Asian Journal of Chemistry Vol. 22, No. 2 (2010), 1118-1124

FTIR Spectroscopic Analysis on Air Dried Serum Films of Diseased Kidney and Healthy Subjects

S. MUTHU*, A. BHASKARAN, N.R. SHEELA and S. SAMBATHKRISHNAN
Department of Physics, Sri Venkateswara College of Engineering, Sriperumbudur-602 105, India
E-mail: muthu@svce.ac.in

In this paper, the FTIR spectra of serum samples from kidney failure and normal subjects have been recorded over the region 4000-400 cm^{-1}. This method involves drying the serum specimen to a film and comparing the absorption level to characterize the diseased kidney from healthy subject. To substantiate the findings univariate statistical analysis is also made. To quantify the spectral differences intensity ratio parameters are introduced. It is shown that the diseased kidney serum is classified with greater sensitivity and normal with higher specificity.

Key Words: Blood, Kidney, FTIR spectrum, Univariate statistical analysis.

INTRODUCTION

The kidneys serve many functions in the body. In addition to filtering the waste from the blood, the kidneys regulate the body's level of chemicals such as sodium and potassium. Kidney damage can happen quickly, but most often develops slowly and silently, often taking years or decades for the injury to become apparent. Recognition of kidney disease as a major national health problem by 2010[1]. There is a wide fluctuation in the number of various formed elements in the blood of a normal human being and a patient, most of which are determined in a clinical laboratory. Spectrophotometry is an effective alternate method and has several advantages over clinical methods like less sample contact and higher information content.

An extensive work has been carried out by several investigators on blood samples in recent years[2-7]. Masilamani *et al.*[2] have carried out the fluorescence spectroscopy as a clinical tool to diagnosis of cancer from blood. Sivagurunathan and Dhinakaran[3] studied variation of protein and lipid content in the cardiovascular diseased blood serum in comparison with that of the healthy human blood serum. Werner *et al.*[4] and Jantsch *et al.*[5] have carried out the mid infrared spectroscopy as a clinical tool to estimate glucose, protein and urea in blood serum. Gunasekaran *et al.*[6] have carried out FTIR spectroscopy analysis on diabetic and healthy human blood sera. Low-Ying *et al.*[7] have carried out the quantization of glucose and urea in whole blood by mid-infrared spectroscopy of dry films. The present work is an attempt to study the variations in the composition of the blood serum in healthy and kidney failure through infrared spectroscopic technique.

EXPERIMENTAL

Blood samples were collected from healthy subjects and kidney diseased patients from Nephrology Department of Government Hospital, Royapettah, Chennai, India. Patients were of both sexes (age between 40 and 50 years). All kidney diseased patients on whom this study is based were under going treatment.

Infrared spectra of the serum samples are recorded in the region 4000-450 cm^{-1} on a Perkin-Elmer spectrum-one FTIR spectrometer equipped with an air cooled DTGs (Deuterated triglycine sulphate) detector at Medopharm, Chennai, India. Infrared transparent KBr material without the sample was scanned as background for each spectrum and 16 scans were co-added at a spectral resolution of 2 cm^{-1}. The collected signal was transferred to the PC. The data were processed by windows based data program-spectrum software and PE Grams Analyst 1000. The spectra were base line corrected and they were normalized to acquire identical area under the curves and the maximum absorbance values of the corresponding characteristic bands were noted.

RESULTS AND DISCUSSION

Assignment of infrared absorption bands of human serum: The absorption infrared spectra of healthy and kidney diseased blood serum samples are shown in Fig. 1 and tentative assignments are given in Table-1. The spectra exhibit only the bands expected from lipids, proteins, glucose, urea and creatinine of the serum samples. Shaw *et al.*[8] have identified the presence of protein, albumin, triglycerides, glucose, urea, creatinine and uric acid in the human blood serum samples. Petibois *et al.*[9] have identified the absorption bands for glucose, explicated with FTIR spectra. They observed the bands due to O-H stretching between 3570 and 3120 cm^{-1}, C-H

