Characterization of biological tissues using Fourier transform near infrared spectroscopy

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Introduction

Near infrared (NIR) spectroscopy has been gaining momentum in the last decade. Most of the earlier work has been done in the agricultural field[1] and recently has been frequently applied in pharmaceutical industry.[2] Lodder *et al*. have used NIR analysis of intact tablets for routine quality control applications and to detect product tampering.[3] Near infrared reflectance in the wavenumber region of 13,000–9000 cm^{-1} has been used as a tool for the estimation of body composition in humans.[4] The most attractive feature of NIR spectroscopy is that it doesn't require any sample preparation and is completely harmless and non-invasive. NIR spectra can be taken directly from a storage bottle or the human body. The NIR region spans the range of 14,000–3300 cm^{-1} of the electromagnetic spectrum. The absorption bands that appear in the NIR region arise from C–H, N–H and O–H groups.

Commercial availability of fiber-optic probes have simplified the non-invasive measurements of spectra in the NIR region. In this paper, the use of Nicolet's Sab-IR probe for the study of biological tissues is discussed. Changes in the skin due to exposure to sun light and other enviromental factors are reported. The resultant spectra are compared with that of fat, protein and water to estimate an approximate percentage of contribution of the above mentioned constituents.

Experimental

The spectrometer used for this experiment was a Nicolet Magna 850 FT-IR spectrometer equipped with a near-IR fiber optic probe. The fiber-optic probe consists of a focusing lens with SMA connectors on a base plate. The detector used is a thermoelectrically cooled lead sulfide (PbS) mounted on the base plate. The optical fibers are one meter long bifurcated NIR fiber which transmit from 11,000 to 4100 cm^{-1}, with a special reflectance probe tip. SabIR is provided with a special sampling station having a background position containing a spectralon sample and a position with polystyrene foam sample for validating the system.

A sensitive, yet simple, sampling technique was developed to obtain reproducible results. Several techniques were explored as possible options. The direct placement of the probe on the skin and the use of a sampling station were the most promising. However, inconsistent data were

obtained when directly placing the probe on the skin. This was probably due to fluctuations in the amount of pressure being applied on skin by the probe. This problem was overcome by the use of the sampling station. The sampling station has adjustable positions for the probe to be placed. The probe was adjusted to a distance of 5/64" from the sample, where the most reproducible spectra were obtained.

Before each experiment a background spectrum was taken from the spectralon sample. All experiments were performed with the SabIR sampling station that has a place where the probe can be mounted and a sample position above the probe. In all of our experiments, the probe was mounted 5/64" below the top surface of the sampling station. This allowed our samples not to touch the sapphire window at the tip of the probe. Fatty acid and protein samples were from the Sigma Chemical Company. The spectra were collected from different subjects on positions between the wrist and the elbow. The spectra from protein and fatty acid were taken with their storage bottles.

Results and discussion

Figure 1 shows NIR spectra taken from skin, water, fat and protein. From the figure it can be inferred that the main spectral features of skin are from water, fat and protein with water being the main contributor. Figure 2 shows the spectra taken from one of the subject's skin before and after exposing the skin to sun over one week. There is a significant increase in absorbance at 8400 cm^{-1}, 6900 cm^{-1} and 5500 cm^{-1}. This is in contrast with what we expected to see. The thought was that exposing the skin to sun over a week might lead to a loss of water and there would be less absorbance. The increase in absorbance might be due to the secretion of some kind of liquid by skin to protect itself from sun. Different combinations of water, fat and protein spectra were produced to achieve the best match with the spectra of skin in Figure 1 and details are given in Table 1. The best match seemed to be 49% water, 38% protein and 13% fat for the spectrum of skin after exposing to sun and 60% water, 30% protein and 10% fat for the spectrum of skin before exposing to sun.

