. Consequently, there is a need for methods to determine the IgG concentration that are simple, rapid, and inexpensive (Hou et al., 2015). The results showed that ATR IR spectroscopy is potentially a simple, quick, and inexpensive method to measure IgG concentrations in human serum samples. The results also showed that it is possible to build a united calibration curve for the umbilical cord and the venous samples. Figure 8A shows the spectra of the combined set of the venous and umbilical cord samples encompassing the wavenumber range of 4000–650 $cm^{-1}$, while Figure 8B shows the averaged spectra (average spectrum for each sample) after truncation to the selected spectral regions.

In addition to immune system disruption, HIV/AIDS infection is also known to cause metabolic abnormalities ranging from dyslipidemia, hyperglycemia, insulin resistance, and diabetes (Omech et al., 2012). ATR-FTIR spectroscopy coupled with chemometrics successfully distinguished sera from HIV infected patients and uninfected controls with distinctions visible in the presence of treatment (Sitole et al., 2014). The study provided original insights for novel systems diagnostics for HIV/AIDS.

Biostructure disorders (e.g., uncontrolled cell division, invasive cell growth into adjacent tissue, and metastatic implantation to other body sites) are called "cancer." Cancer is becoming the leading cause of death all around the world. Cancer-related diseases affect people in all age ranges, but the risk tends to increase with age. It is well known that a precise, accurate diagnostic report is very helpful for drawing up strategies for treatment. Innovative diagnostic methods that provide indications complementary to conventional histopathology, in particular the early biomolecular alterations under malignant conditions are under scrutiny. One such candidate method is IR spectral imaging, which has the potential to provide, in a non-destructive and label-free manner, a biochemical fingerprint of cells and tissues (Khanmohammadi and Garmarudi, 2011; Martin et al., 2010). Several studies were performed in order to compare the serum from healthy persons with the

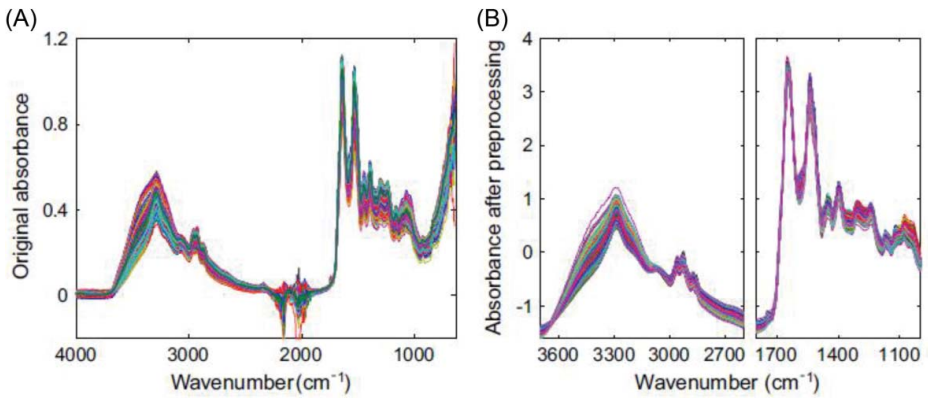


**Figure 8.** (A) Original infrared spectra obtained using a customized 3-bounce total attenuated reflectance spectrometer.(B) Infrared spectra after data pre-processing. Note the average spectra for replicates of each sample are shown in (B) and the spectra have been truncated (Hou et al., 2015).

serum from different types of cancer patients, such as: leukemia (Sutedja, 2003; Sahu et al., 2006; Büttner Mostaço-Guidolin and Bachmann, 2011; Zelig et al., 2011; Sheng et al., 2013), lung (Lewis et al., 2010; Wang et al., 2014), prostate (Baker et al., 2009), ovarian (Gajjar et al., 2013), oral cavity (Giorgini et al., 2013; Menzies et al., 2014; Sahu et al., 2015), cervical (González-Solís et al., 2014), and breast (Backhaus et al., 2010), as well as for monitoring the effectiveness of drugs during chemotherapy (Kazarian and Chan, 2006; Punith and Seetharamappa, 2012; Ostrovsky et al., 2013). The main spectral changes between breast cancer and healthy patients were recorded in the area of the CH stretching vibrations, the C–O ribose, the ribose backbone, and the P–O vibrations (Manavbasi and Suleymanoglu, 2007). The natural detection limit of IR-spectroscopy is in the area of 0.1–0.01%. So the changes which were observed in the spectrum must lie in this area. These examinations show that it is possible to make a qualified diagnosis from a small amount of serum on breast cancer. Breast cancer can also be differentiated from other diseases.

