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# Infrared-based quantification of clinical parameters

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ABSTRACT

Infrared (IR) spectroscopy has become a promising technique for the analysis of clinical samples, and IR-based diagnosis is already a well-established technique in the academic field. This review aims to report the methodologies proposed for IR quantification of different analytes and parameters in a variety of clinical samples; including: liquids, such as whole blood, serum, plasma, urine, human milk, amniotic fluid and cerebrospinal fluid; solids, such as urinary stones, skin, hair and lips; breath; or, microscopic quantifications on tissues. For the application of IR spectroscopy to the clinical field, we also discuss: the benefits, which make IR spectroscopy ideally suited to modern medicine; and, the limitations, which are connected with sensitivity and selectivity issues. The review focuses on recent developments based on modern improvements on chemometric algorithms, sample treatment and direct measurements on samples.



**Contents**



## 1. Introduction

From the discovery of infrared radiation in 1800 by William Herschel, IR spectroscopy has been used as an important source of information for chemical and physical disciplines. The high amount of information provided by the IR spectra has been employed in the analytical field for the determination and the quantification of many compounds in several kinds of sample. Besides, with the development of powerful Fourier transform-IR (FTIR) spectrophotometers, the direct acquisition of the sample spectra can be quickly and easily performed using affordable instrumentation, so IR spectroscopy has been widely applied as an analytical tool to obtain multi-component information.

All the advantages of IR spectroscopy have been applied to the clinical field in order to obtain information about the composition

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**Table 1**
Band assignment in the infrared region to functional groups of biological compounds

| Wavenumber ($cm^{-1}$) | Band assignment | Main source |
|---|---|---|
| 930–1300 | $\nu_s$ (C-O) | Saccharides |
| 1000–1150 | $\nu_s$ (P-O) | Phospholipids, DNA |
| 1150–1300 | $\nu_{as}$ (P-O) | Phospholipids, DNA |
| 1200–1400 | Amide III | Proteins |
| 1430–1480 | $\delta(CH_3)$, $\delta(CH_2)$ | Lipids |
| 1480–1600 | Amide II | Proteins |
| 1600–1720 | Amide I | Proteins |
| 1700–1760 | $\nu_s$ (C = O) | Lipids |
| 2840–2860 | $\nu_s$ ($CH_2$) | Lipids |
| 2860–2870 | $\nu_s$ ($CH_3$) | Lipids |
| 2870–2950 | $\nu_{as}$ ($CH_2$) | Lipids |
| 2950–2990 | $\nu_{as}$ ($CH_3$) | Lipids |
| 3000–3020 | ν (CH) | Lipids |
| 4000–4550 | ν (CH) combinations | Lipids |
| 4550–5000 | ν (NH) ν (OH) combinations | Proteins, saccharides |
| 5600–6050 | 1° overtone ν (CH) | Lipids |
| 6700–7150 | 1° overtone ν (NH) ν (OH) | Proteins, saccharides |
| 8000–9100 | 2° overtone ν (CH) | Lipids |
| 9100–10500 | 2° overtone ν (NH) ν (OH) | Proteins, saccharides |
| 11520–11760 | 3° overtone ν (CH) | Lipids |
| 11765–12900 | 3° overtone ν (NH) | Proteins, saccharides |

of biological fluids and tissues [1]. Biological samples are composed mainly of proteins, lipids, sugars and deoxyribonucleic acid (DNA). All those compounds are active in the IR range and any change produced in the composition or the structure can be evaluated by IR measurements. As an example, Table 1 shows the assignment of different bands related to functional groups present in clinical samples in the mid-IR (MIR) range, related with the fundamental transitions of the vibrational modes, and the near-IR (NIR) region, related with the overtones and combination bands. As can be seen, each family of compounds has different regions of influence, and, in addition, each compound has a different spectral signature so that, using multivariate techniques, we can extract information regarding sample composition, structure of the compounds of the sample and molecular interactions. Hence, IR spectra provide a "snapshot" of the metabolic state of the patient, and biospectroscopists can use changes in the sample spectra, directly related with their composition, for the following.