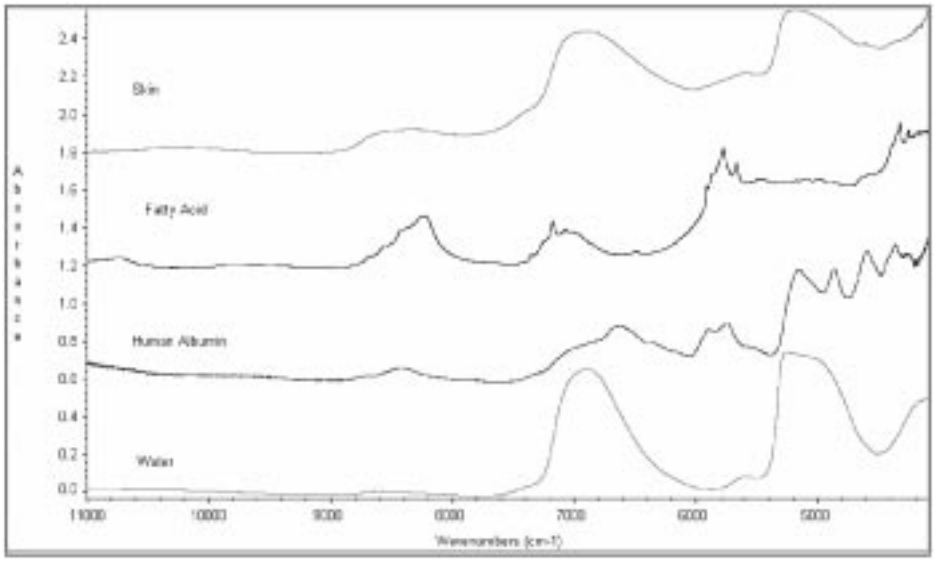

Figure 1. NIR spectra of normal skin, fatty acid, human albumin and water.

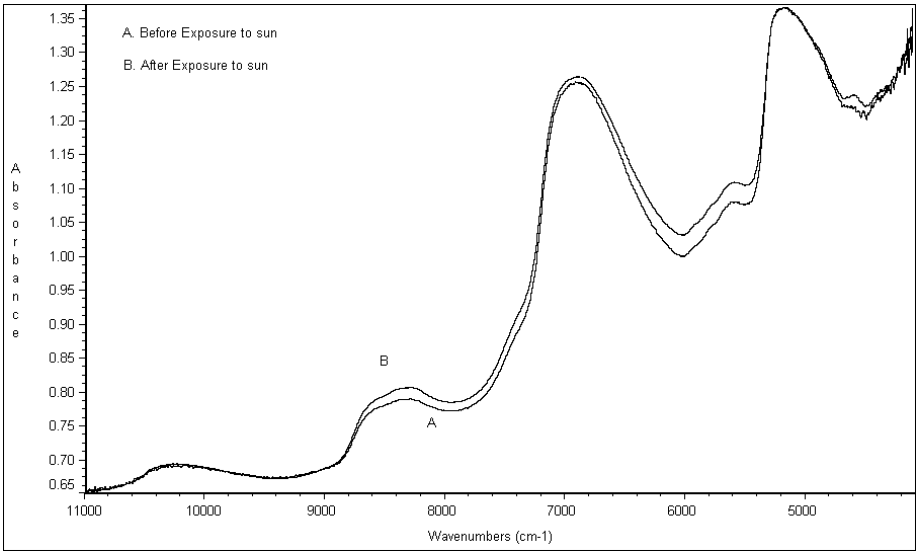

Figure 2. NIR spectra taken before and after exposing the skin to sun.

Table 1.

Combination of water, protein and fat	% Match	
	Before vacation	After vacation
50 + 30 + 20	82.09	86.45
50 + 40 + 10	82.43	86.68
40 + 30 + 30	79.83	84.74
45 + 38 + 17	82.04	86.60
46 + 38 + 16	82.15	86.66
47 + 38 + 15	82.25	86.69
48 + 38 + 12	82.32	86.71
49 + 38 + 13	82.38	86.71
50 + 37 + 13	82.41	86.70
50 + 38 + 12	82.43	86.70
50 + 39 + 11	82.43	86.70
51 + 39 + 10	82.46	86.66
60 + 30 + 10	82.47	86.35

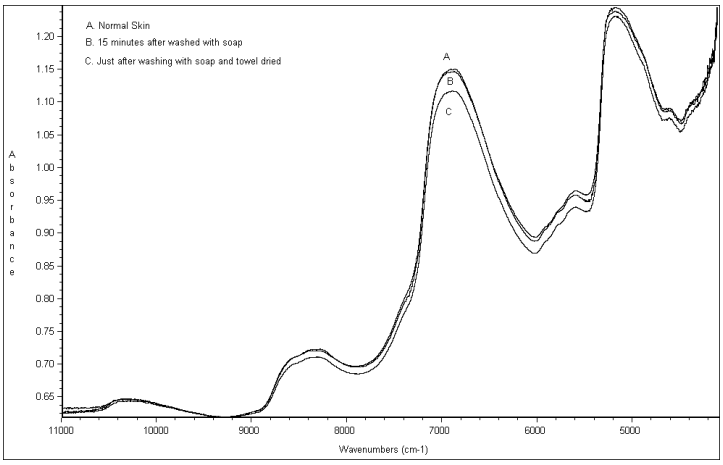

Figure 3. NIR spectra of skin before and after washing with soap.