Cervical cancer screening programs have greatly reduced the burden associated with this disease. However, conventional cervical cytology screening still lacks sensitivity and specificity. There is an urgent need for the development of a low-cost robust screening technique (Purandare et al., 2013). By generating a spectral "biochemical-cell fingerprint", FTIR spectroscopy has been touted as a tool capable of segregating grades of dysplasia. Following FT-IR spectroscopy, derived spectra were examined for segregation between classes in score plots generated with subsequent multivariate analysis. Deeper understanding of the underlying changes in the transition between cervical cytology classes (normal to low-grade to high-grade) is required in order to develop biospectroscopy tools as a screening approach. This will then allow for the development of blind classification algorithms.

IR spectroscopy of blood plasma or serum is a rapid, versatile, and relatively non-invasive approach that could characterize biomolecular alterations due to cancer and has the potential to be utilized as a screening or diagnostic tool (Gajjar et al., 2013). Classification results for ovarian cancer were remarkable, up to 96.7%. This pilot study suggests that ATR-FT-IR spectroscopy of blood is a robust tool for accurate diagnosis and carries the potential to be utilized as a screening test for ovarian cancer in primary care settings. The proposed classification machine is a powerful tool that could be applied to classify the vibrational spectroscopy data of different biological systems (e.g., tissue, urine, saliva), with potential application in clinical practice.

Since serum can reflect human beings' physiological and pathological conditions, FT-IR spectroscopy was used to compare gastric cancer patients' serum with healthy persons' serum (Sheng et al., 2013). The H2959/H2931 peak height ratio might be a standard for distinguishing gastric cancer patients from healthy persons; the result showed that the RNA/DNA ratios of gastric cancer patients' serum were obviously lower than those of healthy persons' serum, as can be seen in Figure 9. The results suggest that FT-IR spectroscopy may be a potentially useful tool for diagnosis of gastric cancer.

Another simple and rapid method for the detection of breast cancer with IR-spectroscopy was developed (Backhaus et al., 2010). The method needs only 1 $\mu$L of a serum sample. The serum sample is dried on a suitable sample carrier such as a Si-plate. After drying the IR spectrum is measured. Every disease leaves a typical fingerprint in the IR spectrum of serum. This typical fingerprint can be used to identify different patient groups, as can be seen in Figure 10. The identification system can be trained by classification methods, using two independent classification methods, cluster analysis and artificial neural networks.

Asthma is a chronic inflammatory disorder of the airways characterized by airway hyperresponsiveness and reversible air flow obstruction that fluctuates over time (Shifren et al., 2012). According to World Health Organization estimates, 300 million

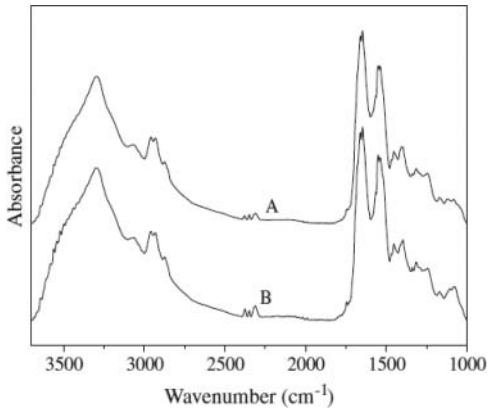


**Figure 9.** Average IR spectra of gastric cancer patients' serum (A) and healthy persons' serum (B) (Sheng et al., 2013).

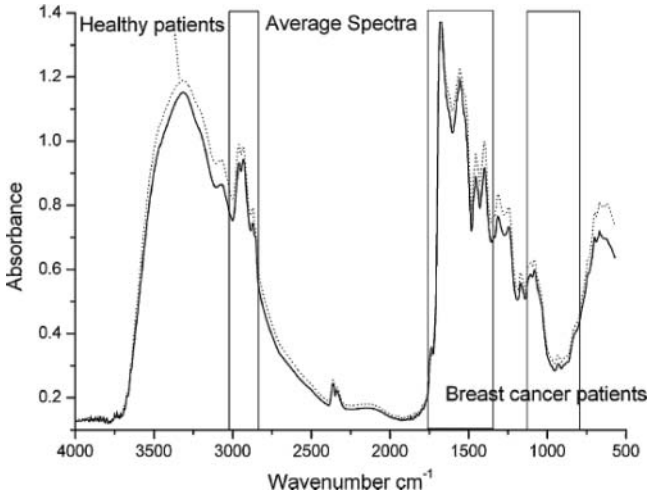


**Figure 10.** Overview spectra in transflection, spectral region 4000–500 $cm^{-1}$ (Backhaus et al., 2010).

people suffer from asthma. In a recent study, was explored the feasibility of detecting asthma and determining treatment response in asthma patients, through Raman spectroscopy of serum (Sahu et al., 2013). Differences like changes in protein structure, increase in DNA specific bands, and increased glycosaminoglycans-like features were more prominent with an increase in asthma severity. No overlap was observed between the treated severe and untreated severe groups, indicating that patient response to treatment could be determined. Overall promising results were obtained, and a large-scale validation study on random subjects is warranted before the routine clinical usage of this technique.