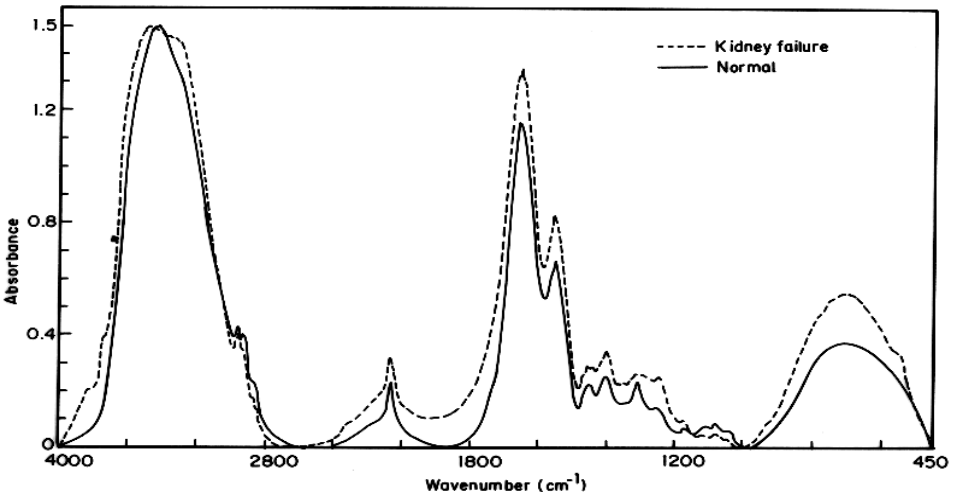

Fig. 1. Absorption infrared spectra of healthy and kidney diseased blood serum samples

TABLE-1
INFRARED BAND ASSIGNMENT OF HUMAN SERUM

Frequency (cm^{-1})	Assignments
700 s	N-H out of plane deformation protein
1084 m	C-O stretching of β anomer (major glucose band)
1126 m	C-O stretch of α anomer glucose
1248 m	PO_2 stretching of phospho lipids
1369 m	CH_2 stretching vibration of β anomer glucose
1401 s	Symmetric CH_3 deformation of protein
1407 m	COO^- stretching of ionized amino acid chains of protein
1460 m	Asymmetric CH_3 deformation of protein
1545 s	C=O stretching and N-H deformation of protein/ urea/creatinine.
1656 s	C=O stretching of creatinine/urea/protein
2869 m	C-H stretching of lipid/protein
2959 m	C-H stretching of lipid/protein
3468 vs	N-H stretching of urea/protein/creatinine
3490 s	N-H stretching of urea/protein/creatinine

stretching between 3085 and 3020 cm^{-1}, the C-O stretching between 1230 and 1000 cm^{-1} and C-O-C deformation between 1275 and 800 cm^{-1}, out of which the C-O region is known to be the most explicit spectral profile, in the intricate spectra of this complex molecule. The specific assignment of bands has been achieved by interpreting the previously well-established IR spectra of blood and serum. For glucose the optimal frequency range of 1250-925 cm^{-1} is used, since the mid IR spectrum of glucose includes several strong absorption bands in this region. In the present work the bands observed at 1369, 1126, 1084 cm^{-1} are assigned to glucose. The bands observed at 2959, 2869, 1248 are assigned to lipids[10]. Because PO_2 and C-H vibrations are very important vibrations in lipid structure.

The bands observed at 1401, 1460 cm^{-1} is due to symmetric and asymmetric bending vibrational modes of CH_3 groups of proteins, respectively. The band observed at 1407 cm^{-1} is assigned to COO^- stretching of ionized amino acid chains of protein. The covalent structure of a protein is essentially linear. Amino acids are connected together to form a chain, the connection being a peptide bond. The peptide bond is simply an amide bond between the carbonyl carbon of one amino acid and the amino nitrogen of another[11]. So the bands observed at spectra of serum samples at 1545, 1656 are due to amide II (N-H bending) and amide I (C=O stretching) vibrations, respectively. The N-H stretching vibrations are identified at 3490 and 3468 cm^{-1}.

