Infrared spectra in monitoring biochemical parameters of human blood

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Abstract. Infrared spectroscopy is gaining recognition as a promising method. The infrared spectra of selected regions (2000-4000cm⁻¹) of blood tissue samples are reported. Present study related to the role of spectral peak fitting in the study of human blood and quantitative interpretations of infrared spectra based on chemometrics. The spectral variations are interpreted in terms of the biochemical and pathological processes involved. The mean RNA/DNA ratio of fitted intensities and analytical area as calculated from the transmittance peaks at 1121cm⁻¹/1020cm⁻¹ is found to be 0.911A.U and 2.00A.U. respectively. The ratio of 1659cm⁻¹/1544cm⁻¹ (amide-I/amide-II) bands is found to shed light on the change in the DNA content. The ratio of amide-I/amide-II is almost unity (≈1.054) for blood spectra. The deviation from unity is an indication of DNA absorption from the RBC cells. The total phosphate content has found to be 25.09A.U. The level for glycogen/phosphate ratio (areas under peaks 1030cm⁻¹/1082cm⁻¹) is found to be 0.286A.U. The ratio of unsaturated and saturated carbonyl compounds (C=O) in blood samples is in form of esters and the analytical areas under the spectral peaks at 1740cm⁻¹ and 1731cm⁻¹ for unsaturated esters and saturated esters respectively found to be 0.618A.U.

1. Introduction
The very broad range of mathematical and statistical methods has provided an excellent opportunity for the quantitative analytical descriptions of experimental results and effects in natural sciences. Derived biological markers from IR spectroscopy used in the identification of disease have been extensively investigated [1]. It is strongly believed that in studies related to spectroscopic techniques, both the reliable experimental procedure and detection of spectral peak positions are important. At present, there is no data available in the literature on FT-IR spectra of whole blood, which may be vital in optical study.

In this study, we examined the phosphate, RNA/DNA, carbohydrate and amide-I/amide-II region using a peak fitting program revealing various new spectral peaks in spectra of human blood. The peak fitting procedure consists of modeling an experimental spectrum with a sum of analytical peak functions. The peak fitting is helpful to resolve spectral peaks in spectra which are often overlapped or making direct measurement of their parameters problematic, unreliable or even impossible.
2. Materials and Methods

2.1. Sample preparation for infrared spectroscopy
In the present work, thirty blood samples were collected. The blood was collected on a sterilized glass slide with frosted end. All the blood smears were kept to be dried in clean, dry and airy place at room temperature, so that the moisture content of the smears was removed [2].

2.2. Infrared Spectra
Thick films on the glass slide were removed and smears were grounded with spectroscopic grade KBr in the powder form. FT-IR spectra were recorded using SHIMADZU 8000 series FT-IR spectrophotometer with diffraction technique. 25 scan were performed in the wavenumber region 2000–400 cm\(^{-1}\) for each sample at the resolution 4 cm\(^{-1}\). Spectral analysis was done using IR solution software. The integrated transmittances were calculated by a band fitting procedure reported earlier [3,4].

3. Observations
The spectra illustrate glycogen bands at 1020 cm\(^{-1}\) and symmetric phosphate stretching band at 1082 cm\(^{-1}\). The region 1300–1000 cm\(^{-1}\) belongs to C–O stretching of alcohols, carboxylic acids, ethers and esters but when the sample is biological, the region is dominated by spectral features of phosphodiester [5]. The band at 1369 cm\(^{-1}\) arise due stretching of C–N of cytosine and guanine [6]. The region 1800–1600 cm\(^{-1}\) belongs to finger print region of C–H phenyl ring substitution overtones [7]. Among several vibrational features, the amide-I band at 1659 cm\(^{-1}\) was due primarily to the C = O stretching vibrational bands of the peptide backbone. The amide-II band at 1544 cm\(^{-1}\) arises due to coupling of CN stretching and in-plane bending of the N-H group.

4. Curve fitting
The curve or peak fitting [8] is mostly used for resolving the overlapped peaks and finding more precise peak parameters e.g., position, height, width and area.

4.1. Initial spectral study
4.1.1 Baseline Correction.
Zero baseline correction was used so that the maximum value of transmittance spectrum is 100% without changing the shape of spectrum.

4.1.2 Smoothening. Smoothening is used on noisy spectrum to reduce noise level. We used Savitsky-Golay smoothening to make spectrum noise because there is some noise at the ends of spectrum.

4.2 Peak fitting
The spectra generated by infrared spectrophotometer are processed before using to monitor RNA/DNA and Protein/DNA contents e.g., curve fitting. Curve fitting includes baseline correction, curve smoothening, SNR correction, spectral normalization.

The blood samples used in this work are rich in vibrational modes. There is more than one vibration mode in some peaks due to the overlapping of the transmittance peaks. These peaks appeared as doublets or triplets. We used PeakFit Version 4.12 programme to allow band fitting and to detect and locate the hidden peaks. The PeakFit locates the peak centroids up to third derivative of the spectrum described [9]. The weak transmittance bands are resolved by FT-IR spectrometer.