- Establishing differences and patterns between the spectra of control and pathological samples, thus detecting "spectral markers" indicative of the pathology under study [2]. The interpretation of those spectral patterns could offer new insights about the effects of the illness on the sample and, most importantly, classification models based on IR spectra could be used for the diagnosis of several illnesses. For these reasons, the potential of IR as a promising tool in diagnosis was recently reviewed [3].
- Quantifying biological compounds in clinical samples. The quantification of those parameters is almost indispensable in hospitals for diagnosing many diseases or monitoring treatments, and in clinical research for acquiring information about the evolution of biological systems under different conditions. While those determinations are currently performed through well-automated enzymatic methods in hospitals of developed countries, several methodologies based on IR have been proposed as alternative or complementary tools, especially bearing in mind the low cost and easily portable solutions for underdeveloped countries or special conditions [4].

We aim to review the proposed methodologies based on IR spectroscopy, focusing on:

- quantifying clinical parameters in human samples, evaluating why IR has become a promising tool in the determination of clinical parameters;
- the advantages that IR spectroscopy provides compared with enzymatic and other reference methods; and,
- a critical review of the limitations of the methodology.

We report on the different methodologies available in the literature for the analysis of liquid, solid and gaseous clinical samples, showing the versatility of the technique. We focus on recent contributions, based on modern chemometric, optical and sample preprocessing techniques, which aim to overcome the limitations of IR and to anticipate the applicability of this technique in modern medicine.

## 2. Advantages of FTIR measurement compared with current methods

Versatility is one of the main strengths of IR methods, because almost all the clinical compounds are active in the IR range and can therefore be quantifiable. In addition, modern spectrophotometers are versatile instruments able to measure directly solid, liquid or gaseous samples using easily interchangeable accessories, including a wide variety of attenuated total reflectance (ATR) modules, transmission cells for liquids and gases, and flow cells, for macro-samples and micro-samples. As an example, Fig. 1 shows some of the compounds and the parameters that have been quantified on different samples of the body according to the studies included in this review. As can be seen, literature reports the determination of a large number of clinical parameters in a wide variety of human samples. As an example, glucose levels in blood can be determined by direct non-invasive measurements through skin [5,6], or on blood [7,8]. Even if microscopy measurements are required, recent development of a powerful focal-array detector enables the fast acquisition of micro-FT-IR images of tissues and, using this system, Majzner et al. attempted to quantify the different secondary structures of proteins in each pixel of vascular wall images [9].

Point-of-Care (PoC) testing is defined as "the ability to move testing closer to the patient" [10] and has the potential to provide fast results, thus accelerating clinical decisions, implying several benefits in community testing, primarily in the pharmacy, self-monitoring, general practice and the emergency department. IR-based methodologies could play an important role in the development of new PoC testing tools. In contrast to enzymatic methodologies, IR might perform the analysis directly without the need for any reagent or pre-processing of samples, and commonly used bench FT-IR spectrophotometers can be replaced by the latest generation of portable spectrophotometers. In addition, the introduction of quantum-cascade lasers (QCLs) as the IR source is a promising improvement in the PoC field, since it avoids the use of a voluminous interferometer, while increasing the sensitivity and the signal-to-noise ratio. The development of compact, portable instrumentation opens up news possibilities to use IR-based methodologies for PoC testing. Brandstetter et al. [11] have built a prototype that integrates a tunable QCL, flow cell and a semi-automated liquid handling system, so it is easy to use for the determination of biochemical parameters on plasma from critically ill patients from an intensive care unit (ICU) (see sub-section 4.1.1 for additional details). Besides, as an example of how IR can help the current PoC and personalized medicine trends, the analysis of breast milk using human-milk analyzers (HMAs) currently permits accurate, personalized fortification of the breast milk, depending on the nutritional deficiencies and strengths of the sample, thus providing the correct amounts of nutrients to the very low-weight infant (see sub-section 4.3).