Creatinine, urea and uric acid are almost observed[12] in the same regions 1800-1400, 3500-2800 and 1600-800 cm^{-1}. In the present work, the bands observed at 1656, 1545 cm^{-1} are assigned to C=O stretching and the bands observed at 3490, 3468 cm^{-1} are assigned to N-H stretching of creatinine and urea present in the serum samples.

Univariate statistical method: Generally, spectral investigations are made using different univariate methods involving ratios of peak intensities, shift in band position and area under the curve[13,14]. According to Beer-Lambert's law, the absorbance of a constituent is directly proportional to its concentration.

Intensity ratio of important peaks: Kidney serves many functions in the body. If kidney is damaged it loses its filtering capacity of blood. So the end products of metabolism are increased in blood and blood serum. The urea, creatinine, lipid and carbohydrates values are increased in the diseased kidney blood compared to healthy blood. The IR spectrum of serum can provide qualitative and quantitative information on such molecules. Considerable spectral differences have been observed between the normal and diseased serum in this study. Based on these differences in the spectral signatures, three intensity ratio parameters were introduced. They were, $R_1 = I_{(1084)}/I_{(1369)}$ in the glucose region, $R_2 = I_{(2959)}/I_{(2869)}$ in the lipid region and $R_3 = I_{(1545)}/I_{(1656)}$ in the urea and creatinine spectral zone.

Region-1 (glucose region): Diabetes is now one of the leading causes of kidney failure. Diabetes that is not well controlled or has been long-term can lead to blood vessels becoming much smaller. As blood vessels decrease in size, they do not allow adequate amount of blood to pass through the kidneys. A decrease in this blood flow can lead to kidney failure. In the present work, the bands observed at 1369, 1126 and 1084 cm^{-1} is assigned to glucose. The intensity of these bands varies considerably indicating glucose profiles get altered in kidney failure serum compared to normal serum. Intensity ratio parameter $R_1 = I_{(1084)}/I_{(1369)}$ is introduced in this spectral window. The mean value between them is 0.91 and it was used as decisive factor to differentiate the kidney failure from the normal samples in the glucose region. It was quiet apparent from the scatter plot (Fig. 2), that the critical ratio value of 0.91 separated the kidney disease from normal with specificity, sensitivity and overall accuracy of 100, 100 and 100 respectively.

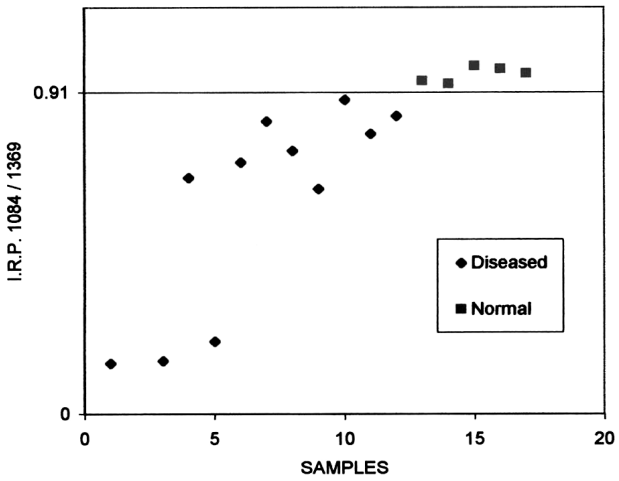

Fig. 2. Scatter plot in the glucose region

Region-2 (lipid region): Lipid metabolism is abnormal in renal failure[15]. Hypertriglyceridemia with a type IV lipoprotein pattern occurs in up to 75 % of patient with renal insufficiency. There are several different classes of lipids but all of them derive their distinct properties due to the hydrocarbon nature of their structure. Hence in the present work, the bands observed at 2956 and 2869 cm^{-1} due to C-H stretching vibrations are assigned to lipids. The normalized and extended spectra of kidney failure and normal serum in the lipid sector. To confirm this report the intensity ratio parameter $R_2 = I_{(2959)}/I_{(2869)}$ was introduced in this spectral zone. The mean value between them is 1.2 and it is used as a criterion to separate kidney failure from normal samples. It was also perceived that from the scatter plot (Fig. 3) the critical ratio 1.2 classifies the kidney failure serum with 91 % sensitivity and normal with 100 % specificity and overall accuracy 96 %. This clearly shows that the lipid metabolism gets altered in the kidney dysfunction.