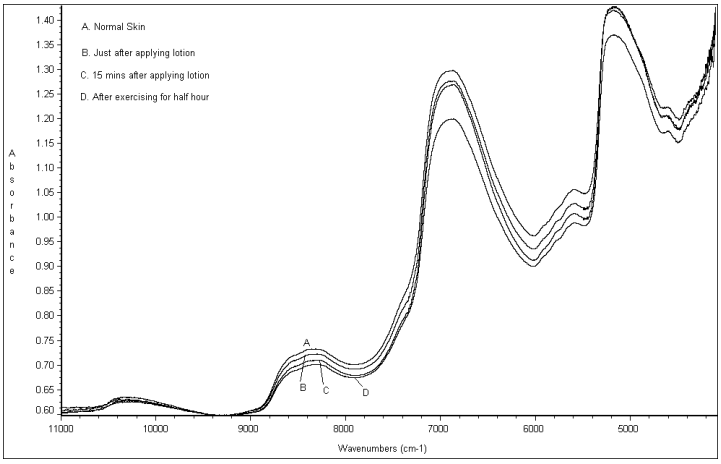

Figure 4. NIR spectra after applying lotion and after exercise.

Another set of experiments were performed on another subject in the lab by washing the skin with soap and towel drying. The resulting spectra are shown in Figure 3. Just after washing the skin there is a noticeable difference in the absorbance. This may be due to the fact that skin becomes dry after washing with soap. Fifteen minutes after washing, the spectra of skin is almost the same as that of normal skin. An obvious explanation is that skin secretes some natural moisturizers to compensate for the dryness. Other experiments conducted on the same subject, include applying moisturizing lotion on the skin and exercising for half an hour outside the lab [walking in the streets for half an hour on a fairly hot day (95°)]. A compilation of the corresponding NIR spectra are given in Figure 4. There is some change in absorbance after applying the moisturizing lotion and there is a marked difference in absorbance for the spectrum taken after exercise. This drop in absorbance might be caused by the changes occurring in the lipids by the application of the moisturizer and exercise. The percentage composition of water, protein and fat and its match with different NIR spectra for the above mentioned experiments are

Table 2.

Combination of water, protein and fat	% Match					
	Normal	Just after washing	1/2 hr. after washing	After exercies	Just after applying lotion	15 mins after applying lotion
50 + 30 + 20	91.37	91.14	90.53	90.12	91.76	92.42
50 + 40 + 10	91.56	91.38	90.81	90.54	92.35	93.05
40 + 30 + 30	90.05	89.99	89.22	88.81	89.5	90.03
45 + 38 + 17	91.50	91.40	90.74	90.45	92.01	92.65
46 + 38 + 16	91.54	91.42	90.78	90.49	92.10	92.76
47 + 38 + 15	91.57	91.43	90.81	90.51	92.18	92.85
48 + 38 + 14	91.58	91.43	90.82	90.52	92.24	92.92
49 + 38 + 13	91.58	91.41	90.82	90.52	92.28	92.96
50 + 39 + 11	91.57	91.39	90.81	90.52	92.33	93.03
51 + 39 + 10	91.54	91.35	90.78	90.49	92.33	93.04
60 + 30 + 10	91.26	90.90	90.41	90.00	91.99	92.72
60 + 25 + 15	91.23	90.85	90.35	89.88	91.82	92.54

summarized in Table 2. A close look at the table reveals the best matches for each spectra. A better than 90% match was obtained for all of the spectra with the spectra made by combining different percentages of water, protein and fat.

Conclusion

The SabIR fiber-optic probe can be used to study the changes occurring in the skin tissues caused by different factors. In this paper, our preliminary studies performed on skin tissues demonstrated the qualitative usefulness of the SabIR probe for observing the differences in biological tissues caused by sun and other enviromental factors. The difficulty in attaining 100% match for different spectra may be due to the fact that the combination spectrum included only water, protein and fat. Addition of other biological components might give a better match. In the future we would like to develop a more quantitative approach to this experiment.

References

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