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# Diagnosis of renal failure by infrared spectrometric analysis of human serum samples and soft independent modeling of class analogy

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ABSTRACT

Attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy has been used in this research for reagent-free discrimination of serum samples obtained from healthy people and those with renal failure. No sample preparation step e.g. drying or pre-concentration is required prior to spectral analysis. Classification was performed based on the spectral variations in patient samples. In the experimental step, 75 blood serum samples, including 40 normal and 35 renal failure cases, were analyzed in 1800–900 $cm^{-1}$ spectral region. Unsupervised pattern recognition of the serum samples using cluster analysis (CA) and principal component analysis (PCA), did not demonstrate any useful capability of these techniques for discrimination aims. Supervised pattern recognition using soft independent modeling of class analogy (SIMCA) was performed. Results showed 95.12% of accuracy in ATR-FTIR diagnostic results being compared with the current clinical methods. The sensitivity and specificity of the proposed method are 100% and 91.3%, respectively.



## 1. Introduction

Clinical diagnostic techniques such as determination of urea and creatinine in serum samples enable physicians to identify probable kidney failure. The kidneys regulate the amount of water and salts in the body by filtering the blood through millions of structures called nephrons. The kidneys also pass out certain waste products from the body. The usual blood test which checks if the kidneys are working properly is to measure the level of urea, creatinine, and certain dissolved salts. A high blood level of urea (uremia) indicates that the kidneys function not properly, or the patient is dehydrated (low water content in body). On the other hand, high blood level of creatinine is a very important symptom in diagnostic tests. It is also noticeable that abnormal level of any electrolyte e.g. sodium, potassium, chloride and bicarbonate dissolved slats in the blood may be due to a kidney problem. Chronic renal failure (CRF) is a late phase clinical syndrome of all kinds of chronic kidney diseases. The main manifestations are the decrease of renal function, i.e. accumulation of metabolites and poisoning substances and the unbalance of water, electrolytes and acid–base equilibrium and some abnormalities of endocrine function. The pathologic factors of CRF include metabolic acidosis, accumulation of end products of protein metabolism, malnutrition, disorder of hormone and uremia toxins that have not been identified up to now. During the last decade, there have been several reports indicating the diagnostic methods of renal failure, the main focusing on quantitative determination of biochemicals in blood samples. Nowadays, analytical methods are developed to help the physician in rapid, accurate and reliable diagnosis of diseases. Infrared spectroscopy has been used by chemists as a powerful tool to characterize inorganic and organic compounds [1]. It has been also applied in biology for studying the structure and conformation of molecules like proteins, nucleic acids and lipids [2,3]. Mid-infrared spectroscopy plays an increasing role in biomedical research [4]. Bio-fluids, such as blood [5], serum [6–9], plasma, urine [10,11], and synovial fluid [12], are ideal candidates for biomedical diagnostics, as most bio-fluids contain rich metabolic information which is associated with different diseases and stages of disease progression. It has been shown that the interpretation of mid-infrared spectra of serum in terms of particular diseases allows for the identification of disease-specific signatures e.g. for diabetes mellitus [13] the metabolic syndrome [14] or rheumatoid arthritis, bovine spongiform encephalopathy (BSE) [15,16] and cancer [17,18]. Fourier transform infrared spectroscopy has been used to the identification of urinary stones and kidney crystal deposits [19]. Based on all of these findings, it seems that analysis of blood and blood components e.g. serum is an easy, rapid and excellent target for detection and identification of various diseases by FTIR spectroscopy. Precise quantification of serum ingredients, such as glucose, total protein, cholesterol and urea has been achieved using mid-IR spectroscopy [12,20]. It is also possible to predict creatinine concentration in whole blood if the concentration exceeds 4 mg $dL^{-1}$ [21]. The diagnosis of diseases via spectral analysis of body fluid consists of some data processing steps.

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The chemometric techniques e.g. multivariate data analysis are the main approach, applied while a research is performed to combine the statistical skills with chemical ones and obtain reliable results. Chemometric efforts appeared to be very useful in solving many analytical problems and are also helpful for classification for identification of illness pattern via pattern recognition. Pattern recognition consists of two general areas; supervised or unsupervised. In unsupervised pattern recognition, information on the individual groupings is likely to be unknown, but is not a pre-requisite. In supervised pattern recognition, however, the groupings of samples must be known to allow predictions to take place [22]. Examples of unsupervised pattern recognition are hierarchical cluster analysis (HCA) and principal component analysis (PCA). Soft independent modeling of class analogy (SIMCA) is one of those methods which are used for supervised pattern recognition. The method developed is based on the pattern recognition decision tree in Chemometrics: A Practical Guide [23]. In the present study, we examined the ATR-FTIR spectra of serum samples obtained from normal people and those patients suffering from acute and chronic renal failure trying to detect the specific biomarker spectral features for discrimination between healthy and patient, utilizing chemometrics.