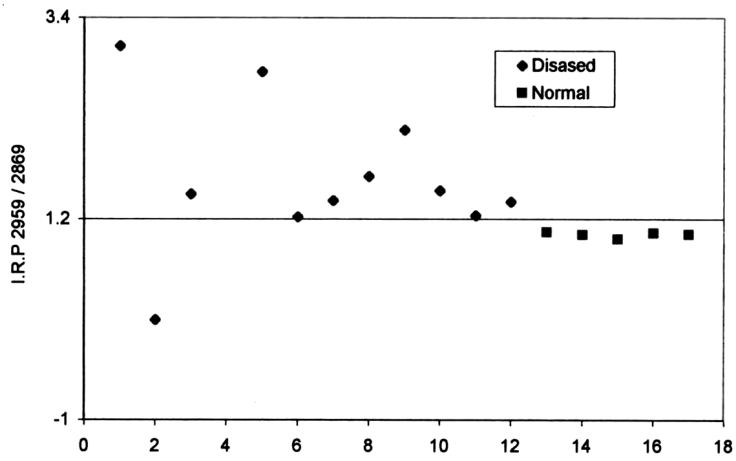

Fig. 3. Scatter plot in the lipid region

Region-3 (urea and creatinine region): A measure of the amount of urea $CO(NH_2)_2$ (waste product) in the blood is used to diagnose the kidney function. Normal blood contains 7 to 20 mg of urea nitrogen per deciliter of blood. If the blood urea nitrogen is more than 20 mg/dl, the kidney may not be working at full strength.

Creatinine ($C_4H_7N_3O$) is an important nitrogenous constituent of urine. It is a waste product from meat protein in the diet and from the muscles of the body. Creatinine is removed from blood by the kidneys, as kidney disease progresses, the level of creatinine in the blood increases. Generally creatinine and urea change in parallel.

In the present work, the bands observed at 1656, 1545 cm^{-1} due to C=O vibrations and at 3490, 3468 cm^{-1} due to N-H stretching vibrations are assigned for blood

serum creatinine and urea[16]. The absorptions of these bands vary considerably indicating blood urea and creatinine gets altered in kidney failure serum compared to healthy serum. To confirm this report the intensity ratio parameter $R_3 = I_{(1548)}/I_{(1653)}$ is used in this spectral zone. The mean value between them is 0.85 and it is used as decisive factor to differentiate the kidney failure from the normal samples in the creatinine and urea region. It is quiet apparent from the scatter plot (Fig. 4) that the critical value 0.85 separated the diseased kidney serum from normal with specificity and sensitivity and overall accuracy of 100, 91 and 96 %, respectively.

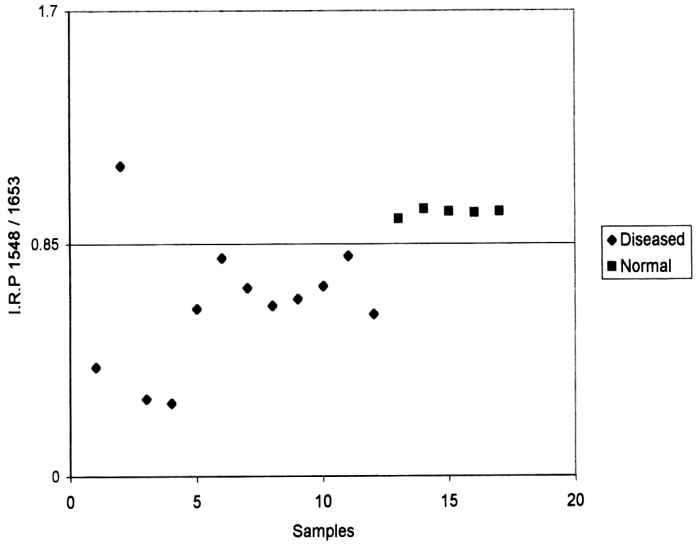

Fig. 4. Scatter plot in the urea and creatinine region

Intensity ratio parameters for the three regions R_1, R_2 and R_3 with their average, standard deviation, sensitivity, specificity and overall accuracy are listed in Table-2.