In our study, the spectra were fitted using standard Gaussian peak shape. The Gaussian curve expression with peak amplitude is given by;

\[
y = a_0 \exp \left[ -\frac{1}{2} \left( \frac{x-a_1}{a_2} \right)^2 \right]
\]

where; \(a_0\) is the Gaussian amplitude, \(a_1\) is position at the centre and \(a_2\) is the FWHM and the Gaussian curve with peak area is expressed as;

\[
y = \frac{A}{\sqrt{2\pi}a_2} \exp \left[ -\frac{1}{2} \left( \frac{x-a_1}{a_2} \right)^2 \right]
\]
where; $A$ is the area of Gaussian peak.

The procedure mathematically enhances the resolution of the spectrum, using an iterative statistical curve fitting of symmetric Gaussian peaks to the straight, baseline corrected spectra. The measured IR spectra of thirty blood samples of groups belongs to A, B, AB and O. The statistical spectral peak fitting of blood groups is shown in Figure 2(a-d).

5. Results and Discussions

5.1 Hidden Peaks
In the entire blood spectrum, an important peak at 1690 cm$^{-1}$ was hidden due to noise and was not seen, but after processing and peak fitting it clearly observed and appeared as a peak in all the spectra of blood.

The peaks at wavelengths; 1167 cm$^{-1}$, 1244 cm$^{-1}$, 1690 cm$^{-1}$ and 1731 cm$^{-1}$ were obtained after peak fitting of blood spectra. The peak at 1167 cm$^{-1}$ arises due to C–O stretching of blood samples [10,11]. Peak at 1244 cm$^{-1}$ arise due to asymmetric $PO_2$ stretching [12]. The peaks at 1690 cm$^{-1}$ and 1731 cm$^{-1}$ arise due to the base carbonyl stretching and ring breathing mode of nucleic acids [13] and fatty acid ester band [14] respectively.

5.2 Biochemical parameters
The analytical area calculated by peak fitting for different peaks have been used to define various biochemical parameters described below;

5.2.1 Total Phosphate Content. The summed phosphate level is obtained under the peaks at 1244 cm$^{-1}$ and 1082 cm$^{-1}$. The average phosphate content under the peaks 1244cm$^{-1}$ and 1082cm$^{-1}$ was obtained 17.669A.U. and 7.426A.U. respectively. The average summed phosphate level for thirty blood samples was found to be 25.096AU for normal blood.

5.2.2 RNA/DNA Level. The RNA/DNA ratio is one of the biological markers [15]. It is the measured ratio of areas under spectral peaks at 1121cm$^{-1}$ and 1020cm$^{-1}$. The individual average of 1121cm$^{-1}$ is
3.939 A.U. and is 1.968 for 1020 cm\(^{-1}\). In the present study the average of RNA/DNA level of thirty blood spectra was 2.001 A.U.

5.2.3 Glycogen/Phosphate Ratio. The spectral assignments show that the region 1350–900 cm\(^{-1}\) belongs to collagen and glycogen [16]. The average glycogen and phosphate content under the peaks 1030 cm\(^{-1}\) and 1082 cm\(^{-1}\) was obtained 2.128 A.U. and 7.426 A.U. respectively. The average ratio of areas under the peaks 1030 cm\(^{-1}\)/1082 cm\(^{-1}\) was found to be 0.286 A.U.

5.2.4 Carbohydrates Level. The ratio of 1045 cm\(^{-1}\)/1544 cm\(^{-1}\) gives an estimate of carbohydrate concentration and is lower in malignant biological samples. We have also taken this ratio as a variable which could be used to discriminate normal and malignant bloods. The average carbohydrate level was obtained 0.114 A.U.

5.2.5 Amide I/II Ratio. The ratio shed light on DNA contents [17]. The contribution of the DNA due to carbonyl group from the bases is quantified by the ratio of integrated transmittance of amide I/II. The contribution of the DNA due to carbonyl group from the bases is quantified by the ratio of amide I/II. It is the measured ratio of areas under spectral peaks at 1659 cm\(^{-1}\) and 1544 cm\(^{-1}\). The individual average of 1659 cm\(^{-1}\) is 35.524 A.U. and is 33.678 A.U. for 1544 cm\(^{-1}\). The average of amide-I/amide-II was found to be 1.054 A.U.

The relative levels of biochemical parameters for normal blood have been shown in Figure 3.

6. Conclusion

This technique enables the study of the state of chemical bonds and the relative concentrations of lipids, proteins, carbohydrates, and phosphorylated compounds. Many peaks like; 1167 cm\(^{-1}\), 1244 cm\(^{-1}\), 1690 cm\(^{-1}\) and 1731 cm\(^{-1}\) which were hidden due to noise are easily seen after spectral processing and peak fitting, it shows the potential role of peak fitting and IR spectral study.

These biochemical parameters derived from IR spectroscopy could be used in identification of different blood related diseases in future.

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