## 2. Experimental

### 2.1. Materials and methods

About 0.5 ml of serum from venous blood was placed in a compartment contact with a sampler horizontal zinc-selenide ATR cell from SpectraTech. Measurements on blood serum samples were performed using the Magna 550 FTIR spectrometer (Nicolet, Madison, WI, USA) equipped with a DTGS detector, an Ever-Glo source and a CsI beam splitter. The used resolution was 4 $cm^{-1}$ and the spectra were recorded by 36 number of scans. Omnic software was used to control the instrument for data acquisition. The main problem in this region is the absorbance of water which consists about 90% of blood. To avoid this problem, we used water as background spectrum. Chemometrics approaches i.e. PCA, CA, SIMCA and all other preprocessing methods were accomplished using Matlab Ver. 7.4 (The MathWorks Inc., MA, USA).

### 2.2. Spectral data obtained by ATR-FTIR spectrometry

The serum samples obtained from healthy and patient persons were examined by FTIR trying to find specific spectroscopic features related to capability of the proposed method for diagnosis of renal failure. Developing specific signal markers for the detection and identification of human disease by FTIR could be of high importance for future rapid and reliable diagnosis of disease. Quick and reliable identification of the disease might be critical, in many cases, for effective treatment. In order to compare the absorbance signals in the FTIR spectra of the serum samples, the baselines of the spectra were initially corrected and normalized by intensity at 2000 $cm^{-1}$.

Fig. 1 shows a typical spectrum of a healthy and a patient sample. The presented spectra are average of 35 patient samples and 40 healthy person samples. Overall, FTIR spectral differences can be used to discriminate healthy and patient classes, although the biological origin of the spectral differences is addressed to the variation in kidney related biochemicals. These spectral differences can provide information that is useful for discrimination aims especially if a multivariate chemometric is also used. It is also mentionable that considering some of spectral differences alone would not be a useful idea for diagnostic classification. Thus according to the signal assignments which are discussed later, it was decided to perform all the chemometric approaches for the total spectral region.

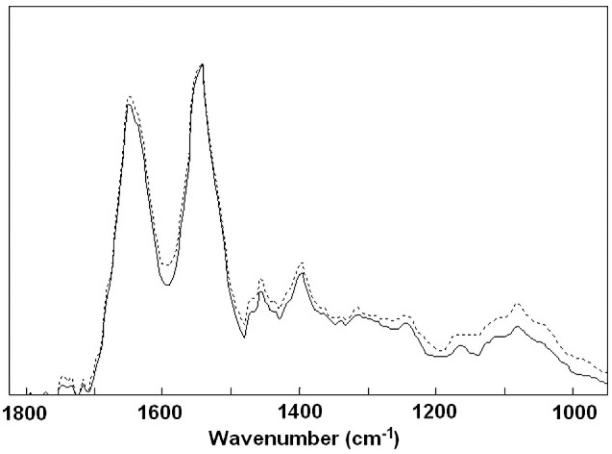


**Fig. 1.** Typical ATR-FTIR spectra of serum sample from a healthy person (solid line) and a renal failure patient (dashed line) in the 1000–1800 $cm^{-1}$ spectral region.

## 3. Results and discussion

### 3.1. Informative assignment of IR spectra

Assigning the IR signals in a sample spectrum, there are several informative spectral features in the investigated spectral region correlated to different biomolecules. Some of the most important assigned bands in this spectral region (1000–1800 $cm^{-1}$) are detailed in below:

1- C═O stretching of lipids at 1739–1732
2- C═O stretching of (amide I) due to proteins at 1720–1600 (α-helix ~1650, β-sheet ~1680 and ~1630, or other proteins)
3- $NH_2$ bending of amino acids at 1630–1560
4- N – H bending of polypeptide bond structures 1600–1480
5- $CH_2$ scissoring, CH deformation and $CH_3$ asymmetric bending due to fatty acids, phospholipids and triglycerides at 1480–1430
6- $COO^-$ symmetric stretching of amino acids at 1430–1360
7- C – O stretching of saccharides at 1300–1000

As mentioned previously, urea and creatinine are the main biochemical targets to be monitored as the investigation of serum samples is conducted by infrared spectrometry. In other words, these are the main 'analytes'. There are some signals due to the functional groups which exist in these analytes. The main probable bands related to the functional groups of urea and creatinine are detailed in Table 1. Clear spectral differences are visually observed between the spectra of healthy person and renal failure patient. The main are at ~1470, ~1370 and ~1320 $cm^{-1}$. Thus it is possible to correlate the spectral differences with the increased concentration of urea and creatinine in serum samples during the renal failure progress. As seen in Table 1, there are about 10 infrared bands due to urea and creatinine while some of them are due to same functional groups in different analytes. According to this consistency, it is difficult to evaluate the effect of single signals in foresaid spectral region as the markers for detection of healthy or patient situation. The complex structure of spectra suggests considering the total region for better diagnosis. In the next step analysis of variance (ANOVA) was performed on spectral data as a widespread approach which is based on simple ideas, comparing the two mean errors. It was proved to be useful to apply classifier chemometric techniques for diagnostic analysis of different classes in chemical researches. Fig. 2 shows box plot archived from ANOVA of two classes of healthy and patient spectra that illustrates the infrared spectrometry as a capable method for diagnosis of renal failure. Totally 75 serum samples, consisting of 40 healthy and 35 patient cases were analyzed.