TABLE-2
RESULTS OF INTENSITY RATIO PARAMETERS IN THE THREE REGIONS OF HEALTHY AND KIDNEY FAILURE SERUM SAMPLES

Region	Intensity ratio parameter	Average ± SD	Sensitivity (%)	Specificity (%)	Overall accuracy (%)
Glucose region	$I_{(1084)}/I_{(1369)}$				
	Normal	0.9595 ± 0.0192	100	100	100
	Kidney Failure	0.6011 ± 0.2762			
Lipid	$I_{(2959)}/I_{(2869)}$				
	Normal	1.0253 ± 0.0253	91	100	96
	Kidney failure	1.9604 ± 0.7991			
Urea and creatinine	$I_{(1548)}/I_{(1653)}$				
	Normal	0.9619 ± 0.0118	91	100	96
	Kidney failure	0.6303 ± 0.2284			

Conclusion

Vibrational spectroscopy is nowadays frequently employed for applications in clinical chemistry or biotechnology, with the major part certainly related to the medical sciences. Since blood serves as the primary metabolic transport system in the body, its composition is the preferred indicator with respect to the health status of the patient. In this study it has been demonstrated that the study of IR spectra of dried serum films on KBr pellet may be used to differentiate between healthy and kidney failure patients. Some remarkable differences are elucidated in terms of spectral profiles, absorption bands, wave numbers and the intensity ratio parameters. Further this is also confirmed by a clear discrimination between healthy and a kidney failure serum samples with the scatter plots drawn using the three important spectral regions namely glucose, lipid and urea and creatinine.

REFERENCES

1. National Kidney Disease Education Program, Department of Health and Human Services, USA (2004).
2. V. Masilamani, N. Sivakumar and K. Vijay Anand, *Asian J. Phys.*, **12**, 125 (2003).
3. P. Sivagurunathan and B. Dhinakaran, *Asian J. Phys.*, **12**, 47 (2003).
4. G.H. Warner, J. Fruch, F. Keller, H. Gerger and B. Dolenko, *Proc. SPIC*, **35**, 3257 (1998).
5. G. Janatsch, Kruse-Jarres, J.D. Marbash and H.M.R. Heise, *Anal. Chem.*, **61**, 2016 (1989).
6. S. Gunasekaran, R.K. Natarajan, V. Renugadevi and R. Rathika, *Asian J. Chem.*, **20**, 2521 (2008).
7. Sarah Low-Ying, R.A. Shaw, M. Leroux and H.H. Mantsch, *Vibrational Spectrosc.*, **28**, 116 (2006).
8. R.A. Shaw, S. Kotowich, M. Leroux and H.H. Mantsh, *Ann. Clin. Biochem.*, **35**, 624 (1998).
9. C. Petibois, V. Rigalleau, A.M. Melin, A. Perromat, G. Cazorla, H. Gin and G. Deleris, *Clin. Chem.*, **45**, 1530 (1999).
10. K.-Z. Liu, R. Bose and H.H. Mantsch, *Vibrational Spectrosc.*, **46**, 959 (1992).
11. E.E. Conn, P.K. Stumpf, G. Bruening and R.H. Doi, Outlines of Biochemistry, John Wiley and Sons, New York (2001).
12. S. Low-Ying, R.A. Shaw, M. Leroux and H.H. Mantsch, *Vibrational Spectrosc.*, **28**, 111 (2002).
13. P. Bhandare, Y. Mendelson and E.P. Stohr, *Vibrational Spectrosc.*, **6**, 363 (1994).
14. N. Vengadesan, D. Koteeswaran, P. Aruna, P. Muthuvel and G. Ganesan, *SPIE Proc. Series*, **3918**, 78 (2000).
15. S.K. Sawhney and R. Singh, Introductory Practical Biochemistry, Narosa Publishing House, New Delhi, India (2000).
16. R.A. Shaw, S.L. Ying, M. Leroux and H.H. Mantsch, *Clin. Chem.*, **46**, 1493 (2000).

(*Received*: 14 January 2009; *Accepted*: 14 October 2009) AJC-7957