Like many other biomedical sciences, the development of the field of haematology has evidently been driven by technology. Haematology is a discipline devoted to understanding and exploiting information in blood in order to understand basic physiological functions and to facilitate the prevention, diagnosis, and treatment of hematological diseases and disorders. IR spectroscopic-based techniques can be used to analyze DNA alterations, secondary structural changes in proteins, and to profile cellular lipids (Liu et al., 2005), as can be seen in Figure 11.

IR-based methods hold several attractions for consideration in the hematology laboratory: (1) No chemical reagents or specific molecular probes are required—the IR "spectral patterns" of the species of interest can provide the basis for detection and quantitation; (2) only small amounts of sample (of the order of microliters of fluids, or small numbers of cells (about $10^3$) are required, leaving ample material for other clinical tests; and (3) it is suited for automation—IR analyzers can yield test results within 15 minutes, with little training required of the operator.

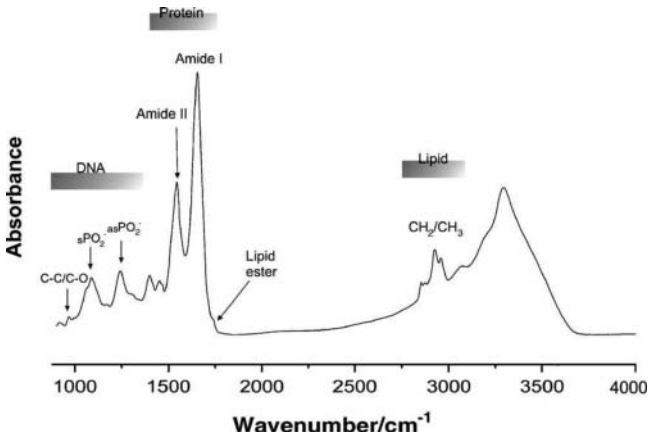


**Figure 11.** Representative IR spectrum of normal lymphocytes revealing basic cellular molecules such as proteins, lipids, and DNA as marked (Liu et al., 2005).

In this way, vibrational spectroscopy can be used for the determination of: Hb oxy-deoxy transition in erythrocytes under stretching conditions (Rusciano, 2010), and biochemical parameters in human serum (Rohleder et al., 2004; Garcia-Garcia et al., 2014; Jensen et al., 2014; Perez-Guita et al., 2014).

## Conclusions

Vibrational spectroscopic methods such as FTIR- and Raman spectroscopy are multipurpose techniques that offer advantages in simplicity, rapidity, low-cost, and minimal sample preparation.

Vibrational spectroscopic techniques, both Raman and IR spectroscopy, have significant potential in the field of biomedical analysis, as they can give molecularly specific biochemical information without the use of extrinsic labels and without being invasive to the system studied.

The greatest benefit of these techniques lies in the high molecular sensitivity combined with a spatial resolution down to a few micrometers. Another advantage is the ability to probe samples under native conditions, which allows new insights into samples without the need for fixation, stains, or an additional marker. Advances in instrumentation have made FTIR spectroscopic imaging the tool of choice for an increasing number of applications.

It was demonstrated that the vibrational properties of water are sensitive to the cellular environment of human tissue and are capable of distinguishing between cancerous and normal human breast tissues. These properties can be treated as hydration fingerprints to discriminate between cancerous and normal tissues, but a definite assignment of the origin and uniqueness of these bands remains and further studies are necessary.

The development of Raman spectroscopic signatures of body fluids has demonstrated the potential for routine spectroscopic examination of biological samples. FT-IR and ATR FT-IR spectroscopic techniques produce spectra containing bands, or peaks, representative of the vibrations of structural bonds and functional groups within biological samples. The positioning of the peaks are specific to particular interactions with molecular bonds and provide specific information relating to the biochemical composition (Petibois et al., 2001).

The proposed methods overcome the problems associated with currently used biochemical methods, which are destructive, time consuming, and expensive.

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